Combat Casualty Care in Ground-Based Tactical Situations: Trauma Technology and Emergency Medical Procedures

(Soins aux blessés au combat dans des situations tactiques : technologies des traumas et procédures médicales d’urgence)

Papers prepared for the RTO Human Factors and Medicine Panel (HFM) Symposium which was held in St. Pete Beach, FL, United States, 16-18 August 2004, in co-operation with the US Department of Defense, Advanced Technology Applications for Combat Casualty Care (ATACCC) Conference.

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The Research and Technology Organisation (RTO) of NATO

RTO is the single focus in NATO for Defence Research and Technology activities. Its mission is to conduct and promote co-operative research and information exchange. The objective is to support the development and effective use of national defence research and technology and to meet the military needs of the Alliance, to maintain a technological lead, and to provide advice to NATO and national decision makers. The RTO performs its mission with the support of an extensive network of national experts. It also ensures effective co-ordination with other NATO bodies involved in R&T activities.

RTO reports both to the Military Committee of NATO and to the Conference of National Armament Directors. It comprises a Research and Technology Board (RTB) as the highest level of national representation and the Research and Technology Agency (RTA), a dedicated staff with its headquarters in Neuilly, near Paris, France. In order to facilitate contacts with the military users and other NATO activities, a small part of the RTA staff is located in NATO Headquarters in Brussels. The Brussels staff also co-ordinates RTO’s co-operation with nations in Middle and Eastern Europe, to which RTO attaches particular importance especially as working together in the field of research is one of the more promising areas of co-operation.

The total spectrum of R&T activities is covered by the following 7 bodies:

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS Studies, Analysis and Simulation Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These bodies are made up of national representatives as well as generally recognised ‘world class’ scientists. They also provide a communication link to military users and other NATO bodies. RTO’s scientific and technological work is carried out by Technical Teams, created for specific activities and with a specific duration. Such Technical Teams can organise workshops, symposia, field trials, lecture series and training courses. An important function of these Technical Teams is to ensure the continuity of the expert networks.

RTO builds upon earlier co-operation in defence research and technology as set-up under the Advisory Group for Aerospace Research and Development (AGARD) and the Defence Research Group (DRG). AGARD and the DRG share common roots in that they were both established at the initiative of Dr Theodore von Kármán, a leading aerospace scientist, who early on recognised the importance of scientific support for the Allied Armed Forces. RTO is capitalising on these common roots in order to provide the Alliance and the NATO nations with a strong scientific and technological basis that will guarantee a solid base for the future.

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Combat Casualty Care in Ground-Based Tactical Situations: Trauma Technology and Emergency Medical Procedures

(RTO-MP-HFM-109)

Executive Summary

The meeting was held jointly with the ATACCC 2004 (Advanced Technology Applications for Combat Casualty Care) meeting. This annual meeting is hosted by the Combat Casualty Care Research Program HQ, USAMRMC, Fort Detrick, Maryland. The remit of this technical evaluation report is confined to the NATO/OTAN component of the meeting.

Theme and Objective

In ground based tactical situations casualties can not be avoided. It is well documented that immediate haemostatic surgery can be life saving, and the most significant factor for survival is the time from injury to surgery. Late complications like septicemia and multi organ failure are in most cases sequelae of the initial hypo perfusion. In situations where evacuation will be delayed, the prehospital handling and management are of critical importance. In recent tactical situations with long distances to hospital, forward surgical teams have been deployed to reduce the time to surgery. Fast and correct decisions in questions of triage, evaluation and initial treatment are life saving and may reduce complications for the individual soldier. New technologies allow rapid location of casualties and advanced diagnostic aid and decision support in the field. The application of sensors to monitor vital signs and computers with embedded knowledge provide such support. Recent technology advances allow for non-invasive and remote monitoring of physiologic parameters and vital signs, thereby increasing the possibility for accurate treatment and management by ground personnel.

The main aim of casualty treatment is to secure oxygenation of critical tissues. Ventilation support, hemorrhage control and organ protection are thus crucial. Haemostatic devices such as improved bandages and tourniquets, haemostatic drugs, and agents such as platelet substitutes and oxygen carrier molecules based on perfluorocarbons or modified haemoglobin address the hemorrhagic challenge. Optimal fluid management, vasoactive drugs and resuscitation fluid additives to promote micro vascular perfusion may protect organ function and prevent organ failure and increase survival. During the last decades micro vascular sensitivity to inflammation has been identified as a major contributor to tissue hypo perfusion and various inhibitors of the inflammatory response have been tried to provide organ protection.

Observations and Conclusions

A substantial number of excellent scientific papers were presented many from world leaders in their domain and all of which had direct short or long-term bearing on combat casualty care. Outstanding science on haemostasis, shock research and resuscitation was dominant but important and cutting-edge presentations on technologies and monitoring with rationale solutions for existing problems were also offered. The Program Committee did an outstanding job in providing a meeting at which any person interested in this domain could obtain substantial information and value.
Recommendations:

- More multi-national presentations at such joint meetings, particularly from research labs in Europe.
- Future focus on:
  - The scientific basis (evidence) for combat and expeditionary injury care.
  - Epidemiology and patterns of severity of injury and databases on the battlefield and post manoeuvre expeditionary force trauma systems.
  - Blast injury research to characterize the scope and nature of primary blast injury in a context of secondary, tertiary, and quaternary injury in contemporary tactics. This particular topic would have significant crosswalk value to civilian terrorist issues.
Soins aux blessés au combat dans des situations tactiques : technologies des traumas et procédures médicales d’urgence

(RTO-MP-HFM-109)

Synthèse

La réunion était organisée conjointement avec la réunion ATACCC 2004 (Applications technologiques avancées pour soins aux blessés au combat). Cette réunion annuelle est organisée dans le cadre du Programme de recherche en soins aux blessés au combat du QG, USAMRMC, Fort Detrick (Maryland, Etats-Unis). Ce rapport d’évaluation technique est consacré uniquement au volet OTAN de la réunion.

Thème et objectif

Les situations tactiques terrestres engendrent toujours des blessés. La littérature indique clairement que des vies peuvent être sauvées si la chirurgie hémostatique est pratiquée immédiatement, et que le facteur le plus important du point de vue de la survie est le délai entre le moment de la blessure et celui de l’intervention chirurgicale. Les complications ultérieures telles que la septicémie et la défaillance multiple d’organes sont, pour la plupart, des séquelles de l’hypoperfusion initiale. Dans des situations où l’évacuation sanitaire est retardée, la gestion de la situation et les soins préhospitaliers sont d’une importance décisive. Dans de récentes situations tactiques, où l’hôpital le plus proche était très éloigné, des équipes chirurgicales de la zone avant étaient déployées afin de réduire les délais d’intervention. La prise de décision de façon rapide et juste en ce qui concerne le triage, l’évaluation et les soins initiaux peut sauver des vies et réduire les complications dans certains cas. Les nouvelles technologies permettent de localiser rapidement les blessés en fournissant aux combattants des aides avancées au diagnostic et à la prise de décision. La mise en œuvre de capteurs pour détecter des signes vitaux, ainsi que d’ordinateurs dotés d’information intégrée en sont des exemples. Les avancées technologiques récentes permettent la surveillance à distance et le contrôle non invasif de paramètres physiologiques et de signes vitaux, favorisant ainsi la dispensation de soins et la gestion de la situation appropriées par le personnel présent sur le champ de bataille.

Les principaux soins dispensés aux blessés ont pour objectif d’oxygéner les tissus vitaux. La ventilation, le contrôle des hémorragies et la protection des organes sont donc des éléments capitaux. Des dispositifs hémostatiques tels que des bandages et des tourniquets améliorés, des médicaments hémostatiques et des agents tels que des plaquettes de remplacement et des molécules transporteurs d’oxygène à base de chlorofluorocarbures et d’hémoglobine modifiés, offrent des solutions au problème des hémorragies. La gestion optimale des fluides, les médicaments vasoactifs et les additifs des fluides de réanimation qui favorisent la perfusion microvasculaire, peuvent contribuer à protéger le fonctionnement des organes, empêcher leur défaillance et améliorer les chances de survie. Au cours des dernières décennies, la sensibilité microvasculaire aux inflammations a été identifiée comme un facteur contributif majeur à l’hypoperfusion des tissus, et différents inhibiteurs de la réaction inflammatoire ont été testés pour la protection des organes.

Observations et conclusions

Un nombre considérable de communications scientifiques de haut niveau ont été présentées par des conférenciers leaders mondiaux dans leurs domaines respectifs. L’ensemble de ces communications
avaient des implications directes à court ou à long terme sur les soins dispensés aux blessés au combat. La réunion était caractérisée par des travaux scientifiques exceptionnels en hémostase, en traumatisme et en réanimation, mais d’importantes communications de pointe sur les technologies de contrôle, offrant des solutions sensées aux problèmes existants, ont également été présentées. Le comité responsable du programme est à féliciter pour avoir organisé une réunion où toute personne intéressée par ce domaine pouvait obtenir de nombreuses informations de grande valeur.

**Recommandations :**

• Présenter davantage de communications multinationales lors de telles réunions conjointes, en particulier des communications faites par des laboratoires de recherche en Europe.

• Mettre l’accent sur :
  • Les bases scientifiques (témoignages) des soins aux blessés au combat et des membres des corps expéditionnaires.
  • L’épidémiologie, la gravité des blessures, les bases de données sur le champ de bataille et les systèmes de traumatisme des corps expéditionnaires après la manœuvre.
  • La recherche dans le domaine des lésions provoquées par l’effet de souffle afin de caractériser la gravité et la nature des lésions dans le contexte de lésions secondaires, tertiaires et quaternaires dans des situations tactiques contemporaines. Cette question aurait des applications intéressantes dans le domaine du terrorisme civil.
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Technical Evaluation Report

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The HFM Panel Symposium was held jointly with the ATACCC 2004 (Advanced Technology Applications for Combat Casualty Care) meeting. This annual meeting is hosted by the Combat Casualty Care Research Program HQ, USAMRMC, Fort Detrick, Maryland and sponsored by the US Department of Defense. The remit of this technical evaluation report is confined to the NATO/OTAN component of the meeting.

The Theme and Objective of the NATO component of the meeting were identified as follows.

1. THEME

In ground based tactical situations casualties can not be avoided. It is well documented that immediate haemostatic surgery can be life saving, and the most significant factor for survival is the time from injury to surgery. Late complications like septicemia and multi organ failure are in most cases sequelae of the initial hypo perfusion. In situations where evacuation will be delayed, the prehospital handling and management are of critical importance. In recent tactical situations with long distances to hospital, forward surgical teams have been deployed to reduce the time to surgery. Fast and correct decisions in questions of triage, evaluation and initial treatment are life saving and may reduce complications for the individual soldier. New technologies allow rapid location of casualties and advanced diagnostic aid and decision support in the field. The application of sensors to monitor vital signs and computers with embedded knowledge provide such support. Recent technology advances allow for non-invasive and remote monitoring of physiologic parameters and vital signs, thereby increasing the possibility for accurate treatment and management by ground personnel.

The main aim of casualty treatment is to secure oxygenation of critical tissues. Ventilation support, hemorrhage control and organ protection are thus crucial. Hemostatic devised such as improved bandages and tourniquets, haemostatic drugs, and agents such as platelet substitutes and oxygen carrier molecules based on per fluorocarbons or modified hemoglobin address the hemorrhagic challenge. Optimal fluid management, vasoactive drugs and resuscitation fluid additives to promote micro vascular perfusion may protect organ function and prevent organ failure and increase survival. During the last decades micro vascular sensitivity to inflammation has been identified as a major contributor to tissue hypo perfusion and various inhibitors of the inflammatory response have been tried to provide organ protection.

2. OBJECTIVE

The main objective of this symposium is to present recent knowledge on war casualty management, based on experience from tactical situations and scientific research. These findings will focus on state of the art and future devices and biological agents for location, triage, monitoring and resuscitation of casualties.”
3. TOPICS TO BE COVERED

During the opening comments and welcoming addresses at the meeting, representative General staff identified further strategic objectives that the meeting should accomplish. Many of these are non-trivial in nature.

After summarizing the scope and nature of the command responsibilities of USAMRMC, Major General Martinez-Lopez, Commanding General of USAMRMC, sought to imbue the some 700 participants of the joint conference with a sense of urgency in addressing the issues of combat casualty care and stressed that the focus should be on the patient -- the combat casualty. He asked participants to examine whether they are partnering, collaborating, cooperating sufficiently to address the urgency of this agenda.

Dr. Robert Angus, Director General of Defense R&D Canada, Chairman of the NATO RTO Human Factors and Medicine Panel, provided the history and current portfolio of the NATO Human Factors in Medicine panel, the goal of which is to optimize the performance of the soldier, through research and technology in human factors (individual, group and systems), human protection and operational medicine.

Major General Roger van Hoof (BE), Chairman of the Committee of Chiefs of the Military Medical Services in NATO (COMEDS) and Brigadier General Roedig (GE), Surgeon General of the German Air Force and HFM Panel Member, stressed the changing environment making great emphasis on the need for interoperability between collaborating national forces providing medical support to military expeditionary units. “Interoperability is the golden standard and requires a global integrated healthcare systems and effective multi-national evacuation system.”

Dr. Erik Fosse (NO), Chairman of the Program Committee and HFM Panel Member, summarized the medical issues in international operations and the process by which the objectives of the meeting had been developed and papers selected.

The first session of the joint NATO/OTAN/ATACCC meeting was concluded with a panel discussion of recent experiences from Operation Iraqi Freedom led by Col. John Holcomb (US), the Trauma Consultant to the US Army Surgeon General and Commander of the US Army Institute of Surgical Research. These panels have been a standard introductory feature to ATACCC, whereat young men and women with recent and relevant experiences provide a generally unstructured account of the injuries encountered, care provided, their problems and frustrations. Representatives from helicopter CASEVAC crews, forward surgical and combat support hospitals were heard from including one young sergeant from the 101st Airborne who had received a bronze star for caring for 18 injured colleagues after himself being wounded. There was much emphasis on the need for flexibility and adaptability in medical support in Operation Iraqi Freedom as the op-tempo changed from rapid, mechanized maneuver phase to early and later insurgency phases, with changing enemy tactics, and the ability to operate from fixed and more sophisticated Combat Support Hospitals rather than FRS and FRSS settings. Persistent “needs” included:

- pediatric equipment to treat wounded and sick children
- address the problems with medical re-supply logistics
- a persistent plea for better and relevant training.

Other issues of significance that were catalogued including coagulopathy and hypothermia, ability to deliver certain blood products, the research challenges associated with duplicating the clear value of fresh whole blood (of which over 500 units had been used in theatre), the need to collect and aggregate data over an
appropriate denominator to achieve a picture of the epidemiology, nature and severity of combat casualties and their contemporary outcome, the need for adaptable trauma systems in operational settings; training, tourniquets, tension pneumothorax (as potentially preventable deaths), mortality and morbidity associated with extremity injuries, eye injuries, limitations of body armor, primary blast injury, diagnostic and monitoring devices (particularly the need for a small, easily-handled, self-contained vital sign monitor), extraction equipment, patient warming equipment, pain medication, the need for CASEVAC helicopters to talk to the ground directly as opposed to through 3 or 4 intermediaries, and the pressing need for an improved medical records system that could travel with the patient.

This panel consistently grounds researchers and others in the realities of combat casualty care, and though unstructured and somewhat unscientific in nature, continues to be of value.

Distribution of topics and papers. The NATO/OTAN component and the joint sessions incorporated some 80 presentations and posters. Although there was significant duplication between some of the presentations and posters, the relative distribution by topic is given in the table below.

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<th>Resuscitation including TBI</th>
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<td>3.</td>
<td>Wounds &amp; Extremities</td>
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**Resuscitation.** As the global deaths from injury continue to rise towards 10 million by the time we are in the second decade of the 21st Century, some 2 to 3 million individuals die per year from post-traumatic hemorrhage and its sequelae improved if not patient specific resuscitation from hemorrhagic shock is a high priority in both civilian and military circles. Low cube weight resuscitation fluid has long been a military research priority. Some twenty years ago (1984) US ARMY MRMC embarked on a mission to provide such a solution. This resulted in Hypertonic Saline Dextran, a product though in use in Europe is still unavailable in the United States. It is fitting that the first scientific paper of this joint symposium should focus on Hypertonic Saline/ Hypertonic Saline Dextran solutions.

Shawn Rhind gave us a masterful summary of the immunological effects observed in series of animal, in vitro and clinical studies, cataloguing beneficial effects on IL CD62L, Beta integrin CD116, the down regulation of PMN/endothelial cell interaction through CD116 CD62L decrease in PMN chemotaxis, increase lymphocyte function, reduction of endothelial cell permeability and improved organ function alterations in cellular signaling and increase in IL10 productivity by macrophages.

Hypertonic Saline Dextran (HSD) has been shown to be safe and effective in eight clinical trials. In recent experiments in the Sunnybrooke, University of Toronto lab, normal saline was shown to up-regulate flow inflammatory lymphocytes. This did not occur with HSD. HSD blindered the inflammatory response to LPS stimulus, enhanced serum-soluble L selectin, inhibited LPS-induced and spontaneous increases in TNF alpha. IL 10 was shown to go up dramatically. The alpha adrenergic inflammatory response to shock found with normal saline was not present with Hypertonic Saline Dextran.
This excellent body of science was further supplemented by a paper contributed by four of the leading scientists involved in research on hypertonic fluid resuscitations (Kramer, Wade, Dubick, and Atkins). George Kramer provided an overview of state of the science on a small volume, hyperosmolar, hyperoncotic resuscitation fluids, and HBOCs. He also noted his recent work on closed-loop resuscitation systems. Eighty-two trials with 7.5% solutions have been conducted, all under powered for survival in shock, but clearly showing that the solution provides early, fast, effective volume replacement and vasodilation with immunomodulation and a mortality decrease of 4.3% when used as HSD. HSD has been shown to be particularly beneficial in head injury and penetrating injury.

It was clear from these discussions that work will continue on optimizing resuscitation fluids. It is indeed fitting that the first injury related, multi-center trial to come from NIHLB/USAMRMMC collaboration will focus on HSD (HS) resuscitation. This 10-center clinical consortium, jointly funded by the National Institute of Health and the US Army MRMC, will eventually provide rapid translational research for the basic sciences devoted to resuscitation, hemostasis and other injury-related research. It is a much needed and urgently required effort that will allow adequate powering and rapid accumulation of appropriate patient populations.

Dr. Pang Shek stressed the considerable value of this effort which he has been promoting for a number of years, stressing that within 5 years substantial results are likely to be able to be taken to the point of wounding provider. Dr. Shek also discussed the NATO Response Force, launched in November 2002 wherein there is a need for a rapid response within 5 days for 30 days – “first force in and out” and thus the urgent need for interoperable medical support systems for such a force.

The ceremonial signing of this agreement occurred at the closing session of the first day of this joint NATO/ATACCC meeting.

Other cutting edge resuscitation strategies included:

**Vasopressin.** Summarizing many years of research funded by the Office of Naval Research, Landry specified the effects of this drug in cardiogenic, septic and late phase (vasodilatory) hemorrhage shock.

Proctor, from the University of Miami also ONR funded, presented data from a fluid percussion, TBI model with shock showing benefits in terms of fluid needs, ICP and cerebral compliance, but expressing some concerns about decreased cardiac index and possible bowel ischemia in this non-vasodilated shock model.

- Another exciting adjuvant is Trans Sodium Crocetinate, which improves O2 consumption in shock by changing the O2 diffusivity by alterations in the structure of water. This tantalizing animal data will hopefully soon result in human tests by Gainer’s lab at UVA.
- Other promising adjuvants presented at the meeting included a world-class summary of the use of complement inhibitor APT070. The use of which would merely require a change in labeling in this already FDA-approved drug.
- A number of papers gave the state of the art on HBOCs. Bovine polymerized hemoglobin (HBOC-201) is being studied in animal models by the Naval Medical Research Center who reported on its hemodynamic, coagulation and immunological effects compared with Hextend® and controls, in hemorrhagic shock models in swine for both controlled and uncontrolled hemorrhage. Kramer and Dubick also updated us on the use of the competing Polyheme®. These oxygen-carrying colloids appear to reduce the need for volume but may still have some issues in terms of nitric oxide
scavenging and increased systemic and pulmonary vascular resistance; though nothing like as bad as those that were found in the Baxter product some years ago. Polyheme® and Hextend® were also studied by Sondeen at the ISR with respect to prolonging resuscitation and preventing “pop-the-clot” rebleeding.

HBOC’s are on the verge of being part of a therapeutic armamentarium but their precise role in the treatment of early shock management remains a little unclear and requires to be defined. Questions also remain regarding the appropriateness of shock models without tissue damage and the complexities and variabilities in uncontrolled hemorrhage shock states.

Hemostasis. This topic is receiving substantial attention from many researchers and is producing results which can be near term fielded. Particularly exciting was the evident interlinkage and crosswalk between immunology and coagulation which is providing a fertile and important field of research focus.

- Recombinant rFVIIa. One of the hottest topics in the whole arena of combat casualty care is the great promise shown by rFVIIa in patients with severe hemorrhage. Dr. Uri Martonovits, the father of rFVIIa use in this domain (the drug was originally created for hemophilia), gave the conference participants an overview of its use in combat casualty care, identifying roles not only for adjuvant use in patients with serious and uncontrolled hemorrhage, but also emerging potential value in patients with intracerebral hematoma from traumatic brain injury and the hemorrhagic consequences of primary blast injury to the lung. Dr. Martonovits deliberately extrapolated the value as being more likely with early use to the point that he suggested that in the future it might be given as a prophylaxis for high risk Special Forces missions.

- Sandro Rizoli reported on a subset of patients from the recently completed controlled trial of use of rFVIIa in severe hemorrhage conducted in a number of European countries, South Africa and Singapore, identified as those who received coagulation factors in the presence of massive transfusion. Dr. Rizoli reported the reduced need of red blood cells and fresh frozen plasma and platelets in those receiving rFVIIa when compared with placebo controls. He also pointed to a trend towards reduction in multiple organ failure and ARDS but no impact on death. Lively discussion identified concerns with respect to:
  - the operational definition of patients by using coagulation factor administration as both a dependant & independant variable
  - disappointment that there was no reduction in death from hemorrhage
  - concerns about cost effectiveness in that the cost of drug administration far outweighed the savings in administrating blood products.

Despite these concerns there was general approbation regarding the fact that a controlled trial had been conducted, the difficulties of which in this patient population cannot be underestimated, and the fact that the good safety record had caused the FDA to withdraw their clinical hold on the use of this exciting and extremely promising drug.

Tourniquets. This topic continues to be the focus of lively discussion and debate. The surgeon’s perspective was ably presented by Dr. Pillgram-Larsen who emphasized the value of compression dressings and urged the limitation of tourniquets to the tactical environment. This approach indeed comports with the pleas of the point of wounding care medics who continue to find value in a tourniquet, but would wish an improved
design. It was somewhat stunning to see a recent picture from Iraq with sticks used to tighten cloth tourniquets—a technology that has been around for centuries. The failure of the one-handed tourniquet to do the job has provoked substantial effort at the ISR to identify the requirements for optimum tourniquet. A later presentation on technologies from Dr. McEven & Inkpen (from one of the leading companies that that makes tourniquets for use in surgery) elaborated on optimal tourniquet designs and their efforts to transport lessons learned in tourniquet use in surgical practice (some 20,000 users per day in the US) to the point of wounding care provider. As surgeons we do not see those that die from lack of tourniquet use in the field. There have been at least 8 such incidents in Operation Iraqi Freedom.

**Dressings.** Many researchers have been developing and evaluating a large number of dressings with hemostatic or other worthy attributes. The Army Hemostatic Research program has tested some eleven dressings with further evaluation on three.

- Martineau (DRDC) presented data on a bi-layer wound dressing. The hydrogel layer prevented drying of the wound and a foam layer provided effective drug delivery. This absorbable, flexible, strong, non-adherent dressing showed promise for both burns and other wounds.

- A formidable array of research from the US Army Institute of Surgical Research presented a comprehensive research program addressing controlled and uncontrolled hemorrhage. These include the hemostasis research program (Ryan), evaluating compressible hemorrhage and strategies to strengthen clot formation using rFVIIa and to manage resuscitation to prevent the “pop-the-clot” phenomena that occurs as the systolic pressure recovers above 90 mmHg, (Sondeen) thus reducing the problems associated with re-bleeding as a consequence of successful initial resuscitation. Dubick gave an overview of the hypotensive research strategies using Hespan, Hextend® and bovine polymerized hemoglobin in controlled and uncontrolled hemorrhage models. This joint Army/Navy program was particularly focused on issues of uncontrolled hemorrhage.

- A structured set of research strategies for non compressible, uncontrolled hemorrhage was presented by both Drs. Sondeen and Kheirabadi. The latter exposed a number of experiments using foam and other agents to induce clotting in closed intra-abdominal hemorrhage. These interesting results are yet to yield a product worthy of clinical consideration but this important research line continues.

- Martini, also from the ISR, presented an elegant and important series of experiments in hemorrhagic shock swine identifying the impact of acidosis and hypothermia individually and collectively on coagulation and thus unveiling separate and complementary strategies for addressing this vexing clinical problem.

**Monitoring & Diagnosis.** A number of works were presented on cutting-edge techniques for monitoring shock states including two papers from the University of Minnesota on cellular energetics and near-infrared spectroscopy to discriminate between likely and unlikely survivors.

Trans-dermal non-invasive monitoring of glucose and lactose was presented using a micro-fluid electro-chemical chip with a wireless transmission range of up to 100 meters. This small monitor could be placed on the individual soldier and function up to 2 hours. Another soldier-centric monitor was the capacitance-coupled ECG monitor which provided ECG trace without skin contact. Other research work discussed the Armored Ballistic Impact Protection System for intended use with the Future Force Warrior together with some very preliminary data. A conceptual presentation arrayed the set of sensors that could be used in the Future Force Warrior detecting vital signs and providing data on the environment for both monitoring and decision support for the medic.
Refinements in triage have been taken very seriously by researchers. A number of posters presented data on sequential analysis of vital signs. Perhaps the most interesting work in this arena comes from the Institute of Surgical Research where continuous prehospital vital signs monitoring in patients has yielded valuable insights into the changes in pulse volume and pulse pressure in early pre-hypotensive shock states. There appears to be little doubt that measurement of the RR interval and information on pulse pressure or pulse volume could substantially improve the ability to detect individuals who are bleeding prior to hypotension developing and thus obviate severe shock states being taken as the threshold for action. In theory, such information could be coupled with time between injury and the identification of sub-clinical shock thresholds, enabling prognostication of the time interval available before definitive therapy must be available and aiding in the titration of resuscitative and coagulation therapies that lengthen this window of opportunity in tactical settings.

The end user (medic) requests for simple, reliable wireless vital signs monitor was heard by Commander Peter Rhee who presented a demonstration of a wireless COTS system that could be made available now and monitor a number of patients at level 1, 2, 3 facilities. Other monitors presented included systems directed at non-invasive monitoring for brain blood flow. A number of others connected sensor suites to decision-support systems to help in triage and treatment decisions. There were at least three solid attempts to integrate vital signs monitoring and ventilator functions. Ultimately these systems will provide closed-loop care with ventilation being driven by end-title C02 and/or compliance, oxygen saturation providing closed-loop direction of FIO2 and measures of shock state guiding intravenous and transfusion therapy. Such devices would ameliorate the risk associated with transfer of patients between level 2 and 3 and would be of particular value in patients that have had damage control therapy at level 2 requiring definitive care at level 3 capabilities.

Researchers also presented data on predictive blood gas analysis for patients needing strategic air evacuation.

Wounds -Fracture Healing

- In Operation Iraqi Freedom, as in all wars in the past 150 years, limb and soft tissue injuries predominate. With increasing interoperability a small light radio-lucent, non-magnetic, low-cost external fixator is sorely needed. NATO has issued a STAMAG for such a transport fixator. New designs responsive to this were presented.
- A number of leading researchers with decades of expertise presented technologies to improve wound healing including photodynamic therapy and low-energy photonic therapy (LEPT). With open fractures constituting the majority of fractures occurring in combat, delayed healing and non-union as result of infection. An antimicrobial bone graft demonstration identified that such technologies were available and indeed could be shortly deployed to address these problems.
- Important contributions were heard on pain management with both basic science research for a morphine replacement and data on use of nasal Ketamine and the Fentanyl lollypop.
- One paper presented the use of training simulators identified substantial skill decrement on performance under stress situations.

The Marine Combat Trauma Registry emphasized the value of systematic data collection in identifying the epidemiology, severity of injury and outcomes of injury and actual workload at various levels of care.
Observations and Conclusions

A substantial number of excellent scientific papers were presented many from world leaders in their domain and all of which had direct short or long-term bearing on combat casualty care. Outstanding science on hemostasis, shock research and resuscitation was dominant but important and cutting-edge presentations on technologies and monitoring with rationale solutions for existing problems were also offered. The Program Committee did an outstanding job in providing a meeting at which any person interested in this domain could obtain substantial information and value.

Had time permitted on an already very full schedule, topics of interest that would have had a ready audience include:

- **Trauma Registries & Trauma Systems.** Contemporary aggregated data on a valid denominator can provide us with information on the epidemiology, severity and nature of wounds and outcome. This would allow for identification of roles of various medical care resources in different phases of combat and more effectively matched need and resources in planning. There have been over 7,000 US casualties in Operation Iraqi Freedom and 935 deaths—every single one of which has received a complete autopsy. Registry data is being collected by the United States Navy and Marines, United States Army and the British Army. Information from these data would surely help guide and prioritize research and primary, secondary and tertiary tactics, techniques and technologies of injury prevention. It would provide a better understanding of systems of care needed and it would thus provide a substrate for precision implementation of the trauma systems that Colonel Holcomb advises us to implement. Trauma registries have played a substantial role in the civilian sector for the past twenty years and are sorely needed for combat settings.

- **The use of IED’s** has been a dominant factor during the insurgency phase of Operation Iraqi Freedom with different tactics and use in combination with small arms causing devastating injuries. The known availability of infantry mobile TBX and the likely use of such weapons mandate a focus on explosive injuries, particularly primary and tertiary blast with a view to protection, prevention, deflection and definitive treatment.

- **Problems associated with the medical resourcing**-accurate and effective matching need with available resources and the failure of the logistic train to adequately supply and re-supply were noted, but few solutions offered.

- **There needs to be a structured approach to transatlantic collaboration.**

Recommendations

- **More Joint Meetings**
- **More multi-national presentations at such joint meetings, particularly from research labs in Europe.**
- **Future focus to include:**
  - The scientific basis (evidence) for combat and expeditionary injury care
  - Epidemiology and patterns of severity of injury and databases on the battlefield and post maneuver expeditionary force trauma systems
  - Blast injury research to characterize the scope and nature of primary blast injury in a context of secondary, tertiary, and quaternary injury in contemporary tactics. This particular topic would have significant crosswalk value to civilian terrorist issues.
- Develop a database using “portal” system to catalog available research and opportunities for collaboration.
- Surgical infection and morbidity with a view to developing multinational clinical trials on primary, secondary, tertiary prevention approaches to the morbidity sequelae of combat injury
The Committee of the Chiefs of Military Medical Services in NATO (COMEDS) and its Relation to RTO/HFM Panel

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COMEDS AND ITS RELATION TO RTO/HFM PANEL

In an attempt to look a few years ahead and sense what current and foreseeable developments in society, medicine and the military could mean for our profession as military medics, future challenges for military medical professionals are described.

Which role COMEDS play to facilitate the adaptation of our multinational military medical community to these evolutions and the support RTO-HFM Panel can give to COMEDS is developed.

1.0 THE SECURITY CHALLENGES OF A PROFOUNDLY CHANGING POLITICO-MILITARY ENVIRONMENT.

Over hardly a decade, the speed of change within our societies has increased dramatically.

Some of the most striking phenomena affect fundamentally, both as individuals and as societies, our perception of the hazards that may hamper our safety, well being and way of life.

One factor is the unprecedented globalisation and interdependence of human activity. This leads to increased mutual contacts and mobility of people, goods and ideas. People are well aware that lingering crises in remote parts of the world may indirectly affect supply lines of energy, stock markets, weather patterns, global economy and finally their jobs and families. But at the same time, it may directly confront them with terrorism, when taking their next business or holiday flight.

This evolution had already been accelerated by the media revolution. The so-called CNN factor became famous during the first Gulf war. What has been added to that is the incredible expansion of web accessible information and Email contacts over the last years. Today hundreds of millions of almost instantaneous individual information exchanges take place, without any state authority controlling this flow. This fact is an incredible factor of freedom, as well as an enormous risk factor. This is because the quality of the information can be quite poor and the mass magnitude of this medium can turn it into a real psycho shock wave, which is an identified prime terrorist goal. Let alone the so called “cyber attacks”,

which can virtually paralyse entire sectors of society. Defending against these hazards has become a multi billion business.

Add to this the fact that there is a diagnosed “democratic deficit” in an increasing number of inter- and multinational organisations. This phenomenon is exacerbated by the lack of accountability for decisions taken by multinationals and by the global networking of organised crime.

All these factors lead to a de facto decreasing influence by the individual States on the course of action in crucial domains, such as defence, finance, and even provision of welfare.

At the same time, this situation increases the likelihood that relatively small states, or non-state actors, organisations or groups of extremist use asymmetric means, in order to impose their political or financial aims on a global scale.

The sociological fact that aging western societies face growing immigration pressures, due to demographic evolutions, mainly in the south, fits in this anxiety model.

Sociologist predict increasing competition for shrinking natural resources, such as water and energy will boost instability in major parts of the globe. Even without further proliferation, some states in these regions are known to have weapons of mass destruction (WMD) today.

While conventional war will remain the means of last resort to resolve inter-state confrontations, the majority of future conflicts will be asymmetrical.

The rise of non-state threats is a tremendous problem for Western governments and militaries, because we are legally and behaviourally prepared to fight other legal-basis states. Furthermore, although the human suffering is the same, one could cynically say that personnel casualties within typical four member families have greater society impact than in societies with a traditional demographic overflow. The reluctance within our western type nations to cope with the inevitable casualties of conflict is a well-known weakness known worldwide. This makes terrorist or guerrilla type actions causing lots of casualties the more attractive for those who are technically in the underdog situation.

It obviously has made it crucial to “win the media war”. Media coverage now has a dramatic effect on public opinion, morale of troops and finally on political sustainability within world-wide coalitions.

2.0 HOW ABOUT NATO?

NATO has spent decades in the so-called “Cold War era”. This period was characterised by the mutual nuclear deterrent and a vast geographical spread of numerous army divisions along a static border. The enemy was “doctrinally templatable” and Alliances were fixed. Nations had draft systems in place, so manpower was not a problem. Plans anticipated huge casualty rates in case of a major combat between these heavily armed and mechanised forces. The mutual nuclear deterrent, a predictable way to mutually assured destruction, worked.

Since the collapse of the former WARSAW Pact, which culminated in the fall off the BERLIN Wall and the whole Iron Curtain, the strategic environment has dramatically changed.
This period was followed by the NATO led operations in the BALKANS, which started in the early nineties and are still ongoing. This is the era of the Peace Support Operations. Its characteristics are quite different. After having agreed, or forced to agree by the multinational community to a peaceful end of a war situation, a multinational force separates belligerents. They gradually evolve under military presence and political guidance towards a peaceful cohabitation. These ongoing peace support operations continue, as a shared burden, whilst NATO Nations continue to downsize their post Cold War Forces and the Alliance will have expanded to twenty-six Nations.

Yet, a new historical trend has profoundly changed the transatlantic Alliance, since the nine-eleven terrorist attacks on the United States.

NATO was focussed on the state-centred threat and was not geared for non-state menaces.

In fact, the NATO summit in PRAGUE set out the new beacons for NATO in the new security environment. It stated “NATO needs the capability to field forces that can move quickly to wherever they are needed and sustain operations over great distance, including in an environment where they might be faced with biological, chemical and nuclear weapons.”

This declaration ended the “out of area” debate in NATO. NATO is now involved in its first out of area operation in AFGHANISTAN.

Key evolutions within this new approach are the creation of the NATO Response Force, which is designed to operate in a high intensity environment. It will be kept at short notice, will be sustainable on its own for at least 30 days and will be able to draw on designated specialist capabilities, including a dedicated NBCR battalion.

A permanent matter of concern in NATO is the capability gap between the United States and its European Allies. This could lead to a “de facto” role for the US and coalitions of the willing in high intensity warfare and a specialisation of European Nations in “mop-up operations”.

Indeed, the European Rapid Reaction Force is more oriented towards peace and stability operations around Europe.

### 3.0 THE CHANGING MILITARY IN NATIONS.

Many European Allies are now underway in creating smaller, lighter, more mobile Forces, which are sustainable over longer periods.

The war in IRAQ showed us again the increasing use of remotely controlled precision weapons, and a dramatic evolution towards network-centric warfare techniques. It also shows us that despite a quick dismantling of the classical military means of the adversary, keeping the peace in regions where the population is traditionally armed, can pose a serious and costly challenge in both forces needed and casualties suffered.

The US Joint Forces Command and other NATO nations staffs currently develop new joint missions doctrines. These are meant to provide the services with a blueprint for joint missions in an age of unpredictable enemies and undefined battlefields. Major combat, stabilisation, homeland security and strategic deterrence are the main doctrinal components.
In the end, what matters most in war is what is in the mind of one’s adversary. It is clear that the asymmetric warfare is one aspect emerging from the superpower unbalance in classical conflict. Modern western technology created the intelligent weapon. Ironically, this million-dollar weapon is also to strike pre-emptively to destroy or deter so-called “rogue states”, but also terrorist groups of all kind.

These groups easily recruit amid thousands fanatic, frustrated and religiously determined young people. Once “loaded” with some explosives, they become cheap and deadly accurate “intelligent human bombs”.

Our armed forces are faced with three pressures: falling defence budgets; rising costs of both equipment and personnel; and demands for more spending for increasing new roles and missions.

The transition in most Nations to an all-volunteer force has driven the manpower cost. Most armed forces have a massive shopping list for both new equipment and personnel, which far exceeds the available means. As a result, not all these requirements can be met and harsh choices are to be made.

For equipment, the choice might be cancellation or delays for new programmes and smaller orders. For military personnel, the result is usually over-stretch, longer periods away from home, shortages, and a greater willingness to substitute reserves and civilians.

Today, the individual casualty is a substantial loss for any deployed force.

One consequence is that the pressure to improve the so-called “tooth to tail” ratio will ever increase. The ratio was one to one during the Cold War.

As an example: it is currently 70% in favour of non-combat support and infrastructure functions in the US Defence budget.

It is precisely this trend to make logistics, medical and general infrastructure pay more within shrinking defence budgets that pushes these support functions to adopt ever “lighter” and more flexible solutions.

We also see the implementation of the civilian” just in time delivery” principle on military logistics. In civilian life, this has dramatically increased the number of trucks on our roads and decreased the number of warehouses. In transit visibility is now the main challenge for the operational manager. Thus the trend to reduce the “logistics footprint” in operations continues.

It is also the main driver behind options for outsourcing (example: third party logistics support) and off the shelf commercial solutions. So the “lean and mean” force, focussed mainly on the combatant core business of the future is coming up quickly.

Quite obviously this evolution pushes medical support further on the road of stabilising techniques and consequent early aero medical evacuation. Medical support installations providing the comprehensive package of specialist care in theatre will become ever more rare an asset. They will increasingly be found in modular and containerised task tailored formats on board of support ships or as host nation support facilities in adjacent countries.

In the civilian sector, rationalisation through automation is often the option. Ultimately the question of the replacement of some of the combat functions by remotely controlled drones and robots comes nearer.
4.0 THE CHANGING MEDICAL PROFESSION

In civilian health care, ever more emphasis is put on preventive action in order to reduce morbidity as a whole. Great effort is performed to restore social functionality of the individual patient.

Budgetary pressure will continue to promote new techniques, which should produce more outpatient care and shorten hospitalisation periods.

A well-established emergency care system continues to enhance its responsiveness, even in remote areas. Helicopter evacuation and emergency intervention care teams bringing advanced trauma support techniques to the spot of the traffic accident are quite common standard in most of our Nations.

Telemedicine and teleconsulting techniques as well as medical data transmissions continue to develop. These means offer “off the shelf solutions” for situations where scarce medical staffs should be engaged in more cost-efficient ways.

Although considered as disparate and still inadequate by many, civilian authorities have recently made efforts to enhance the responsiveness of the civilian medical infrastructure in dealing with the potential consequences of WMD.

5.0 WHAT DOES IT ALL MEAN FOR THE MEDICAL SERVICES?

The inevitable trend to do more with less is likely to continue. At the same time, the political leadership, public opinion, the operational commander and the individual soldier and his family expect nothing less than the top performance to reduce the number and the consequences of casualties. Ever more effective body armour reduces fatal casualty numbers but increases significantly the medical challenge to save the survivor’s limbs. As a result, a greater percentage of wounded may need longer hospitalisation and rehabilitation. The ratio of disabled veterans may well increase.

Senior medical staffs will have to continue to make the case for their function. This is to be done in an environment where budget constraints boost interservice competition and concurrence amongst functions.

Military decision-makers quite naturally tend to minimise health and medical support matters, especially in the planning phase of operations. Taking into account that casualties mean some degree of defeat or failure and medical support installations are a considerable logistical burden to move and support, operational planners generally tend to see things too optimistically.

A positive evolution is a better understanding within the military of the importance to take care of pre and post deployment health issues. Preventive and post deployment reconnaissance and screening can substantially reduce both the occurrence and consequences of deployment related health issues. The myth of the young, healthy, well-trained and invulnerable fighter has happily vanished in favour of a more prudent approach of preventive health and veteran issues.

Medical support concepts will have to focus ever more on supporting smaller, more mobile units, equipped with greater precision firepower. This might imply the need to push forward life saving techniques to smaller unit levels, especially in Special Forces type operations. It will speed up the debate
of which medical, paramedical and non-medical personnel should be trained and equipped to perform which medical life-saving acts in operational crises situations. Asymmetrical conflict situations means that medical installations, road and air ambulances may loose the theoretical Geneva Convention protection they might have had in interstate conflict.

In the new asymmetric environment, the current clear-cut line between military and civilian medical care will increasingly prove artificial. Press and interpreters accompanying troops, urban warfare in a chaotic civilian environment, guerrilla type suicide attacks on troops in such an environment. The co-ordination with NGO in the post-conflict environment. The potential for WMD type attacks on troops and civilians in a “mixed environment” and how a restriction of movement policy would affect the overall sanitary situation. All these situations need to be addressed within the broader civil-military dialogue.

Let’s not forget the evolution to the use of Non Lethal Weapons. We look at laser blinding, high energy beam shocking, foam immobilising, psycho vapours or rash-provoking means. All these will not be lethal, but surely will have some strong “incapacitating” medical effect. This means that medical specialised surveillance and care for the users and for those incapacitated will be needed. If this trend continues, we might need the ophthalmologists, psychiatrists, dermatologists and others back. These are the specialists we are dismissing today in trying to further downscale our military medical services.

In the longer run, we will need to move towards common capabilities. The greater size medical support (such as role 3 field hospitals and strategic aero-medical evacuation) are certainly up for potential pooling of efforts; certainly if we want these facilities to be able to function in CBRN environments.

The ability to medically support troops in a CBRN operational environment is key to the overall credibility of WMD defence. The medical function should dispose of the staffing and means to handle this kind of challenge. Too often concepts and plans dismiss CBRN as a prevention, warning and decontamination problem. Whilst this may be true for the CRN aspect, Bio defence will not be credible without capable medical support.

All this enhances the need for sound medical staffing and the profile of medical advisors within our organisations. Unfortunately, there is currently no consensus amongst nations about where to fit adequately the medical function within multinational staff structures and at which level it should be allowed to sit at decision- makers tables. It is clear that the new type of operations will increase the need for direct near real time web-centric co-ordination between the operational manager and the combat medical support elements at all levels.

6.0 WHICH ARE THE MEDICAL PLANNING PARAMETERS NEEDED?

For more than a decade, NATO and NATO nation’s medical planners have used the medical planning parameters contained in the so-called ACE Directive 85-8. These generic casualty rates had been predominantly based on World War and Cold war statistical data. They were focussed on a deliberate presumption, which was the so-called 75 percentile. The choice was to generically plan for a medical support system which should be able to cope at all support levels with 75% of all historically known casualty data. This made it a robust planning tool for planning medical support for major mechanised combat operations. Its strength was the fact that NATO and NATO staffs had consensus on this approach and it also supported the force planning for robust medical support within nations. (Despite substantial erosion in recent years, we still draw today on these resources).
The need for an adaptation of these planning figures arose when NATO started the so called Peace Support Operations (PSO) in the BALKANS, which dealt with post conflict operations. Although the robust generic capacities were more than sufficient, justified criticisms mentioned the lack of fine-tuned figures and a capacity rather than a capability driven approach. In these days, the “small footprint” for logistics and the “zero casualty option” were the new benchmarks for all planners.

In fact, today’s medical support should be predominantly driven by the capabilities needed to provide top level medical support to the individual casualty. So, the question of when at latest (so where?) should we be able to provide life and limb saving surgery becomes more important than the total number of beds we can deploy. Of course this approach points quite logically to more flexible and smaller units, rather to pushed forward quality than to mass capacity for big military battle support.

Currently, efforts are underway, mainly by the supreme Allied Command Transformation medical staffs and the COMEDS work group on structures, operations and procedures, to develop a new planning tool.

Nations collected data from the past decade operations should form a sound bases for a capability driven analyses and a consensus about a common planning tool. A broader understanding of the individual staff section’s responsibilities in planning medical support should benefit to their credibility and Command support for the consequences of the findings. Once available, reliable NBCR planning factors should be added to the tool. In fact, one cannot currently plan for credible medical support, ignoring the possibility of NBCR consequence management.

7.0 NOW, WHAT IS COMEDS AND WHAT COULD IT CONTRIBUTE TO ADDRESS THESE NEW CHALLENGES?

7.1 COMEDS, the history

For more than two decades before the creation of COMEDS existed EUROMED, which was the forum for the Chiefs of Medical Services of the EUROGROUP (founded in November 1968). The value of such a forum was also recognised by the highest medical authorities of the US, CA and FR. The delegates of these Nations actively participated as observers in the work of EUROMED, together with the medical representatives of the Major NATO Commanders. So, even before its transfer to NATO, EUROMED had already become an unofficial authority on military medical matters in NATO. It was meant as a forum to promote mutual understanding, co-ordination of operational principles and procedures and exchange of medical information between the member medical services.

Although initially EUROGROUP Ministers had agreed in Dec 92 to the transfer of all EUROGROUP activities to the Western European Union, it was subsequently decided on 22 May 92 to make EUROMED available to NATO as the most appropriate medical umbrella organisation.

The MC approved on 22 Oct 93 with MC 335(military Decision) the establishment of COMEDS including Terms of Reference and the Council noted that on 6 Dec 93.

7.2 COMEDS, the structure

As you know, today COMEDS represents the Nations and NATO medical communities at the most senior level. It is composed of the 19 Surgeon Generals, both Strategic Commands medical advisors and the IMS medical staff officer. The West European Union medical staff officer, the Military Agency for Standardisation and the Civil Emergency Planning Directorate Joint Medical Committee attend as non-
voting observers. Belgium provides the chairman, the staff officer and the secretary for COMEDS work from their national positions. The secretariat is located in the infrastructure of the Belgian Chief of Defence Staff. COMEDS has no budget; it has a liaison desk at the NATO International Military Staff.

COMEDS meets in plenary meetings twice a year (spring and autumn meeting at NATO HQ).

It has 9 specialised working groups, chaired by a Nation. These WG normally meet once a year (only MMSOP meets twice a year).

These working groups are the following:

- WG on dental service (DS) : chaired by GERMANY
- WG on emergency medicine (EM): chaired by ITALY
- WG on food hygiene and food technology (FHTVS): chaired by GREECE
- WG on medical materiel and military pharmacy (MMMP): chaired by UK
- WG on military medical structures, operations and procedures (MMSOP): chaired by GERMANY
- WG on military psychiatry (MP): chaired by UK
- WG on military preventive medicine (MPM): chaired by US
- WG on medical training (MT) : chaired by DK
- Standing group of partner medical experts: chaired by IRELAND

COMEDS is the Senior Committee responsible for co-ordination of military medical matters in NATO. Its direct reporting to the Military Committee symbolises the direct access of the medical advisor to the Commander at all times and at all levels. The reason for this sound principal is the sensitivity of the very specific, human resources related activities, and the medical communities are responsible for. This specificity does not interfere with the fact that, in NATO, medical is part of the broad definition of logistics and its activities should always be closely harmonised within the logistics family as well, of course.

I think we all realise that the ever expanding and multi-faceted challenge and risk environment our troops are confronted with can only enhance the need for sound medical advice to the Commander at all levels and well prepared, capable and flexible medical support to the men in the field.

COMEDS and the NATO Agency for Standardisation are currently underway in developing a new architecture for the military medical working groups. When accepted by the Military Committee, this new structure should increase effectiveness and transparency and allow a more streamlined management of activities.

### 7.3 Co-ordination within the medical community

The fact that the Medical Advisors of both Allied Command Transformation and Allied Command Operations and the International Military Staff medical staff officer participate as full members in the COMEDS plenaries, guarantees the best circumstances for coherent consensus building and decision making at the policy making, doctrinal and conceptual levels. The sound principles of multinational medical support, coherently embedded within the broad logistics policies, have been created over the last years. Both Military Committee 326/2 and Allied Joint Publication 4-10 form the cornerstones of multinational medical planning, accepted by the Nations and NATO. COMEDS receives liaison reports from NATO Standardisation Agency and the Senior Civil Emergency Planning Committee/Joint Medical Committee. The COMEDS chairman reports to the Military Committee and provides a liaison report to the Senior NATO Logistics Conference.
7.4 COMEDS, the way ahead

Facing shrinking defence budgets and manpower ceilings, many NATO Nations tend to progressively transfer manpower from combat service support functions, including medical, to combat and combat support functions.

Nations last decades Crises Response Operations experiences, fortunately, confirm the impression that recent casualty rates are strikingly low, although local mass casualty situations can never be excluded.

Disease and non-operational injuries (like traffic accidents) form the bulk of today’s operational medical workload.

As a result, peacetime standards of timely evacuation and adequate treatment of the individual casualty govern the number and locations of medical installations deployed and the evacuation means needed.

This « new » situation increasingly drives Nations tendency to rely more heavily on reserve forces with lower readiness status for the Art 5 situations. The scarce remaining active duty units cover the minimum needs to support CRO contributions.

The scarceness of medical personnel has now become the driving factor to speed up the multinational integration of medical support structures.

In fact, the scarce, highly qualified and difficult to recruit medical personnel is facing more and more frequent operational tours of duty, interfering with their normal high workload in peacetime hospitals. In most cases they must experience the frustrating situation of technical underemployment during their deployment tours.

On the other hand, we observe an increasing need for medical preventive actions, before, during and after deployment and the growing sensitivities of the troops and the public opinion for the long-term health hazards of the operational environment.

In the coming years, the inevitable trend « to do more with less » will continue. We should, however be aware of the trap. Greater efficiency due to multinational co-operation is a positive evolution, but should not become the national excuse to further erode national medical capabilities into real operational showstoppers.

NATO and Nations operational and medical planners must find the right balance between enhanced multinational effectiveness in the field, increased demands for preventive medicine and the paramount need to maintain sufficient national surge capabilities for combat operations. All these challenges will enhance the need to form capable, multinationally oriented medical advisors, technicians and planners.

8.0 HOW CAN RTO/HFM BE SUPPORTIVE IN OUR COMMON EFFORT

Surgeon generals and senior medical advisors are responsible towards the highest military and political decision-maker. They increasingly have to co-ordinate multinational medical support policies. To be able to do that, they themselves need sound staffing and technical advise. These should be based on the best available scientific and operational data and state of the art techniques and principles.
Quite obviously, RTO/HFM should continue to play a key role in supporting this process, since it can provide coherent bases for operationally oriented research initiatives.

A few good examples are the following:

RTO/HFM could support efficiently the current effort of ACT medical branch in reviewing and updating casualty rates for combined joint operations. ACT has requested Nations to provide their epidemiological morbidity data and will produce outsource analyses of these data. The scientifically statistical results should form the bases of a new casualty-planning tool to replace the current ACE Directive 85-8 model.

Another example is the Canadian study you performed about EPINATO and disease surveillance. This work has been of great help for the COMEDS to clarify the way ahead in this crucial field.

Because COMEDS wanted to exclude overlapping activities, it decided a few years ago to disband its research workgroup. It has become increasingly clear that RTO/HFM has an important role to play in supporting the COMEDS scientifically and meet its scientific research-oriented needs.

9.0 CONCLUSION

Our analysis showed that the medical community has already made considerable progress over the past years. The standardisation base for a more coherent multinational medical support structure is well on track. This structure should be flexibly based on national contingents as well as on multinational solutions.

However, reality learns, that when it comes to concrete force generation and force planning issues, despite encouraging individual examples, Nations generally show reluctance to put into practice what was previously agreed.

The reason is obvious. The scarce role 1 and 2 and 3 assets are, quite normally and in first priority, highly visible integrated parts of the national contingents. The real problem areas occur at Corps or Force levels, where multinational integration is the real challenge and visibility and national sensitivities are less predominant. Our political and military leaders have recently experienced how medical support can become the unexpected operational showstopper. It would be unfair to look at the Surgeon Generals, certainly if they lack the means and power to prevent that from happening. Often, personnel and budget caps drive the force generation process and medical is genuinely ignored, until the support gaps show. Medical units, although theoretically present, usually turn out to be costly and hard to sustain.

Time will show if our political and military masters had the vision to give us the means and flexibility we need to confront the new challenges with the appropriate medical responses the public expects from us.

A lot of question marks remain also and the need to answer them together, sooner rather than later. Within its Terms of Reference, COMEDS is certainly trying to push these matters to the highest decision making levels. But sound advises to the highest NATO authorities by COMEDS must rely on strong scientific based data. The RTO/HFM Panel is therefore one of COMEDS preferred partners.
INTRODUCTION

The principal Cold War role of NATO's medical services was to be prepared for the treatment and evacuation of large numbers of battle casualties. Multinational solutions to medical support were not considered necessary or practical. The new NATO force structures and strategic concepts emphasise mobility, interoperability, sustainability, jointness and multinationality; i.e. deployment of multinational forces to any area for any mission. NATO now faces the threat of asymmetric conflict and terrorism, with the civilian society, rather than just the military, at risk of attack. Therefore appropriate Force Health Protection is a core competency. An effective and reliable military medical support system helps maintain the trust of military personnel and the wider public in the military and its political leadership. Furthermore military medicine has broadened beyond the purely clinical to areas such as preventive medicine, medical intelligence, epidemiological surveillance and screenings, and patient regulation.

Unfortunately in many nations, medical shortfalls have become a severe limitation upon their operational capability. Consequently, multinational medical support options become increasingly necessary and require more complex co-ordination at each staff level, especially after the change from long-established Cold War planning to current strategic and operational planning. Health and medical care in operations have increasingly become a responsibility of the Alliance's operational commanders and, at times, may even become the commander's main concern.

MC 326/2: NATO PRINCIPLES AND POLICIES OF OPERATIONAL MEDICAL SUPPORT outlines the principles of operational medical support and presents NATO medical policies that are derived from them. This important document seeks to identify the aspects of NATO operational policies unique to medical support. The intention is to guide nations in developing compatible medical support concepts, plans, structures and procedures.

The fundamental principles and policies set out in this document apply to the full spectrum of NATO operations in peace, crisis and conflict, including the Alliance military response to unconventional threats.

This operational medical policy document does not stand alone and is linked to other NATO policy in a number of areas. The key documents concerned are listed in the References section.

In the following the key points of MC 326/2 are outlined.
PRINCIPLES

A. HEALTH AS AN ASPECT OF MILITARY OPERATIONS

Health is a key force multiplier of fighting power. In an operational context, health is the ability to carry out duties unimpeded by physical, psychological or social problems.

At all times, nations retain their legal duty of care as an employer of their military personnel. However, upon transfer of authority, the NATO commander shares that responsibility. Increasingly, due to national shortfalls, medical support, and particularly secondary health care, is delivered by a multinational solution, therefore becoming more the responsibility of the NATO commander. The medical services will advise on health matters and nations deliver the medical care required, but only the commander can balance the health and medical risks involved in his plan and decide if they are acceptable.

B. HEALTHCARE STANDARDS

Medical Ethics and Legal Constraints. Whilst all military personnel are bound by military laws and regulations, medical personnel also have additional individual responsibilities to the ethical and national legal requirements of their own clinical profession. The Geneva Conventions, in granting special rights to medical personnel, is one example.

Standard of Medical Care. Military medicine is highly specialised due to the environment and conditions it is frequently practised in and the procedures will not always be the same as practised during peacetime. The four main aspects affecting clinical quality are organisation, training, environment and equipment. The aim of military medical care in operations is to achieve outcomes of treatment equating to best medical practice. The application of this principle must be guided by the principles embodied in the concepts of Clinical Governance and Evidence Based Medicine.

C. OPERATIONAL PRINCIPLES

Timeliness of Treatment. Time is a fundamental factor in the effectiveness of medical care. It is conducted in the knowledge that immediate clinical care for acute conditions will decisively improve the patient’s prognosis on mortality, invalidity and the development of posttraumatic stress conditions. Therefore, military medical assets must be accessible in a timely manner. Speed of medical evacuation to a stable intensive care environment and, where necessary, surgery is essential to the survival of severely injured casualties and their quality of outcome. The guideline for NATO operations is that advanced trauma care should be available within one hour of injury.

Continuity of Care. A casualty's recovery will depend on the continuance of appropriate care throughout the medical chain. Standards of equipment and staff expertise should be maintained and, in most areas, improve the further up the chain a casualty progresses. Patients passing through an operational medical system must be given care that is continuous, appropriate and progressive. In-transit care must be available during evacuation, which is a medical responsibility as part of the treatment continuum.

Medical Influence Upon Operational Planning. Medical support planning is an integral part of the operational planning process from the outset and has to be performed in close co-operation with all other general staff divisions. Commanders and their staff should consider the impact of casualties on the operational plan and
how they are to be cared for. This requires not only for them to be supported by an able medical staff, but also that they have an understanding of the principles that underpin the delivery of medical support.

Principal Components of Deployed Health Care. A deployed medical system comprises: a command and control structure (C4SRI), an integrated system of treatment and evacuation, and medical logistics. The principal components of operational health care, around which the medical system is built, are Medical Force Protection, Emergency Medicine, Primary Care, Secondary Care and Evacuation. The required medical capabilities and their locations will be principally determined by the time-related constraints of medical care, the commander's campaign plan and casualty estimates.

Medical Force Protection (MFP). Historically, forces have suffered considerably more casualties in operations due to Disease and Non-Battle Injuries (DNBI) than to combat. Preventive Medicine and easy access to Primary Health Care are fundamental aspects of maintaining the health of personnel and the sustainability of forces. Disease and non-battle injury (DNBI) will be an ever-present health risk to personnel. A primary responsibility of medical support is the maintenance of health through the prevention of disease.

Readiness of the Medical Support System. Adequate medical support is a fundamental element of any operational package and medical elements need to be as well prepared and as available for deployment as the forces they support.

Multinationality. Medical assets are scarce and of high value. Multinational medical solutions have considerable potential to reduce the burden of their provision upon individual nations. However, the existence of national differences, such as varying clinical protocols, different languages and legal restrictions, can make this complex. Joint multinational training in peace will pay many dividends for NATO operations in the future.

THE ORGANISATION OF OPERATIONAL MEDICAL SUPPORT

COMPONENTS OF DEPLOYED HEALTH CARE

THE CAPABILITIES REQUIRED COMPRIS THE FOLLOWING MAIN FUNCTIONAL AREAS:

- MEDICAL COMMAND AND CONTROL SYSTEM (C4ISR)
- MEDICAL FORCE PROTECTION
- AN INTEGRATED TREATMENT AND EVACUATION SYSTEM
- A MEDICAL LOGISTIC SYSTEM
- SPECIALIST AREAS

A. MEDICAL COMMAND AND CONTROL (C4ISR)

To operate effectively, medical advisors require direct access to their NATO commanders and other key command staff elements. Medical personnel must be fully integrated into the staff and operational planning processes and appropriately represented on reconnaissance teams. The medical staff must be adequate in size, training and experience to undertake appropriate and timely medical planning. The Medical Director is
responsible for timely medical planning and coordination. A dedicated and structured command and control system is the essential foundation of an efficient medical support structure. This system, supported by a dedicated communications and information management system, must be capable of planning, executing, controlling, supporting and auditing the full range of medical support functions. The medical command system should provide seamless resources to support treatment, evacuation and passage of information from the initial point of injury or sickness to evacuation to definitive treatment and final disposition.

An essential requirement of medical support is the availability of reliable, timely and current medical intelligence, from the initial planning stage throughout the operation as well as during and after redeployment. Such intelligence will form the basis of qualified recommendations to the Force Commander as an integral part of the overall force protection concept.

The efficient management of medical information, particularly regarding patients, is a vital element of competent medical support planning. It is essential that this information is standardized and distributed rapidly to all authorized personnel with a need for it. Principal areas of medical concern will be:

a. **Passage of Information.** Medical decision-making is dependent on the efficient, speedy processing of environmental, tactical, and casualty data.

b. **Patient Tracking and Regulation.** Both Patient Tracking and Regulation require up-to-date and accurate information about individual casualties and the availability of treatment and evacuation assets. The key requirement is the maintenance of an accurate database.

c. **Clinical Records.** Medical documentation should be interoperable throughout the theatre of operations and in all national contingents. Copies of patient documents must move with the patient throughout the evacuation system to definitive care.

d. **Tele-consultation.** Tele-consultation can be a useful tool, particularly when the area of operations is remote and medical resources are limited. Planning should take into account that the use of telemedicine systems will be governed and may be restricted by operational electromagnetic security measures.

B. MEDICAL FORCE PROTECTION

Medical Force Protection (MFP) measures are an essential element of every contingency plan. The plan will continue throughout the deployment and must extend well into the post-deployment period.

A particular aspect of MFP is defense against Weapons of Mass Destruction (WMD). Protection against WMD requires a comprehensive and integrated approach including vaccination, chemoprophylaxis, and personal protection.

C. INTEGRATED TREATMENT AND EVACUATION

**Roles of Care Capabilities.** Deployable Medical Treatment Facilities (MTFs) are classified according to their treatment capability in a system of roles, progressively numbered from 1 to 4. Most of the care capabilities of each Role are intrinsic to the next higher Role.

a. **Role 1.** Role 1 medical support provides for routine primary health care, specialized first aid, triage, resuscitation and stabilization.
b. **Role 2.** Role 2 provides an intermediate capability for the reception and triage of casualties, as well as being able to perform resuscitation and treatment of shock to a higher technical level than Role 1. It will routinely include Damage Control Surgery (DCS) and may include a limited holding facility for the short term holding of casualties until they can be returned to duty or be evacuated. Role 2 may also include Dentistry, Environmental Health and Psychiatry or Psychology.

c. **Role 3.** Role 3 is designed to provide secondary care within the restrictions of the Theatre Holding Policy. Role 3 medical support is deployed hospitalization and the elements required to support it. This includes a mission-tailored variety of clinical specialities including primary surgery and diagnostic support.

d. **Role 4.** Role 4 medical support provides the full spectrum of definitive medical care that cannot be deployed to theatre or is too time consuming to be conducted there. It includes the provision of specialist surgical and medical procedures, reconstructive surgery and rehabilitation. It will normally be provided in the country of origin or the home country of another Alliance member. In many member nations Role 4 care is provided for within the national civilian health system.

The movement (EVACUATION) of the seriously ill is a high-risk activity. The task of transferring casualties is further complicated in military operations by factors such as the operational environment, the weather, the length and quality of evacuation routes and the availability of suitable evacuation assets. Medical evacuation is the movement of patients under medical supervision to MTFs as an integral part of the treatment continuum. There are three categories of medical evacuation, which apply to sea, land and air systems. They are forward, tactical (within theatre) and strategic (out-of-theatre) evacuation. The evacuation plan is closely interrelated to both the medical footprint and holding policy. The robustness of the evacuation chain is proportionally related to the quantity and capability of the treatment assets that will be required in theatre. A medical evacuation system requires the following capabilities:

a. **Availability.** The aim of the medical system should be to evacuate casualties 24 hours a day, in all weather and sea-states, over all terrain and in any operational scenario. Clearly, this will not be possible in all operational scenarios, particularly with special forces or at sea. In such instances contingency plans are required.

b. **Continuity.** The crew of medical evacuation assets must be trained and equipped to provide continuity of care of the casualty throughout the evacuation.

c. **Casualty Regulation.** There are two main aspects to casualty regulation: the management of the flow of casualties, particularly at times of high flow, and the direction of individual patients through the system primarily according to their clinical need. The Casualty Regulation System should be able to provide timely and accurate tracking information throughout the chain of evacuation.

Planning and executing an effective medical evacuation system is a medical responsibility. Since national medical evacuation doctrines and capabilities may differ substantially, only properly coordinated procedures can assure the smooth transfer of patients within a multinational medical support structure.

**Aeromedical Evacuation.** Aeromedical Evacuation will join many parts of the medical chain together and will normally provide the crucial link out of theatre for those casualties being set to Role 4. A centrally controlled multinational aeromedical evacuation system, for combined joint NATO operations, offers the ability to optimise the assets and provide for economy of scale by utilising Role Specialist and Lead Nation concepts.
D. MEDICAL LOGISTICS
The medical logistic system should be well regulated, efficient and cost-effective. Notwithstanding national policies and overall responsibilities, the coordinating responsibility and authority for planning and executing an effective medical logistic system lies with the NATO Commander. The unique characteristics of medical material set it apart from other commodities (Geneva Conventions, national and international regulations, handling requirements, etc.).

Blood products and medical gases are two supply items that require particular consideration when planning medical logistics. The availability of safe blood and blood products is essential and their supply is a complex and sensitive matter.

E. SPECIALIST AREAS
Dental care is an integral element of operational medical support. Prevention and correction of dental disease will ensure a higher availability of forces to deploy and reduce the number that will subsequently need to be removed from theatre for such problems. Pre-deployment dental care is a national responsibility and must be a priority for forces held at high readiness for NATO deployments. Deployed dental support is important both to help maintain force availability and also to deal with dental trauma.

Psychological casualties may include battle shock and a range of other anxiety and depressive disorders, some of which will be complicated by physical injury. If correctly managed many can be returned to duty quickly. The aim is not to "medicalise" the management of these cases but, where possible, rehabilitate individuals within their own units. Psychiatry teams may generally be based within medical units, for administrative and control reasons, but should be prepared to conduct their clinical business on a peripatetic community basis. Psychiatric briefing is an element of MFP and should be utilized, as appropriate, before, during and after an operation.

DISCUSSION
The transformation of the world security environment has lead to a dramatic change of NATO’s strategic concept. Alliance members need to have a way to rapidly form military coalitions which can be deployed to any area for any mission.

The integration of NATO Response Forces (NRF) in the new military strategy of NATO which is determined by the basic characteristics: of concentration of forces, flexibility, global mobility and rapid augmentation requires an efficient medical service with small medical footprints. It should provide a standard of medical care which is as close as possible to prevailing peacetime standards. In addition to the conduct of national sovereign tasks, it must have the capability for international cooperation in the sense of interoperability. In this context, the military requirement is that the mobility and flexibility of medical support during operations must correspond to the mobility and flexibility of the units to be supported. Thus the evacuation of wounded and sick patients receives a new status as far as quality and quantity are concerned. This alltogether requires from the Alliance a huge medical reorganization and/or upgrade programmes which are in various stages of completion. Unfortunately regular forces have found it increasingly difficult to establish an effective medical care providing system and expensive medical equipment has been more difficult to fund from restricted budgets.
In some nations, medical shortfalls have become a severe limitation to their operational capability. Consequently, multinational support options become increasingly necessary to establish and maintain the high level of medical care and to release the burden for those countries which permanently carried out the medical mission at level 2 and 3 all over the years in ongoing UN and NATO missions. With MC 326/2 NATO has available an excellent document which applies to the full spectrum of NATO operations in peace, crisis and conflict, including the Alliance military responses to unconventional threats to guarantee effective medical support for NATO forces or NATO-led operations. It is of utmost importance that the NATO nations integrate the fundamental medical principles and policies set out in MC 326/2 in their national concepts and it is paramount that the NATO Strategic Commands translate the content of this document into their doctrine. Joint medical support is an operational issue, rather than a logistic one.

Operationally, NATO’s recent involvement has shown its ability to react flexibly beyond its borders. NATO provides as lead organisation since June 2004 the C2 structure and most of the 6500 troops serving with the International Security Assistance Force (ISAF) in Kabul and protects up to five Provincial Reconstruction Teams (PRT). However to date medical support is a critical resource. Among other items this is why a push to “whip capitals into quicker action” is under way by NATO Secretary General Jaap de Hoop Scheffer.

To sum it up, NATO is on its way to accept more responsibility in terms of Joint Medical Support. With MC 326/2 NATO increases the speed and efficiency of the Alliance approach to the way to provide full spectrum healthcare response capability to meet the entire range of joint military operations. This asks for an integrated, global healthcare system able to establish and to maintain high quality preventive, primary, restorative and trauma healthcare to all NATO troops and an effective evacuation system... anytime ......anywhere.

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MC 477 (FINAL), 18 Jun 03, Military Concept for the NATO Response Force.

AMedP-13, Nov 00, NATO Glossary of Medical Terms and Definitions.
Hypertonic Saline Resuscitation Restores Inflammatory Cytokine Balance in Post-Traumatic Hemorrhagic Shock Patients

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SUMMARY

Fluid resuscitation can often exacerbate injury sustained during hemorrhagic shock and is associated with altered immuno-inflammatory events. Early monocyte dysregulation and excessive proinflammatory cytokine production are thought to play a key role in the development of post-traumatic multiorgan dysfunction in resuscitated trauma patients. Compared with standard isotonic crystalloid resuscitation using 0.9% normal saline (NS), 7.5% hypertonic saline with 6% dextran-70 (HSD) has been shown in experimental studies to reduce shock/resuscitation-induced inflammatory reactions and lessen organ dysfunction. However, the immunomodulatory capacity of HSD, has not been evaluated in clinical human trials. In this prospective, randomized controlled trial we show that a single (250 mL) bolus infusion of HSD in hemorrhagic trauma patients restores the balance between pro and antiinflammatory mediators in the early post-resuscitative period. Flow cytometric single-cell analyses revealed that, compared to standard resuscitation with NS, which selectively expands the proinflammatory CD14+CD16+ monocyte phenotype, initial treatment with HSD elicits selective depletion of CD14+CD16+ cells and down-regulates monocytic adhesion molecule expression. Moreover, HSD significantly inhibits intracellular TNF-α production by CD14+CD16+ monocytes, while upregulating both IL-10 and IL-1ra by CD14++CD16– monocytes. This differential profile of monocytic cytokine expression in response to HSD appears to be mediated, at least partly, by attenuation of post-resuscitation noradrenergic-stimulated signaling pathways. These findings demonstrate that HSD promotes a more balanced early inflammatory response in resuscitated hemorrhagic shock patients.

1.0 INTRODUCTION

Hemorrhagic shock is the major cause of death on the battlefield [1]. Of those soldiers wounded in combat who die of wounds, it is estimated that 20% could be salvaged before exsanguination if provided with appropriate medical care [2]. Along with urgent control of bleeding, intravenous fluid replacement therapy for intravascular volume restoration and organ perfusion is routinely required to treat combat casualties [3]. Similarly, despite advances in modern surgical care, major trauma with associated hemorrhagic shock remains a leading cause of civilian mortality in young adults [4,5]. Those patients who survive the initial tissue injury and circulatory shock are at high risk for development of the systemic inflammatory response and delayed multiorgan dysfunction syndromes [6]. Although research findings document a link between post-traumatic complications and immune dysregulation [7], the underlying cellular and molecular defects associated with the pathophysiology of resuscitated hemorrhagic shock are not fully elucidated [8].
Adequate intravenous fluid administration for restoration of intravascular volume and maintenance of tissue perfusion is essential for successful management of hemorrhagic shock [9]. Yet, considerable controversy persists over the ideal fluid formulation [10]. The concept that early, aggressive high-volume resuscitation is critical to the optimal treatment of hemorrhagic shock has been widely accepted and practiced since the Vietnam War. Subsequently, the practice of large volume isotonic crystalloid resuscitation became the standard-of-care for civilian trauma. However, the impact of different fluid replacement strategies on shock/resuscitation-induced immunological perturbations has not been prospectively studied in trauma patients [11]. In particular, the early post-resuscitation dysfunctional period represents a critical time point that remains under-investigated, but amenable to appropriate therapeutic interventions aimed at restoring physiologic hemodynamics and reestablishing inflammatory equilibrium [12].

Various immunoinflammatory alterations have been described in clinical and experimental investigations of post-traumatic hemorrhagic shock [13]. The initial immunological response to trauma/hemorrhage is characterized by excessive innate immune stimulation, with intense monocyte/macrophage activation [7] and production of numerous inflammatory mediators [14]. The proinflammatory cytokines TNF-α and IL-1β are the primary endogenous mediators of acute inflammation, eliciting endothelial activation, capillary leak and circulatory collapse [15]. While localized inflammatory cytokine release is considered a protective host response to injury or infection [16], overproduction and loss of compartmentalization leads to unabated systemic inflammation and subsequent multiorgan dysfunction [7]. Consequently, the proinflammatory response is tightly regulated by an intricate network of endogenous counter-inflammatory molecules [16]. Chief among these are the antiinflammatory cytokines and specific receptor antagonists, including, IL-10 and IL-1(ra) receptor antagonist [17].

In addition to direct feedback loops within the immune system, a central role of the neuroendocrine system in regulating the magnitude and duration of the post-traumatic inflammatory cascade is strongly indicated [18]. The sympathoadrenal system is rapidly activated in response to severe trauma/hemorrhage, resulting in greatly augmented (50- to 100-fold) concentrations of circulating catecholamines [19], which are themselves potent regulators of inflammatory cytokine production [18]. Monocyte/macrophages are a main target of both epinephrine (Epi) and norepinephrine (NE) induced sympathetic effects, and differentially modulate cellular activities via alterations in α and β-adrenoreceptor-stimulated intracellular signaling pathways [20].

Standard-of-care resuscitation of hemorrhagic trauma patients calls for prompt replacement of intravascular fluid using large volumes (2–3 times blood loss) of isotonic crystalloids, such as normal saline (0.9% NaCl) [21]. While aggressive isotonic fluid administration adequately restores systemic blood pressure and is lifesaving in many patients, it often leads to volume overload and post-resuscitative complications [22]. These include increased blood loss and pulmonary edema, ultimately causing greater morbidity and mortality [4]. Moreover, convincing experimental evidence indicates that conventional large-volume fluid resuscitation can exacerbate shock-induced microcirculatory dysfunction and inflammatory tissue injury [3,12]. Thus, the search continues for fluid replacement regimens, which avoid intravascular fluid overload and help prevent pathophysiologic alterations induced by shock/resuscitation [9].

Small-volume hypertonic/hyperoncotic solutions appear promising as initial fluid replacements [5,23]. One of the most common formulations used for resuscitation of hemorrhagic shock is 7.5% hypertonic saline, in combination with the hyperoncotic colloid, 6% dextran-70 (HSD). Although hypertonic saline has been investigated for many years [24], contemporary interest arose in 1980 when Velasco et al. [25] demonstrated that a bolus injection of 7.5% hypertonic saline (4 mL/kg) rapidly restored intravascular volume and central hemodynamics in severely hemorrhaged dogs. Since then, the physiological responses to hypertonic resuscitation have been extensively studied in experimental animals and humans [26,27]. The immediate
circulatory effects of hypertonic saline derive from rapid osmotic mobilization of endogenous fluids from the extravascular to the intravascular compartment; the resultant 3–4-fold expansion of plasma volume improves mean arterial pressure, cardiac output and peripheral tissue perfusion [23,27]. Overall hypertonic resuscitation results in lower fluid requirements and normalization of physiological parameters.

Since the original human study by De Filippe et al. [28] revealed the substantial hemodynamic benefits of hypertonic saline infusion in refractory hypovolemic trauma patients, numerous clinical reports have confirmed the efficacy and safety of hypertonic solutions for primary resuscitation of hemorrhagic shock [26]. Although limited by small sample size, the majority of prospective randomized controlled trials where patients received initial treatment with HSD have shown a tendency for improved early (24 h) and long-term survival to discharge, as compared to standard-of-care [5,23,29]. Similarly, a meta-analysis [30] and cohort analysis [31] of individual patient data performed by Wade et al., demonstrated enhanced patient survival using HSD for post-traumatic resuscitation. Moreover, other studies have shown that HSD reduces both fluid requirements [32] and incidence of post-resuscitation complications, such as renal failure, coagulopathies, and acute respiratory distress syndrome (ARDS) [33].

More recently, the focus of shock/resuscitation research has shifted from hemodynamic restoration to the potential role of hypertonic solutions as immunomodulatory agents [5,34]. Experimental evidence from in vitro studies and animal models of hemorrhagic shock suggest that physiological increases of plasma tonicity can attenuate inflammatory reactions and improve post-resuscitative outcome [12,34,35]. For example, hypertonic saline suppresses multiple leukocyte functions, including, neutrophil-endothelial cell adhesion, cellular rolling/transmigration, and oxidative burst [36]. Consequently, hypertonic saline is more effective than isotonic fluids in protecting against neutrophil-mediated pulmonary and hepatic injury in animals after resuscitation [37,38]. Less is known concerning the modulation of monocyte/macrophage function by hypertonicity, but recent studies indicate that hypertonic resuscitation can dampen unrestrained proinflammatory cytokine cascades, while augmenting counter-inflammatory reactions [37,39].

Much of the past research on hypertonic saline has been conducted on animals or in isolated human blood cultures. Unfortunately, extrapolation from animal studies and laboratory experiments to the clinical situation is difficult as animal responses do not wholly parallel those of humans, and since in vivo immunocompetant cells experience bidirectional communication with hormones and cytokines [35,40]. Thus, despite compelling experimental findings, HSD has not yet received widespread clinical acceptance, and its potential immunomodulatory actions remain untested in trauma patients. The present study was designed to prospectively compare the immunoinflammatory effects of initial resuscitation with a single 250-mL infusion of HSD followed by isotonic crystalloids versus standard-of-care only. Specifically we evaluated serial changes in: (1) intracellular expression of pro (IL-1β, TNF-α) and antiinflammatory cytokines (IL-1ra and IL-10) by blood monocytes; (2) surface expression of monocytic adhesion molecules (CD11b and CD62L); and (3) circulating catecholamine concentrations (Epi and NE) during the early resuscitative course. We hypothesized that primary fluid replacement with HSD would promote a more balanced early inflammatory response in resuscitated trauma patients.

2.0 METHODS

2.1 Patient Selection and Study Design

This single-centre, prospective, randomized controlled trial enrolled 20 severely injured trauma patients, admitted to the surgical emergency department of Sunnybrook and Women’s College Health Sciences Centre
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over a 14-month period, under a policy of ‘delayed informed consent’ with the approval of the Institutional Review Board. Eligible patients were entered into the study at the time of presentation to the trauma centre from the scene of injury. All patients or their next of kin were then informed of their participation as soon as possible after study enrollment and permission were obtained for continued data collection and inclusion of these data into the study database.

Patients were eligible for inclusion in the study if they sustained severe trauma; had at least one recorded episode of hypotension (systolic blood pressure ≤ 90 mm Hg), were 16 years of age or older, had evidence of blood loss (external, thorax, abdomen, retroperitoneum), had an injury severity score (ISS) ≥ 15, and as per the investigator’s judgment, were expected to survive for at least 24 h. Patients were excluded if they refused to participate, were admitted ≥ 6 h after trauma, vital signs were absent, were pregnant, or had stigmata of chronic disease. The patient characteristics at the time of admission are summarized in Table 1.

Table 1: Demographic characteristics and clinical outcomes of the study patients

<table>
<thead>
<tr>
<th></th>
<th>0.9% NS</th>
<th>7.5% HSD</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>47.5 (15.9)</td>
<td>49.3 (16.7)</td>
<td>.75</td>
</tr>
<tr>
<td>Gender, male, no. (%)</td>
<td>9 (90%)</td>
<td>7 (70%)</td>
<td>.76</td>
</tr>
<tr>
<td>ISS</td>
<td>25.9 (10.3)</td>
<td>26.3 (11.4)</td>
<td>.83</td>
</tr>
<tr>
<td>Crystalloid - pre-hospital, mL</td>
<td>835 (855)</td>
<td>2144 (1343)</td>
<td>.048*</td>
</tr>
<tr>
<td>ER, mL</td>
<td>4542 (2758)</td>
<td>3689 (1865)</td>
<td>.28</td>
</tr>
<tr>
<td>total first 24h, mL</td>
<td>8080 (2736)</td>
<td>7796 (3189)</td>
<td>.75</td>
</tr>
<tr>
<td>Blood - pre-hospital, units</td>
<td>0.5 (1.16)</td>
<td>1.22 (1.7)</td>
<td>.27</td>
</tr>
<tr>
<td>ER, units</td>
<td>1.56</td>
<td>1.5</td>
<td>.62</td>
</tr>
<tr>
<td>total first 24h, units</td>
<td>4.36 (6.77)</td>
<td>2.2 (2.9)</td>
<td>.38</td>
</tr>
<tr>
<td>Colloids total first 24h, mL</td>
<td>696 (773)</td>
<td>361 (377)</td>
<td>.02*</td>
</tr>
<tr>
<td>Death</td>
<td>2 (14.3)</td>
<td>0</td>
<td>.21</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

Upon hospital admission, patients randomly received a either single dose of 250-mL of 7.5% hypertonic saline with 6% dextran-70 (HSD) or the same volume of standard 0.9% isotonic crystalloid (normal saline, NS), administered intravenously as a bolus infusion from identical unidentified bags. Initial resuscitation was followed by further administration of isotonic fluids dosed according to individual patient needs, as recommended by Advanced Trauma Life Support® (ATLS) guidelines [21]. No patients received vasopressors or ionotropes throughout the study period. Patients were followed until hospital discharge or death.

2.2 Antibodies and Reagents

Fluorescent mouse anti-human monoclonal antibodies (mAbs) against the cell-surface epitopes CD16–FITC, CD45–PerCP and CD14–APC, cellular adhesion molecules CD11b (Mac-1 /β2 subunit)–FITC and CD62L (L-selectin)– PE, and intracellular Fast Immune™ anti-Human-cytokine mAbs, specific for IL-1-PE, IL-1ra-PE, IL-10-PE and TNF-α-PE, along with their respective isotype-matched (IgG1 and IgG2a) control antibodies,
were all obtained from Becton Dickinson Biosciences (BD Biosciences, San José, CA). FACS® brand Erythrocyte Lysing Solution, Permeabilizing Solution™, and CellWASH™ (optimized PBS containing 0.1% sodium azide) were also obtained from BD Biosciences. LPS (Escherichia coli 055:B5), paraformaldehyde and brefeldin A (BFA) were purchased from Sigma Chemical Company (St. Louis, MO). Tissue culture reagents, including complete RPMI 1640 medium and Dulbecco’s PBS (pH 7.4), were purchased from GibcoBRL/Life Technologies (Grand Island, NY). All culture flasks and 12 x 75-mm polystyrene and polypropylene tubes were obtained from Falcon (BD Biosciences). Standardized preparations of HSD and NS solutions were purchased from the hospital pharmacy.

2.3 Blood Collection, Cell Culture and Stimulation Conditions

Venous blood samples (totalling 15 mL) were withdrawn serially from each patient using existing intravenous lines over five time-points: at baseline (hospital admission), and again at 1, 3, 6, and 24-h post-resuscitation. Samples for hematological and flow cytometric analyses were collected into 3-mL sterile glass Vacutainers™ (BD, Franklin Lakes, NJ), containing EDTA and sodium heparin, respectively. After sampling, specimens were kept at room temperature and transported to the lab for culture within 3 h. All blood work was performed in a laminar flow hood using sterile technique. A 2-mL aliquot of freshly sampled sodium heparin anticoagulated whole blood was treated with the Golgi transport inhibitor BFA at a final concentration of 10 µg/mL to enable intracellular cytokine accumulation. For determination of ex vivo, de novo cytokine producing activity, BFA-treated heparinized whole blood was further subdivided into two 1-mL aliquots, which were incubated in the presence of vehicle (RPMI) for unstimulated cytokine expression, or with an optimal concentration (1 µg/mL) of LPS for stimulated cytokine expression. Non-BFA-treated heparinized whole blood was processed in parallel for determination of unstimulated and stimulated cellular adhesion molecule expression. All samples were then cultured for 22 h at 37°C in a 5%-CO₂ humidified atmosphere.

2.4 Cell Surface and Intracellular Immunofluorescence Staining

Due to limited blood available from trauma patients and because cellular isolation techniques can artifactually activate leukocytes resulting in changes in antigen expression, a whole-blood method was selected for measuring cell-surface and intracellular antigen expression. For phenotypic characterization of monocyte subsets, 100-µL aliquots of unstimulated and LPS-stimulated blood were incubated for 15 min at room temp in the dark with saturating dilutions of fluorochrome-conjugated anti-CD45, anti-CD14 and anti-CD16 mAbs in 12 x 75-mm polystyrene tubes. Monocyte cellular adhesion molecule expression was identified by a direct immunofluorescence technique, using anti-CD11b and anti-CD62L mAbs in conjunction with anti-CD45/CD14. Appropriate class-matched isotype immunoglobulin negative control mAbs were added simultaneously to separate tubes, at identical concentrations for each assay, to detect non-specific binding. Immediately following surface staining, cells were treated for 10 min with 2 mL of 1x FACS™ Lysing Solution, resulting in simultaneous erythrocyte lysis and partial fixation of leukocytes. After centrifugation (500 x g, 5 min) and aspiration of the supernatant, specimens for surface staining were washed with 2 mL of PBS CellWASH™ (containing 1% sodium azide and 1% bovine serum albumin). Stained cells were then fixed with 300 µL of 1% paraformaldehyde and stored at 4°C until data acquisition by flow cytometer. Thereafter, specimens for intracellular cytokine detection were further treated with 500 µL of 1x FACS Permeabilizing Solution™ and incubated for 30 min at room temp with 20 µL of the corresponding fluorescent-labeled anti-Hu-cytokine mAbs [41]. After incubation, cells were washed, aspirated and resuspended in 2% paraformaldehyde. Samples were kept at 4°C in the dark until flow cytometric acquisition.
2.5 Flow Cytometric Data Acquisition and Analysis

Stained cell suspensions were acquired on a dual-laser FACSCalibur flow cytometer (BD Biosciences) calibrated for four-colour analysis. An electronic acquisition gate was set to include all monocytes according to regionalization on the basis of anti-CD45/CD14 fluorescence emission characteristics using bivariate dotplots in CellQuest™ Pro software (BD) (Figure 1a). Further sequential gating was performed to identify two major subpopulations of monocytes on the basis of their coexpression of the CD16 antigen (FcRγIII) as shown in Figure 1b. The phenotypic frequencies of CD14⁺CD16⁻ and CD14⁺CD16⁺ monocyte subsets were expressed as percentages of total CD14⁺ monocytes. Typically, ≥5,000 CD14⁺ monocyte-gated events were acquired for assessment of the frequency of cell-associated cytokines and cellular adhesion molecule expression. Relative mean fluorescence intensities [i.e., MFI of sample antigen minus MFI of isotype control, in arbitrary units (au) scaled form 0 to 10,000] of the selected monocyte adhesion molecules and intracellular cytokines, in unstimulated and LPS-stimulated cultures, were quantified using fluorescence histogram data (Figure 1c). Analysis gates and quadrant markers were set to define positive and negative populations according to the non-specific staining of isotype-matched negative controls. Instrument optical alignment and fluidics were verified for each cytometer run using CaliBRITE™ beads and day-to-day variability in instrument settings were monitored and adjusted with AutoCOMP™ software (BD). Absolute cell counts were obtained by multiplying the corresponding percentages of cells derived from FACS analysis by total leukocyte counts obtained from a hematology analyzer (Coulter Electronics, Hialeah, FL).

Figure 1: Representative flow cytometric immunofluorescence data: illustrating the four-colour sequential gating method used to identify whole blood monocyte subpopulations and quantify intracellular cytokine production. Leukocyte subsets (monocytes, granulocytes, lymphocytes) were distinguished according to cell-surface staining characteristics, using CD45-PerCP and CD14-APC (dotplot a); total CD14⁺ monocytes were further classified as CD14⁺bright and CD14⁺dim according to their intensity of CD14 staining. Additional staining with CD16-FITC (dotplot b) allowed definition of CD14⁺CD16⁻ and CD14⁺CD16⁺ monocyte subpopulations. Panel c displays a corresponding single parameter fluorescence histogram (FL2) used to evaluate the %cytokine-positive and mean fluorescence intensity (MFI) of cells showing the differential pattern of TNF-α expression typical of CD14⁺CD16⁻ and CD14⁺CD16⁺ monocyte subsets (broken-line: isotype control antibody; solid-line: CD14⁺CD16⁻ subset; shaded histogram: CD14⁺CD16⁺ subset). The data shown are representative of a single trauma patient after LPS stimulation at 1 µg/mL for 22 h in the presence of brefeldin A (10 µg/mL).
2.6 Hormonal Analysis

Specimens for catecholamine determination were drawn into 4.5-mL vacuum tubes containing EDTA and reduced glutathione (Amersham, Arlington Heights, IL) and stored briefly on ice. Plasma was separated in a refrigerated centrifuge for 15 min (4°C; 3000 x g) and the supernatant frozen at –80°C until assay. Unbound catecholamine concentrations were quantitated by gas chromatography-mass spectrometry.

2.7 Statistical Analysis

Serial changes in intracellular cytokines, adhesion molecules, and hormones were evaluated by two-way ANOVA for repeated measures. Differences between resuscitation strategies were indicated by a significant treatment group x time interaction effect; the Newman-Keuls post-hoc multiple comparison test was performed to isolate specific group and time differences among treatment means using a Huynh-Feldt correction for multiple comparisons. Data within the groups were compared at each data point by a factorial ANOVA and Scheffé multiple comparison test further established detected differences. The chi-square test was used for intergroup comparisons of baseline characteristics. Unless specified otherwise, variables are expressed as means ± SE. For all comparisons, a probability of less than .05 was considered to be statistically significant.

3.0 RESULTS

3.1 HSD Prevents Expansion of the CD14⁺CD16⁺ Inflammatory Monocyte Phenotype

Peripheral blood monocytes consist of two principal subsets [42], which display distinct phenotypical and functional properties, including their cytokine production profiles [43]. We sought to determine if initial HSD resuscitation would alter the frequency of these key cellular subsets in blood samples obtained from resuscitated trauma patients. Multi-colour immunofluorescence analysis, based on the coexpression of the LPS receptor (CD14) and Fcγ receptor III (CD16), allowed the two types of monocytes to be defined: a major subset termed ‘classical’ monocytes, that are strongly CD14-positive but negative for CD16 (CD14⁺CD16⁻ monocytes) and a minor subset that are weakly CD14-positive which co-express CD16 (CD14⁺CD16⁺ monocytes). CD14⁺CD16⁺ monocytes are more mature cells that readily express proinflammatory cytokines, including TNF-α and IL-1β, but typically fail to produce significant amounts of the antiinflammatory cytokines, such as IL-10 [44]. Based on this pattern of cytokine expression, CD14⁺CD16⁻ cells have been termed ‘proinflammatory’ monocytes and their concentration is expanded in various pathological inflammatory states [45]. Although still within the circulation, CD14⁺CD16⁺ monocytes possess functional activities that are analogous to mature tissue macrophages [43]. A representative example of the differential monocyte subset gating procedure used for the current analysis and the staining pattern of surface expression determined from flow cytometry data is shown in Figure 1, a–b.

The absolute monocyte counts and relative subset distributions (as a percentage of total leukocytes) are summarized in Table 2. A comparison of the composition of total CD14⁺ blood monocytes, as well as CD14⁺⁺CD16⁻ and CD14⁺⁺CD16⁺ monocyte subsets, in trauma patients resuscitated with HSD, demonstrated a differential pattern of monocyte subset distribution as compared to standard resuscitation with NS. Our results indicate that the mean (± SE) frequency (counts and proportions) of CD14⁺ monocytes were similar at baseline and remained relatively constant throughout the experimental period in both HSD and NS resuscitated patient groups. As a percentage of total circulating monocytes, the classical CD14⁺⁺CD16⁻ subset comprised the majority of circulating monocytes in both HSD and NS patient arms at baseline (77.12 ± 6.4% vs. 79.54 ± 2.24%, respectively), and the proinflammatory CD14⁺⁺CD16⁺ subset constituted a smaller population (24.89 ±
5.29% vs. 22.32 ± 2.24%, respectively) (Table 2); nonetheless, the fraction of CD14+CD16+ cells was still expanded in all patients relative to reported values (~10% of monocytes) for healthy individuals [43].

Table 2: Whole Blood Monocyte Counts and Subset Distribution by Resuscitation Group

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Total Leukocytes</th>
<th>CD14+CD16+</th>
<th>CD14++CD16–</th>
<th>CD14+CD16+ Subset</th>
<th>CD14++CD16– Subset</th>
<th>Monocyte Subset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counts</td>
<td>% Leukocytes</td>
<td>Counts</td>
<td>% Monocytes</td>
<td>Counts</td>
<td>% Monocytes</td>
</tr>
<tr>
<td>Normal Saline (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>14.75 ± 0.92</td>
<td>0.56 ± 0.11</td>
<td>3.79 ± 0.44</td>
<td>0.40 ± 0.10</td>
<td>77.12 ± 7.40</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>1h</td>
<td>14.41 ± 1.82</td>
<td>0.58 ± 0.12</td>
<td>3.72 ± 0.43</td>
<td>0.41 ± 0.09</td>
<td>72.27 ± 1.60</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>3h</td>
<td>12.51 ± 1.59</td>
<td>0.57 ± 0.09</td>
<td>4.86 ± 0.75</td>
<td>0.37 ± 0.07</td>
<td>67.42 ± 4.14</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>6h</td>
<td>10.87 ± 1.07</td>
<td>0.50 ± 0.08</td>
<td>4.87 ± 0.64</td>
<td>0.31 ± 0.06</td>
<td>62.48 ± 5.12</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>24h</td>
<td>11.08 ± 0.84</td>
<td>0.58 ± 0.13</td>
<td>5.26 ± 1.13</td>
<td>0.33 ± 0.10</td>
<td>61.15 ± 3.35</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Hypertonic Saline (n=10)</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.09 ± 0.96</td>
<td>0.50 ± 0.08</td>
<td>3.85 ± 0.57</td>
<td>0.39 ± 0.05</td>
<td>79.54 ± 2.24</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>1h</td>
<td>12.50 ± 1.07</td>
<td>0.46 ± 0.05</td>
<td>3.00 ± 0.56</td>
<td>0.37 ± 0.04</td>
<td>84.53 ± 1.55</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>3h</td>
<td>12.26 ± 0.89</td>
<td>0.42 ± 0.06</td>
<td>3.48 ± 0.56</td>
<td>0.36 ± 0.09</td>
<td>85.22 ± 2.29</td>
<td>0.06 ± 0.02</td>
</tr>
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<td>6h</td>
<td>10.83 ± 0.67</td>
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<td>3.99 ± 0.48</td>
<td>0.35 ± 0.03</td>
<td>82.76 ± 3.10</td>
<td>0.07 ± 0.01</td>
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<tr>
<td>24h</td>
<td>9.55 ± 0.75</td>
<td>0.44 ± 0.07</td>
<td>4.62 ± 0.65</td>
<td>0.40 ± 0.02</td>
<td>83.48 ± 7.35</td>
<td>0.07 ± 0.01</td>
</tr>
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</table>

*a* Whole blood samples were stained simultaneously with anti-CD14 and anti-CD16 antibodies, and the percentage of CD14+CD16+ and CD14+CD16- cells were determined by flow cytometric analysis within the monocyte gate. Absolute monocyte counts were obtained by automated leukocyte count, and the cell numbers of monocyte subpopulations were calculated with the percentage distribution obtained from flow cytometry. Results are expressed as mean ± SE, and statistical comparisons between treatment groups were conducted by repeated measures ANOVA.

*b* Monocyte subset ratio calculated as [(%CD14+CD16+ / %CD14++CD16–) x 100].

* p ≤ 0.05 within trial vs. baseline; † P ≤ 0.05 between trial vs. normal saline; all others, P > 0.05 by ANOVA.

As shown in Table 2, the frequency and absolute counts of circulating CD14+CD16+ proinflammatory monocytes were differentially modulated by the type of resuscitation strategy; initial resuscitation with NS provoked a significant (P < 0.05) expansion of the CD14+CD16+ subpopulation at 3, 6, and 24-h post-resuscitation, whereas, HSD-resuscitated patients exhibited a marked reduction in this subset over the same time-course. Concomitantly, the percentage and number of CD14+CD16- monocytes dropped significantly (P < 0.05) in NS treated patients, while the proportion of these cells was modestly increased after HSD resuscitation. These opposing alterations in CD14+CD16- and CD14+CD16+ monocyte subsets were reflected by parallel changes in the monocyte subset ratio (i.e., CD14+CD16+ / CD14+CD16-), which showed a progressive increase (from ~ 32 to 57) in NS treated patients and a decrease (from ~ 29 to 20) in HSD resuscitated patients (Table 2). These results demonstrate that NS resuscitation leads to a selective expansion of the proinflammatory CD14+CD16+ monocyte phenotype in the peripheral blood during the post-resuscitation period, while HSD elicits less overall monocyte redistribution, with a bias towards selective depletion of the CD14+CD16+ subpopulation.
3.2 **HSD Resuscitation Inhibits Intracellular TNF-α Production by CD14⁺CD16⁺ Monocytes**

To assess the impact of resuscitation strategy on the production of intracellular cytokines, a whole-blood multiparameter flow-cytometric assay was used to measure changes in the spontaneous and LPS-stimulated expression of pro (TNF-α, IL-1β) and antiinflammatory (IL-1ra, IL-10) cytokines in monocytes of trauma patients (Figure 1c). Compared to conventional cytokine assay methods (e.g., immunoassays of serum or culture supernatants), flow cytometric intracellular cytokine detection offers the unique advantages of whole blood analysis, which does not require cellular purification or isolation, and allows detection of cytokine production at the single-cell level rather than bulk systemic cytokine release [41]. For the present analysis, changes in monocytic intracellular cytokine expression were calculated as both the percentage of cytokine-positive cells (i.e., % of cells expressing cytokine of interest) and as relative MFI values (i.e., amount of cytokine produced per positive cell).

Post-traumatic shock/resuscitation-induced alterations in the spontaneous and LPS-stimulated percentage of cytokine-producing monocytes and their fluorescence intensity values are presented in **Table 3** and **Figure 2**. Upon admission, intracellular immunofluorescence analysis of unstimulated whole-blood cultures from all trauma patients measured at baseline, revealed that on average approximately 15% of CD14⁺ blood monocytes spontaneously expressed the proinflammatory cytokines TNF-α and IL-1β with no differences between treatment arms (Table 3). Neither resuscitation regimen significantly affected spontaneous or stimulated expression of IL-1β, although our findings did show a trend towards higher expression of this cytokine by CD14⁺ monocytes after NS resuscitation. On the other hand, spontaneous shock/resuscitation-induced expression of TNF-α by unstimulated CD14⁺ monocytes increased significantly ($P < .05$) over time in NS resuscitated patients, with the % TNF-α-positive monocytes more than doubling and the MFI of positive cells increasing by almost 20% above baseline by 24-h. Importantly, **HSD** resuscitation totally reversed ($P < .05$) the spontaneous increases in TNF-α expression by unstimulated CD14⁺ monocytes.
Hypertonic Saline Resuscitation Restores Inflammatory Cytokine Balance in Post-Traumatic Hemorrhagic Shock Patients

Table 3: Percentage (%) and mean fluorescence intensity (MFI) of cytokine-positive CD14+ monocytes in unstimulated whole blood according to resuscitation treatment group

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>IL-1β %</th>
<th>IL-1β MFI</th>
<th>TNF-α %</th>
<th>TNF-α MFI</th>
<th>IL-1ra %</th>
<th>IL-1ra MFI</th>
<th>IL-10 %</th>
<th>IL-10 MFI</th>
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<tr>
<td><strong>Normal Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>14.8 ± 2.0</td>
<td>377.6 ± 20.9</td>
<td>14.4 ± 2.5</td>
<td>338.2 ± 12.1</td>
<td>11.9 ± 2.1</td>
<td>372.8 ± 17.2</td>
<td>10.1 ± 1.8</td>
<td>325.2 ± 14.1</td>
</tr>
<tr>
<td>1 h</td>
<td>22.1 ± 2.4</td>
<td>373.1 ± 18.3</td>
<td>17.7 ± 3.2</td>
<td>341.3 ± 10.4</td>
<td>13.3 ± 3.0</td>
<td>379.2 ± 16.1</td>
<td>11.5 ± 2.9</td>
<td>324.4 ± 13.5</td>
</tr>
<tr>
<td>3 h</td>
<td>22.0 ± 2.8</td>
<td>382.1 ± 20.1</td>
<td>20.0 ± 2.8</td>
<td>351.8 ± 19.4*</td>
<td>11.6 ± 2.9</td>
<td>365.6 ± 15.9</td>
<td>11.9 ± 2.4</td>
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<tr>
<td>6 h</td>
<td>22.9 ± 2.4</td>
<td>380.2 ± 27.9</td>
<td>21.7 ± 2.8</td>
<td>354.9 ± 17.6*</td>
<td>15.4 ± 3.3</td>
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<td>24 h</td>
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<td>31.4 ± 3.9*</td>
<td>399.7 ± 19.7*</td>
<td>13.8 ± 3.4</td>
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<tr>
<td>Baseline</td>
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<td>371.4 ± 11.8</td>
<td>13.3 ± 2.9</td>
<td>344.3 ± 14.1</td>
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<td>389.1 ± 20.4</td>
<td>9.5 ± 2.4</td>
<td>325.9 ± 10.1</td>
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<tr>
<td>1 h</td>
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<td>16.6 ± 3.1</td>
<td>341.7 ± 15.8</td>
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<td>417.1 ± 21.9</td>
<td>20.1 ± 3.8*</td>
<td>321.5 ± 8.5</td>
</tr>
<tr>
<td>3 h</td>
<td>17.24 ± 3.02</td>
<td>376.6 ± 11.8</td>
<td>17.3 ± 3.5</td>
<td>338.7 ± 20.4†</td>
<td>29.2 ± 4.8†</td>
<td>415.7 ± 22.3</td>
<td>25.5 ± 3.0*†</td>
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<td>24.7 ± 2.0*†</td>
<td>357.1 ± 17.1*†</td>
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<td>375.6 ± 20.2</td>
<td>21.5 ± 2.8*</td>
<td>349.8 ± 16.7*</td>
<td>26.8 ± 3.3†</td>
<td>447.7 ± 24.9*</td>
<td>21.1 ± 2.9*†</td>
<td>354.0 ± 25.4‡†</td>
</tr>
</tbody>
</table>

Evaluation of monocytic cytokine production was based on the percentage and relative mean fluorescence intensity (MFI; channel ± SE) from 5,000 events within each monocyte subset. Differences in MFI for all cytokines were measured on a single parameter (FL2) histogram using a linear scale (arbitrary units scaled from 0–10,000) after subtracting MFI values from corresponding isotype-matched negative controls.

*P < 0.05 within trial vs. baseline; †P < 0.05 between trial vs. normal saline; all others, P > 0.05 by ANOVA

Examination of LPS-stimulated cytokine production showed a similar pattern of TNF-α expression to that observed in spontaneous cultures, with substantially augmented (>150%) production with NS resuscitation, as measured by changes in MFI from baseline (Figure 2A–B). Detailed monocyte subset analysis revealed that, although both CD14+CD16- and CD14+CD16+ subpopulations were capable of expressing TNF-α, the expanded CD14+CD16+ inflammatory subset was primarily responsible for both spontaneous and LPS-induced upregulation of TNF-α production after NS treatment. Moreover, as shown in Figure 2B HSD resuscitation specifically inhibited TNF-α-production by CD14+CD16+ monocytes. These data demonstrate that HSD resuscitation not only reduces the mobilization of CD14+CD16+ monocytes to the peripheral blood of resuscitated patients, but also effectively inhibits both the spontaneous and stimulated capacity of these proinflammatory cells to produce TNF-α in response to inflammatory stimuli.
Figure 2: Changes in intracellular cytokine expression by blood monocyte subsets of resuscitated patients. Normal saline (NS) and hypertonic saline/dextran (HSD)-treated patients exhibited differential expression of tumor necrosis factor (TNF)-α (A, B), IL-10 (C, D), and IL-1ra (E, F) by CD14⁺CD16⁻ and CD14⁺CD16⁺ subsets, respectively. Blood was cultured without (spontaneous: ◦, NS; ○, HSD) or with lipopolysaccharide (LPS-stimulated: ■, NS; □, HSD) at 1µg/mL for 20-h in the presence of brefeldin A. Intracellular cytokines were stained as described in the Methods. Data are expressed as change in mean fluorescence intensity (ΔMFI) compared to baseline (set at 100%). Significant differences: *P < .01, post-resuscitation values vs. baseline within treatment group; †P < .01, HSD vs. time-matched NS values.
3.3 HSD Resuscitation Enhances IL-10 and IL-1ra Production by CD14\(^{++}\)CD16\(^{-}\) Monocytes

To determine the effect of hypertonicity on the ability of circulating blood monocytes to mount a counter-inflammatory response to shock/resuscitation, spontaneous and LPS-induced intracellular expression of the antiinflammatory cytokines IL-10 and IL-1ra was measured in whole blood monocyte subsets obtained from resuscitated trauma patients. As shown in Table 3, standard resuscitation with NS did not significantly influence the acute (1–6-h) spontaneous monocytic expression of either antiinflammatory cytokine, but did significantly \((P < .05)\) suppress IL-1ra expression 24-h post-resuscitation. Compared to standard resuscitation, HSD treatment led to an early and sustained rise in spontaneous production (% and MFI) of both IL-10 and IL-1ra. In the case of IL-10, we found that the %IL-10-positive CD14\(^{+}\) monocytes more than doubled by 1-h post-resuscitation and remained significantly \((P < .05)\) above baseline levels after 24-h; this response was closely matched by a corresponding increase in IL-10 MFI values (Table 3). Importantly, monocyte subset analysis traced the HSD-induced increase in spontaneous IL-10 expression exclusively to augmented production by the CD14\(^{++}\)CD16\(^{-}\) subset; this response was further potentiated after LPS-stimulation (Figure 2C). These results demonstrate the inability of CD14\(^{+}\)CD16\(^{+}\) monocytes to produce IL-10 (Figure 2D) and are in concurrence with previously reported findings indicating that these cells fail to express significant amounts of IL-10 [44].

Correspondingly, HSD resuscitation also amplified IL-1ra expression in LPS-stimulated monocytes; however, in contrast to IL-10 production, both CD14\(^{++}\)CD16\(^{-}\) and CD14\(^{+}\)CD16\(^{+}\) subsets contributed to the overall increase in IL-1ra production (Figure 2E-F). Together, these observations are consistent with recent experimental animal studies [37,46] demonstrating that HSD can profoundly augment early post-traumatic compensatory antiinflammatory cytokine production, and that this may promote a more balanced inflammatory response, by favorably shifting the equilibrium of pro vs. antiinflammatory cytokines.

3.4 HSD resuscitation downregulates monocytic cellular adhesion molecule expression

Leukocyte recruitment and adhesion to the vascular endothelium is a pivotal event in acute inflammation and tissue damage [47]. This process requires the sequential interaction of cell-surface L-selectin (CD62L) and \(\beta_2\) integrins (CD11b), with their complementary endothelial ligands. Monocyte/macrophages constitutively express low levels of these molecules, which mediate adherence (rolling and firm attachment) to activated or damaged endothelium and subsequent migration into adjacent inflamed tissues. Inappropriate upregulation of these molecules after shock resuscitation can lead to microvascular injury and the development of post-traumatic organ dysfunction [48]. In the present investigation, between 98% and 100% of all monocyte populations expressed the selected adhesion molecules at baseline, and that this proportion was not significantly altered over the sampling period by either resuscitation strategy (data not shown). Changes in monocyte surface density (expressed as % change in MFI over baseline) of CD11b and CD62L by LPS-stimulated and unstimulated monocytes was, however, significantly affected by the experimental treatment. On average, LPS-stimulation augmented monocytic CD11b expression by 14%. Notably, LPS-induced up-regulation of CD11b expression was significantly inhibited \((P < .05)\) by treatment with HSD between 3 and 24-h post-resuscitation, as compared to NS (Figure 3A). Despite an apparent early rise in unstimulated CD11b expression by monocytes after NS resuscitation, differences were not statistically significant over time or between treatment arms.

By comparison, unstimulated CD62L expression began to rise 1-h after standard NS resuscitation, reaching statistical significance by 24-h \((p < .01)\) (Figure 3B). Unstimulated CD62L levels remained unchanged following HSD resuscitation; however, as with CD11b, the expression of CD62L was significantly inhibited by HSD at 3-h, 6-h and 24-h compared with NS. LPS-stimulation elicited an average of 25% loss of CD62L surface expression as compared to unstimulated levels. However, no significant inter-trial treatment effects
were observed for LPS-stimulated CD62L expression over the sampling period. In sum, these findings demonstrate that both stimulated and unstimulated monocytic adhesion molecule expression is down-regulated in response to HSD as compared to NS. This suggests that HSD resuscitation elicits less overall monocyte activation and that at the microvascular level, HSD treatment may have the capacity to modulate monocyte trafficking, possibly limiting leukocyte-endothelial interactions in acute inflammatory reactions.

Figure 3: Changes in cellular adhesion molecule expression by blood monocytes of resuscitated trauma patients. Normal saline (NS) and hypertonic saline-dextran (HSD)-treated patients showed differential expression of CD11b (A) and CD62L (B) by unstimulated (■, NS; □, HSD) and lipopolysaccharide-stimulated (●, NS; ▴, HSD) blood monocytes. Data are expressed as percentage change in mean fluorescence intensity (ΔMFI) compared to baseline conditions (set at 100%). Significant differences: *P < .05, post-resuscitation vs. baseline values within a treatment group; †P < 0.05, HSD vs. time-matched NS control values.
3.5 Circulating NE Secretion is Inhibited by HSD Resuscitation

Changes in circulating concentrations of NE and Epi are shown in Figure 4A-B, respectively. On admission, pooled mean baseline concentrations of NE and Epi (907.9 ± 115.5 and 1207.3 ± 13.9, respectively) were substantially elevated in hemorrhagic shock patients as compared to expected basal values (reference ranges, 100–400 and 60–90 pg/mL, respectively) reported for healthy adults. Compared to baseline values, resuscitation with NS quickly augmented circulating NE levels up to 60% \((P < .05)\) between 1 and 6-h; remarkably, HSD resuscitation completely abrogated \((p < .05)\) the post-resuscitative elevation of NE. In contrast, Epi levels did not differ between treatment arms, but fell significantly over the post-resuscitation period in both patient groups.

![Figure 4](image)

**Figure 4:** Changes in circulating catecholamine concentrations of resuscitated trauma patients. Normal saline (NS, □) and hypertonic saline-dextran (HSD, ■)-treated patients exhibit unique hormone secretion profiles. Data are expressed as the mean (± SE) circulating concentration of norepinephrine (A) and epinephrine (B). Significant differences: *\(P < .05\), post-resuscitation vs. baseline values within a treatment group; †\(P < 0.05\), HSD vs. time-matched NS control values.
4.0 DISCUSSION

A growing awareness of the limitations of conventional isotonic crystalloid resuscitation fluids and the potential advantages of hypertonic/hyperoncotic solutions, has led to the reemergence of experimental and clinical interest in the role and mechanism of hypertonic saline resuscitation of hemorrhagic shock [9]. Increasingly, experimental evidence indicates that treatment with hypertonic saline/dextran (HSD) exerts profound immunoinflammatory activities, influencing post-injury cellular and molecular inflammatory reactions to shock/resuscitation leading to an improved host response [34,35]. The present clinical trial demonstrates, for the first time in resuscitated hemorrhagic shock patients, that compared with standard isotonic fluid therapy, supplementing initial resuscitation with a small-volume (250-mL) of HSD differentially modulates inflammatory cytokine and cellular adhesion molecule expression by blood monocytes of treated patients. Our results provide direct evidence that HSD infusion in the early stage following hemorrhagic shock helps reestablish the critical balance between pro and antiinflammatory mediators, which could lead to a reduction in post-traumatic complications and improved patient outcome as documented in animal models [5]. Additionally, this study suggests that HSD’s immunoregulatory activities are linked to altered sympathetic activation.

The pathophysiology of resuscitated hemorrhagic shock is characterized by an uncontrolled whole-body hyper-inflammatory reaction [8] that is triggered by a number of hostile stimuli, including, injured tissue, ischemia, hypoxia, and reperfusion damage [49]. Strong activation of the monocyte/macrophage system with excessive synthesis of the primary proinflammatory cytokines TNF-α and IL-1β, are thought to play a crucial role in the early pathogenesis of hemorrhagic shock [8]. These intercellular signalling molecules elicit their biological responses by activation of intracellular signal transduction pathways via binding cell-surface receptors, which are coupled to multiple downstream cytosolic intermediates and ultimately transmitted to nuclear regulatory factors (e.g., nuclear factor (NF)-κB), leading to the expression of target genes and regulation of target cell function [16]. Many normal physiologic processes, including, endocrine, immune and inflammatory responses are regulated by endogenous cytokine secretion. Therefore, homeostatic control of the balance between pro and antiinflammatory cytokines is critical for the maintenance of health [15]. Overwhelming insults such as severe hemorrhagic shock constitute a formidable challenge to the patient’s ability to maintain this balance [13].

Blood monocytes are a heterogeneous population of mononuclear leukocytes that, like lymphocytes, comprise phenotypically and functionally distinct subsets [50]. They play a pivotal role in the innate immune response, serving as nonspecific effector cells, secreting cytokines and regulating tissue inflammation. At least two monocyte subpopulations are distinguishable on the basis of their expression of membrane antigens, migratory properties and cytokine production profiles [42,43]. In healthy individuals, the majority of circulating monocytes are strongly CD14-positive, but lack coexpression of CD16 (CD14+CD16-, classical monocytes). A minor subpopulation coexpress CD16 and are weakly CD14-positive (CD14+CD16+); these cells usually account for about 5–10% (~0.05 x10^9 cells/L) of all circulating monocytes under normal physiological conditions [43]. A significant finding of the present study was the marked differential redistribution of blood monocyte subpopulations observed in HSD-resuscitated trauma patients, as compared with patients who received just standard isotonic resuscitation.

CD14+CD16+ cells constitute a proinflammatory subtype that exhibit several features of inflammatory tissue macrophages, including, a distinct pattern of cytokine expression as compared with classical monocytes [43,50]. Specifically, the CD14+CD16+ subset have an enhanced capacity for TNF-α production [51], while they produce little or no IL-10 [44]. Circulating levels of CD14+CD16+ monocytes are known to increase dramatically (up to 50% of total monocytes) in patients with severe infectious diseases or other clinical
Hypertonic Saline Resuscitation Restores Inflammatory Cytokine Balance in Post-Traumatic Hemorrhagic Shock Patients

disorders with features of systemic inflammation [43,45]. Distinct alterations in the phenotype and function of blood monocytes have also been noted in patients undergoing major surgery [52]. Here, we demonstrate that both the mean proportion (~23%) and absolute counts (~0.12 x10^9 cells/L) of circulating CD14^+CD16^+ monocytes were already elevated in patients upon arrival at the trauma centre. Those patients resuscitated by standard methods experienced a continued expansion of the inflammatory subset (up to 35%) during the subsequent post-resuscitation sampling period. Remarkably, HSD-resuscitated patients displayed a significant reduction in CD14^+CD16^+ cells (<15%) over the same time-course. These results show clearly that HSD prevents post-traumatic expansion of the proinflammatory CD14^+CD16^+ monocyte phenotype. Considering that the frequency of CD14^+CD16^+ monocytes varies in association with several inflammatory states, and is directly related to the cytokine production potential of blood monocytes, our findings strongly suggest that HSD has the capacity to modulate monocytic inflammatory cytokine production.

Although the mechanism for this distinct pattern of monocyte subset redistribution is presently unknown, it can be speculated that enhanced post-traumatic sympathetic activation may be involved [19], since CD14^+CD16^+ monocytes are known to be selectively mobilized from the marginal pool in a rapid catecholamine-dependant fashion after extreme forms of physical stress [53]. The current study also demonstrates that HSD resuscitation elicits significant alterations in cell-surface expression of L-selectin (CD62L,) and β integrins (CD11b) by circulating monocytes. Most notably, initial treatment with HSD was found to significantly blunt post-resuscitation expression of these adhesion molecules. Previous clinical studies, involving polytrauma patients, have shown that acute up-regulation of monocytic CD62L and CD11b occurs after injury and is related to the development of post-traumatic organ failure [54]. Furthermore, it has been shown that under inflammatory conditions, up to 70% of alveolar macrophages are may be derived from circulating CD14^+CD16^+ monocytes [55]. This finding raises the possibility that standard resuscitation fluids may promote the egress of inflammatory monocytes from the blood into the lungs through their enhancement of the CD14^+CD16^+ phenotype. Thus, our results suggest that HSD treatment may help avert widespread inflammatory monocyte activation upon injury via its capacity to modulate monocyte trafficking and by limiting leukocyte-endothelial interactions in the early post-traumatic period. Further studies are required to definitively elucidate the mechanism of differential shock/resuscitation-induced monocyte redistribution.

The major finding of the present investigation substantiates that supplementing initial resuscitation with a small quantity of HSD not only selectively influences the mobilization of functionally heterogeneous monocyte subsets, but also differentially modulates their intracellular expression of pro and antiinflammatory cytokines. With respect to TNF-α, we found that standard resuscitation induced a marked increase in the spontaneous and LPS-stimulated cytokine-producing activity of blood monocytes. Impressively, treatment with HSD effectively halted the upregulation of TNF-α expression at the single-cell level. Detailed monocyte subset analysis indicated that the expanded CD14^+CD16^+ subset was principally responsible for the enhanced TNF-α production. These findings indicate that specific blood monocytes are an important source of enhanced TNF-α production in resuscitated hemorrhagic shock patients, and that HSD resuscitation down-regulates this excessive inflammatory response. Our results are consistent with a recent study by Belge et al. [51], which provided evidence that the minor population of CD14^+CD16^+ inflammatory monocytes is a major source of intracellular TNF-α production in humans. The current findings are also in accordance with previous clinical reports showing significantly elevated monocytic gene expression and systemic concentrations of TNF-α immediately following major surgery [56], severe trauma or hemorrhagic shock [57].

To our knowledge, there are no other clinical reports evaluating the effects of hypertonic resuscitation on cytokine production in humans; however, available data from experimental studies support the current findings. Several reports indicate that hypertonic saline effectively inhibits spontaneous and LPS-stimulated cytokine production by a variety of tissues following hemorrhagic shock and ischemia-reperfusion [58].
Hypertonic Saline Resuscitation Restores Inflammatory Cytokine Balance in Post-Traumatic Hemorrhagic Shock Patients

Recent studies using a two-hit model of shock resuscitation found that hypertonic preconditioning inhibits LPS-induced TNF-\(\alpha\) production by isolated alveolar and peritoneal macrophages [37,46,59]. Moreover, studies using cDNA microarray analysis to characterize inflammatory gene expression in major organs and human leukocytes after shock/resuscitation reveal that manipulation of fluid tonicity modulates cytokine gene transcription, including TNF-\(\alpha\) expression [60]. A strength of the current investigation is the use of intracellular flow cytometric analysis, which has the advantage over conventional cytokine assays, in that it allows the immunophenotype, frequency and functional characteristics of individual cytokine-producing cells to be determined simultaneously within heterogeneous whole-blood cell populations, thus avoiding many problems inherent to soluble cytokine measurements [41].

An appropriate balance between pro and antiinflammatory cytokines is critical in the resolution of many pathological inflammatory conditions [16]. If unchecked, sustained dysfunctional inflammation contributes to sequential multiorgan failure and is a major cause of late trauma deaths [6,12]. Therefore, the immune system closely regulates proinflammatory cytokine production via upregulation of counter-inflammatory mediators. IL-10 is a prototypical endogenous antiinflammatory and immunosuppressive cytokine [61], which inhibits monocyte/macrophage activation and down-regulates the biosynthesis of TNF-\(\alpha\) and IL-1\(\beta\), while preventing their biologic actions via up-regulation of IL-1ra [17]. TNF-\(\alpha\) is the primary inducer of IL-10 synthesis, and IL-10 downregulates its own production via an autoregulatory feedback mechanism [62]. The antiinflammatory and immunosuppressive actions of IL-10 derive from its capacity to inhibit the translocation of NF-\(\kappa\)B, leading to reduced transcription of a number of inducible genes involved in immune and inflammatory responses [63]. IL-10 also decreases leukocyte adhesion and recruitment to sites of inflammation [61]. Together these mediators serve to limit the potentially injurious effects of excessive inflammatory reactions [17].

Our results show that specific blood monocytes from patients resuscitated with HSD have an enhanced capacity for IL-10 and IL-1ra production, as compared with patients resuscitated by standard means alone. We determined that antiinflammatory cytokine expression exhibits a monocyte subset-specific production profile. That is, IL-1ra expression was detected in significant amounts in both CD14\(^++\)CD16\(^-\) and CD14\(^+\)CD16\(^+\) monocytes subsets, whereas, enhanced IL-10 expression was confined exclusively to CD14\(^++\)CD16\(^-\) cells. This observation agrees with earlier findings demonstrating that CD14\(^+\)CD16\(^+\) monocytes do not produce significant amounts of IL-10 [44], while IL-1ra is more ubiquitously synthesized [64]. These findings are also in agreement with recent animal studies showing that alveolar and peritoneal macrophages exposed to hypertonic conditions have significantly upregulated IL-10 synthesis [37,46]. Collectively, these observations strengthen the hypothesis that hypertonic resuscitation may exert its beneficial effects through early upregulation of counter-inflammatory mediators.

Nevertheless, previous experimental and clinical studies have produced conflicting results concerning the beneficial versus deleterious effects of endogenously elevated or systemically administered IL-10 [65]. In a number of studies, increased circulating concentrations of IL-10 have been associated with adverse clinical outcome in patients with sepsis syndromes. However, several recent investigations have shown that early therapeutic administration of IL-10 is effective in preventing the initial surge in TNF-\(\alpha\) observed after traumatic hemorrhagic shock [66], and also in reducing the systemic inflammatory response and lethality in murine models of sepsis and reperfusion injury [67]. Indeed, Miller-Graziano et al. [68] suggested that trauma-mediated downregulation of IL-10 production contributes to the overproduction of TNF-\(\alpha\) and increased occurrences of end organ failure in severely injured patients. Similarly, Donnelly and Colleagues observed that both IL-10 and IL-1ra were significantly lower in non-surviving ARDS patients [69]. These authors concluded that the failure of patients to mount an effective early antiinflammatory cytokine response contributed to the adverse outcome.
These results imply that when administered early in the post-trauma period, HSD has the capacity to rapidly up-regulate the counter-inflammatory cytokines, which are capable of attenuating hyper-inflammatory reactions, thereby preventing exacerbated pathology. It follows that initial resuscitation with HSD may also help avert a subsequent exaggerated immunosuppressive compensatory antiinflammatory response. This hypothesis is supported by laboratory evidence showing that HSD reduces the risk of delayed immunosuppression in mice after hemorrhagic shock by restoring suppressed lymphocyte function [70].

While the physiologic responses to HSD resuscitation are reasonably well defined [27], the mechanisms by which hypertonicity alters cellular and intracellular signaling pathways involved acute immunomodulation are not yet fully elucidated [11,35]. Potential targets include modification of various cell surface receptors, cytoplasmic second messengers and/or nuclear transcription factors [5,34]. Although the current study was not designed to directly evaluate the impact of hypertonicity on cytokine signaling, our finding that HSD greatly diminishes post-resuscitation concentrations of NE implies a sympathetic neuroendocrine mechanism for such alterations. The current findings also corroborate earlier studies showing that HSD resuscitation significantly attenuates the sympathoadrenal response to trauma and hemorrhage, with substantial reductions in circulating NE [71]. This is particularly relevant since catecholamines are recognized as key regulators of inflammatory cytokine production [18,20].

**Figure 5:** Potential sympathetic noradrenergic mechanisms of inflammatory cytokine modulation by hypertonic saline (solid lines indicate stimulation, dashed lines indicate inhibition; see text for details).
of catecholamines are mediated via α- and β-adrenergic cell-surface receptors coupled to intracellular cAMP levels. NE-induced α-adrenergic stimulation has a cAMP-decreasing effect that enhances the formation of IL-10, but inhibits TNF-α [72] (see Figure 5). On the other hand, β-adrenergic stimulation by Epi mediates a cAMP-enhancing effect, which inhibits the production of TNF-α. Based on these observations and the present findings, it can be postulated that the upregulation of NE (mediating reduced cAMP) observed in patients resuscitated by standard means, contributes to excessive production of TNF-α and insufficient IL-10 induction in the early post-resuscitative period. Thus, it appears that through its specific capacity to inhibit NE secretion while preserving circulating Epi levels, HSD shifts the balance in favor of enhanced cAMP levels, thereby, mediating down-regulation of TNF-α and upregulation of IL-10.

The concept of HSD-induced differential sympathetic adrenergic cytokine regulation is supported by a number of experimental observations from previous studies. For example, in rats endogenous gut-derived NE has been shown to play a crucial role in the early systemic inflammatory response via enhanced hepatic α-adrenoreceptor stimulated TNF-α production [73]. Correspondingly, hemorrhage-induced pulmonary TNF-α expression is prevented by α-adrenergic blockade in mice [74]. Moreover, recent experimental findings demonstrate that exposure of human leukocytes to moderate hypertonicity triggers rapid accumulation of cAMP and suppression of their inflammatory activity [75]. Collectively, these findings suggest HSD resuscitation, through its capacity to stabilize shock/resuscitation induced NE concentrations, can dampen α-adrenergic-mediated TNF-α production, leading to reduced inflammation, ischemia and organ damage.

5.0 CONCLUSION

Hemorrhagic shock/resuscitation-induced immunoinflammatory alterations are clearly a complex phenomenon, likely due to the interaction of a variety of endogenous mediators. The present results demonstrate that, compared to standard treatment, supplementing initial resuscitation of hemorrhagic shock patients with 250-mL of HSD exerts a subset-specific immunomodulatory effect on peripheral blood monocytes, resulting in a shift in the dynamic balance between pro and antiinflammatory responses in the early post-resuscitation period. In particular, HSD seems to restore the balance between pro and antiinflammatory cytokines, by reducing initial TNF-α production by CD14⁺CD16⁺ monocytes, while simultaneously enhancing early antiinflammatory IL-10 and IL-1ra production by CD14⁺CD16⁻ monocytes. HSD’s beneficial therapeutic effects may, therefore, also derive from its ability to avoid extensive delayed counter-regulation by reducing the magnitude of the initial systemic inflammatory response. Although further research is required to identify specific intracellular mechanisms involved in HSD’s immunomodulatory actions, our results suggest that the differential cytokine expression in response to HSD may be mediated, at least partly, by preferential inhibition of post-resuscitation noradrenergic-induced TNF-α production and concomitant enhancement of IL-10 production via down-regulation of α-adrenergic-stimulated signaling pathways. These findings reinforce the notion that hypertonic/hyperoncotic solutions are not simply benign volume enhancers, but instead also act as potent pharmacological agents. As such, small-volume HSD resuscitation strategies should be designed to supplement, not supplant current fluid replacement modalities and prompt surgical intervention. Overall, HSD offers new promise as a simple, yet highly effective therapeutic approach for prevention of the excessive hyper-inflammatory state associated with resuscitated hemorrhagic shock and the risk of organ damage. These findings should influence the design of future clinical trials of hypertonic solutions in pre-hospital and hospital trauma care.
Acknowledgements
We thank Sheila Petrongolo for expert technical assistance. This work was supported by Defence R&D Canada.

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Hypertonic Saline Resuscitation Restores Inflammatory Cytokine Balance in Post-Traumatic Hemorrhagic Shock Patients


Compression Bandage, not Tourniquet. Experience in 68 Patients with Traumatic Amputation after Mine Injuries

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TOURNIQUET, A SUBOPTIMAL TREATMENT

Sixty eight patients with traumatic amputations after mine injuries were treated in the demilitarized zone between Iraq and Kuwait. Most were seen during a three week period of Iraqi mine harvesting. During the first days, continuous bleeding distally to applied tourniquets were frequently observed. Orders were issued to remove any applied tourniquets and dress the wounds with a tight elastic bandage. Three out of 18 patients died during the first part of the period compared to 1 of 50 during the last part. The new directives led to visibly less hemorrhaging. Hemoglobin at admission increased and fewer patients needed transfusions. In extensive crush injuries and traumatic amputations a tight bandage applied from the end of the extremity and in proximal direction should be used. Tourniquet should not be used.

1.0 INTRODUCTION

Stopping of external hemorrhage has the highest priority in combat casualty care. ”Hemostasis” with a tourniquet has a strong position with the public. It is effectiveness is questionable. In the fall of 1991, the effect of tourniquets were observed in patients injured by antipersonnel mines in the demilitarized zone between Iraq and Kuwait.

2.0 MATERIAL

In the fall of 1991, 157 patients were taken care of after mine explosions by the military medical unit in the United Nation mission in the demilitarized zone between Iraq and Kuwait. The injured patients were brought to one of two first aid posts in the desert and 109 were evacuated to a role 2+ field hospital. One patient had an open chest wound, two had tracheal puncture wounds, while one had penetrating head injury. Twenty seven patients had eye injuries, 13 being penetrating. Sixty eight patients had major amputations, seven of them had two extremities blown off. Sixty four major surgical procedures were performed.

One hundred and forty eight of the patients were seen during a three week period of Iraqi mine harvesting in the antitank mine fields in the desert. These fields consisted of more rows of antitank mines several meters apart. Small antipersonnel mines had been placed around the antitank mines. The patients had mostly stepped on the antipersonnel mines or picked them up. In some cases the fuse of an antitank mines had exploded after being dismantled.

After first aid treatment the patients were either evacuated by helicopter or by ambulance to the field hospital. Helicopter was used during daylight and when there was not a sandstorm. Evacuation time from injury to hospital was regularly four to six hours or more.

3.0 METHODS

The surgical detachment worked according to the principles of a light field hospital. The patients were operated upon if necessary, stabilized and evacuated to Iraqi hospitals some 50 miles away. Operated patients were kept until the following day. All amputations were open with planned primary delayed closure five days post injury.

In amputation cases tourniquets had always been put on by the patients’ comrades in the field. Additional tourniquets were in the initial period applied by the medical corpsmen. Rapid intravenous infusion was started with one liter of Dextran and continued with Ringer’s acetate. With this treatment the patients were often found circulatory unstable upon hospital admission. Continuous bleeding distally to applied tourniquets was frequently observed (figure 1). Hemoglobin was often low. On September 28th orders were issued to remove any applied tourniquets in the field, pack the damaged soft tissue and cover the wounds with a very tight elastic bandage. Rapid intravenous infusion was given only to patients with altered consciousness due to low blood pressure. As a routine only a slow intravenous drip was started. Patients received blood transfusions when hemoglobin was lower than 7 g/100ml and there was need of volume replacement. When hemoglobin was lower than 5 g/100ml, transfusion was given for the anemia.

![Figure 1: Traumatic amputation of right lower extremity after stepping on an antipersonnel mine. There is bleeding through the bandages in spite of tourniquets put on both below and above the knee. There was substantial blood soiling on the litter.](image)

The 68 patients of the 109 patients evacuated to the field hospital suffering traumatic major amputations are analyzed. Patients were followed as long as they were under the administration of the surgical unit. Registrations before and after September 28th, when the new first aid routines came into use, were compared. The effect of tourniquet and infusions is evaluated by comparing hemoglobin and transfusion need and initial clinical course. Chi-squared test with Yate's correction for small numbers, Fisher exact test or Student's t-test have been used.

4.0 RESULTS

Twenty patients altogether (13%) died during observation. Four of the 109 patients evacuated to the field hospital died (4%). One patient with amputated lower extremity was stabilized and evacuated to rear
hospital, but he died in the ambulance, the tourniquet obviously not being effective. An other patient died two days postoperatively from brain damage caused by anemia, fall in blood pressure and consequent low output cardiac failure.

Two patients had extensive hemorrhages from large wounds in the groins and thighs. They died during transport before they reached the field hospital. The last of these were in deep shock without peripheral circulation when found. Intravenous lines could not be established in the field. He was the only patient among those who were evacuated to the hospital, that died in the last part of the observation period. Of the hospitalized 68 amputees, 3 of 18 (17%) died during the first period, 1 of 50 (2%) during the last period (p<0.05) (table 1).

The new routines led to less hemorrhaging observed by less soiling of blood on the litters (figure 2). Hemoglobin value at admission was higher in the last period (mean 10.5 g/100ml versus 8.6 g/100ml, p<0.05). The three patients that died in the first period were infused to low hemoglobin values: 5.6 and 4.6 gram/100ml. Patients in the first period were often described as having "watery bleeding from the wounds". Fewer patients needed blood transfusions after the use of tourniquet was disbanded (13/50 (26%) as compared to 10/18 (56%), p<0.05). Hemoglobin value at admission was higher in the last period (table 2).

<table>
<thead>
<tr>
<th>Dates 1991</th>
<th>Treatment</th>
<th>Number</th>
<th>Transfusions</th>
</tr>
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<tbody>
<tr>
<td>July 31&lt;sup&gt;th&lt;/sup&gt; – September 27&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Tourniquet</td>
<td>18</td>
<td>3 (17%)</td>
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<tr>
<td>September 28&lt;sup&gt;th&lt;/sup&gt; – October 14&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Not tourniquet</td>
<td>50</td>
<td>1 ( 2%) *)</td>
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*) p<0.05 chi-squared test

Table 1: Mortality in 68 patients with major amputations after mine injuries before and after the use of tourniquets was disbanded.

<table>
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<th>Transfusions</th>
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<tr>
<td>July 31&lt;sup&gt;th&lt;/sup&gt; – September 27&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Tourniquet</td>
<td>17</td>
<td>10 (56%)</td>
</tr>
<tr>
<td>September 28&lt;sup&gt;th&lt;/sup&gt; – October 14&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Not tourniquet</td>
<td>50</td>
<td>13 (27%) *)</td>
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*) p<0.05 Student’s t-test

Table 2: Hemoglobin values at hospital admission before and after the use of tourniquets in amputations was disbanded.

In the first part of the period the patients were often very unstable. In three cases immediate operation with clamping of the femoral artery through a medial incision on the femur, was performed. With the new routines there was time for complementation of further evacuation or operation without hurry.

Blood transfusions were given to 24 patients, 23 with amputations. Fewer patients needed transfusions after the use of tourniquet was disbanded (table 3), but the mean number of transfusions to those who received any, remained the same, 2.3 units.
<table>
<thead>
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<td>50</td>
<td>13 (27%) *)</td>
</tr>
</tbody>
</table>

*) p<0.05 chi-squared test

Table 3: Patients needing blood transfusions before and after the use of tourniquets in amputations was disbanded. The median number of transfusions to those who needed it was 2.3 in both groups.

Urine output was low in several patients, but normalized in all shortly after admission. Renal function could not be further monitored.

The age of the patients in the first and last part of the observations period was mean 33,2 +- 9 and 29,9 +- 7 years (p=0,13). Range was 9 to 51 years. They were all men.

Figure 2: Correct treatment in traumatic amputation. Complete hemostasis has been achieved by packing the soft tissue and applying a very tight compressing bandage from the end and in proximal direction.

5.0 DISCUSSION

A tourniquet will not stop bleeding from the bone marrow of a crushed extremity. A tourniquet is usually made from a piece of cloth. It compresses the artery over a short length. Some blood will seep by. This will lead to venous stasis and increased bleeding. Tourniquet below the knee will not work. One of the arteries here passes between bones (figure 3).
In wounds the arteries will usually contract, in some cases after the blood pressures has dropped. Copious intravenous infusions will elevate the blood pressure and bleeding may restart. More infusions are needed to maintain the blood pressure and the patient is hemodiluted. If cardiac output falls due to rebleeding, the patient is in a worse situation with combined hypovolemia and anemia. In cases with short evacuation time, the documentation from urban traumatology is convincing. In long evacuation time, the subsequent anemia from seeping hemorrhage over a long time, is probably as dangerous as some hypotension.

Prehospital blood pressure and the exact amount of intravenous fluid given was not recorded sufficiently well to be analyzed in this study. Less and more careful infusions were used during the latter period. That weakens the conclusion somewhat as hypotensive resuscitation may have been responsible for some of the observed improvement.

Hemostasis with a tourniquet as the hemostatic remedy has a strong position with the public. It is not excluded in some first aid books [1]. It is, however, emphasized that it should be applied only when satisfactorily control of the bleeding has not been achieved with a tight bandage. To us that will only be a situation with a trapped, crushed extremity and ongoing arterial bleeding. A tourniquet is a risk to the survival of remaining stump of the limb and may lead to unnecessary loss of knee or elbow.

A tourniquet left on for a long time may lead to reperfusion injury. Inflammation induced injury caused by reperfusion of hypoperfused tissues are greater than the hypoperfusion in itself. The reperfusion damage with massive destruction of the microcirculation in the injured limb may occur after 60 minutes of local low flow [2,3,4]. The reperfusion injury is, however, not only a microvascular catastrophe affecting the hypoperfused limb. Inflammatory mediators affect vital organs as well, especially the gut mucosa seems to be vulnerable [5,6,7,8,9]. In hypotensive patients where the splanchnic bed is poorly perfused, we should regard tourniquets as a considerable risk not only to the limb, but also to the life of the victim.

The argument that tourniquets as last resorts should be allowed when “effective pressure dressing” does not control the bleeding rises problems. It is not easier to teach correct application of effective tourniquets...
than to teach application of compressive bandages, as we learnt by our medical corpsmen in Iraq. Quite contrary, results are better when tourniquets are avoided [10,11]. Effective wound packing may be thought successfully to a variety of personnel [12]. Studies indicating improved control of limb bleeding by tourniquet in certain situations are not always all that convincing as it may be difficult to ascertain that the protocol indications were met [13] (figure 4).

![Figure 4: By stressing the use tourniquet all sorts of misconceptions arise. (Photo: Norwegian Air Ambulance)](image)

Battlefield tourniquets in situations where meticulous wound packing and dressing can not be undertaken, “tactical tourniquets”, may be used under fire or in evacuation from a mine field. The tactical tourniquet should as soon as possible be replaced by a compressive bandage before reperfusion injury poses a problem (figure 5).

We were able only to register the early mortality and early complications. All patients with decreased urine output initially, regained normal output within few hours after admission. All had good oxygenation after restoration of the circulation. One patient with double amputation and tracheal puncture was septic when evacuated to the rear hospital on the third day.

In mine injuries the mortality is considerable [14]. With long evacuation times to hospital, only patients with lacerations and extremity injuries can be expected to reach hospital alive. Among the 109 patient evacuated to the field hospital, one had a chest wound, two tracheal puncture wound, and none abdominal injuries. Among patients hemorrhaging to death before transport could be arranged, at least two had intrathoracic and intraabdominal injuries. Postmortem examinations were not performed.

6.0 CONCLUSION

Tourniquet has no place in the treatment of hemorrhaging in traumatic amputations after mine injuries. A tight compressing bandage should be applied from distal end and in proximal direction.
Figure 5: It is probably easier to learn to apply a compressive bandage than make an effective tourniquet. In this case great innovation is shown, but little understanding.
(Photo: Tromsoe Mine Victim Resource Center, Norway)

7.0 REFERENCES


The Potential Role of Recombinant Activated Factor VIIa (rFVIIa) in Military Pre-Hospital Setting

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ABSTRACT

Hemorrhage is a major cause of death of combat casualties in the battlefield. Coagulopathy may develop soon after trauma and plays an important role in the development of uncontrolled bleeding. Thus, introduction of potent hemostatic agents that can overcome the complex coagulopathy of trauma may decrease mortality from exsanguinations. Recombinant factor VIIa (rFVIIa) has been shown to overcome a variety of coagulation and platelet disorders including trauma-related coagulopathy.

Controlled animal trials, small case series and anecdotal case reports have suggested that the use of rFVIIa may slow down and even control massive bleeding in trauma and hence prolong survival and reduce mortality. In most cases rFVIIa was used as an adjunct treatment to surgical hemostasis. However, in some, cessation of bleeding with reduction of early mortality was achieved by administration of rFVIIa alone. The accumulating efficacy data together with the high safety of rFVIIa suggest that "fielding" of rFVIIa to the combat setting should be considered with the aim of widening the "survival window" of exsanguinating casualties. As controlled trials in the combat setting are not feasible further assessments will have to be based on data from civilian trauma.

INTRODUCTION

Hemorrhage accounts for 40-50% of combat mortality [1]. Over 80% of those killed in action (KIA) died within the first hour of injury on the battlefield (immediate, first and second echelon mortality) before definitive medical care could be administered. It is postulated that shortening the time interval from injury to advanced medical treatment ("golden hour") could reduce mortality. Therefore, armed forces worldwide are endeavouring to provide early basic resuscitative and surgical interventions by deployment of surgical units as close as possible to the battlefield (Far Forward Surgery [FFS]) and by improvement of prehospital transport systems that provide better treatment during evacuation (Transport Treatment Systems [TTS]).

However, these modalities alone may not be sufficient to reduce hemorrhagic-induced mortality. This is evident by the experience from urban trauma, where 65% of exsanguinations occur after admission to the hospital [2] and most (82%) intraoperative trauma-related mortality is caused by uncontrolled hemorrhage [3]. Therefore, undoubtedly there is a clinical need for new potent hemostatic agents to supplement the limited armamentarium of therapeutic options for this frequently lethal complication [4]. This need in particular is as yet unmet in the combat setting, where evacuation as well as advanced hemostasis may be delayed for hours [5].

Accumulating anecdotal evidence and several studies indicate that rFVIIa may overcome the complex trauma-related coagulopathy [6-8] and serve as an adjunct therapy to surgical hemostasis. Such concomitant use may achieve control of the bleeding in a large proportion (50-75%) of exsanguinating patients [6,9,10]. Limited data from animal studies [11] and some clinical cases [9] suggested that treatment with rFVIIa alone can completely, or temporarily control coagulopathic bleeding. Therefore, it is possible that if administered early in the prehospital settings, rFVIIa may prolong the "golden hour" for exsanguinating military and civilian trauma casualties. However, the data to support prehospital administration of rFVIIa is vague at the present time. Nonetheless, early mortality of combat casualties from hemorrhage prior to administration of any advanced medical help, the lack of appropriate treatment modalities, and the promising potential of rFVIIa as a hemostatic agent, has led some military forces to equip themselves with rFVIIa.

In Israel the use of rFVIIa in soldiers is fully covered by the army, and in some special scenarios the drug is available at the level of the battlefield [12]. To the best of our knowledge there have been sporadic uses of rFVIIa in the recent wars of Afghanistan and Iraq.

Coagulopathy in Trauma

Coagulopathy (diagnosed by prolonged plasma clotting assays) develops early in 25-36% of trauma victims [13,14], correlates to the severity of trauma and is associated with increased mortality, over and above that of injury severity [13,15]. The real incidence of early coagulopathy is underestimated, since the clotting assays do not reflect the effects of hypothermia, acidosis and hyperfibrinolysis on hemostasis, as explained below. The mechanism of coagulopathy in trauma is complex and multifactorial:

1. **Consumption Coagulopathy** is induced by exposure of tissue factor (TF) at the site of injury that activates the coagulation cascade, as well as the fibrinolytic system, leading to the consumption and degradation of platelets and coagulation factors. The term disseminated intravascular coagulation “DIC” is frequently used to describe trauma-related coagulopathy. It is important, however, to realize that in most cases this does not reflect a true DIC, since there are no diffuse micro-thrombi such as those found in a true DIC [6,11].

2. **Hyperfibrinolysis** may be more common than realized. The reason for underestimation of its role in trauma stems from the lack of routine laboratory tests for fibrinolysis. Recently, rotation thromboelastograph (ROTEG) performed on multi-trauma victims suggested that early marked hyperfibrinolysis is common in massively bleeding patients [Vorweg M & Doehn M. Personal communication, 2004, unpublished data]. The reproduction of these findings in larger patient series would support the theory that early administration of antifibrinolytic agents may be of benefit in some of these patients.
3. **Hypothermia** is a common complication of combat injury leading to severe combined platelet and coagulation defects [16-18]. The effect of hypothermia on coagulation is also underestimated since the blood samples are rewarmed to 37°C before testing and platelet functions are not routinely monitored. In combat settings, coagulation tests are not performed at all. The capacity to prevent and treat hypothermia in the combat setting is limited, thus, there is a need for a hemostatic agent, such as rFVIIa, that can bypass the coagulopathic effect of hypothermia [8,19].

4. **Dilutional Coagulopathy** ensues from the dilution of coagulation factors and platelets by crystalloids, colloids, or blood products. The severity of dilutional coagulopathy is determined by both volume and type of fluids [20,21].

5. **Anemia-Induced Coagulopathy.** Red blood cells (RBCs) play an important mechanical and biochemical role in the coagulation process in addition to their role in oxygen delivery. Anemia causes prolongation of bleeding time which can be corrected by either RBC transfusions, or erythropoietin administration [22-24]. Reduction of hematocrit (Hct) inhibits platelet adhesion and aggregation e.g., at Hct of 20 aggregation and adhesion are decreased to a level similar to that of 20,000 platelets [25].

6. **Acidosis** compromises both coagulation enzymes and platelet functions [8,16], its contribution to coagulopathy is also underestimated, since the routine plasma clotting assays (PT and PTT) do not reflect the coagulopathic effect of acidosis. Measurements of thrombin generation on cell surfaces, which reflect the real in vivo coagulation process, revealed a marked inhibition of thrombin production with the decrease of pH [8]. Acidosis also decreases the response to rFVIIa [8]. Therefore, correction of acidosis is important.

These multifactorial mechanisms of coagulopathy form the rationale for introducing an effective hemostatic agent that overcomes coagulopathy and thus may play an important role in the reduction of hemorrhagic mortality and morbidity in both combat and civilian settings.

Recombinant activated factor VIIa has been approved by the U.S. Food and Drug Administration (FDA) for nearly a decade for the prevention and treatment of bleeding episodes in hemophilic patients with inhibitors (neutralizing antibodies) to coagulation factor VIII (in hemophilia A) or factor IX (in hemophilia B). Recently, it has been approved by the European Regulatory Authorities (EMEA) for use in Glanzman’s thrombasthenia and FVII deficiency. Despite its beneficial effect in hemophilia and a variety of congenital and acquired coagulation and platelet defects [26,27], its use in trauma has been avoided until recently, due to the theoretical concern of increased risk of thromboembolic complications.

The mechanism of action of rFVIIa suggests enhancement of hemostasis at the site of injury without activation of the systemic coagulation cascade. Naturally-occurring FVIIa circulating in small quantities has a very weak enzymatic activity until it binds to TF, that normally does not come in contact with the circulating blood. When TF is exposed at the site of injury the complex TF-VIIa locally initiates activation of the coagulation cascade (on the surface of TF-bearing cells) by activating FX and FIX. Activated FIX (IXa) forms a complex with its cofactor FVIIa on the phospholipid membrane of activated platelets (adhered at the site of injury), and activates FX much faster than the TF-VIIa complex). FXa forms a complex with its cofactor FV (also on the phospholipid membrane of activated platelets), which stimulates prothrombin to produce a small amount of thrombin. The small concentration of thrombin is insufficient to convert fibrinogen to a fibrin clot, but further accelerates the coagulation cascade by activating FV, VIII, FXI, and additional platelets. Following this acceleration, a large amount of thrombin is formed that subsequently changes fibrinogen to fibrin clots. Administration of a "therapeutic" high dose of rFVIIa results in a huge increase of VIIa level, compared to the physiological state, leading to faster and higher thrombin generation [28]. High concentration of rFVIIa can also directly activate FX on membranes of activated platelets (adhered at the site of injury), independently of TF, further enhancing thrombin generation [29].

To summarize, FVIIa initiates the coagulation cascade on the TF-bearing cells at the site of injury which thereafter continues on the surfaces of activated platelets adhered at this site. This process is the physiological “ TF dependent pathway”. In the presence of a therapeutic high dose of rFVIIa thrombin
generation is higher and faster. In addition, a therapeutic high dose rFVIIa also initiates a unique pathway, which does not exist during physiological activation of coagulation - the "TF independent pathway". This pathway initiates the coagulation cascade on the activated platelet membranes directly, without the need of TF, adding to the formation of high and fast thrombin generation.

**Improved clot quality by rFVIIa.** In vitro analysis of the fibrin clots formed in the presence of a high thrombin concentration has shown that such clots have a different type of architecture that is stronger and far more resistant to degradation by fibrinolytic enzymes compared to normal clots [30-31]. This is explained by the activation of thrombin-activatable-fibrinolytic inhibitor (TAFI) by the high thrombin burst [32].

An animal model of uncontrolled arterial hemorrhage demonstrated that resuscitation-induced rebleeding occurred at a much higher mean arterial pressure (MAP) in the rFVIIa-treated group than in the placebo group [33]. This provides in vivo evidence for the stronger architecture and adherence force of the clot to the site of injury following administration of a therapeutic dose of rFVIIa.

A growing number of preliminary studies, case series and reports, describing the efficacious and safe use of rFVIIa in a large array of uncontrolled bleeding episodes in surgical and medical patients have recently been published [34-38].

**The role of rFVIIa in hemorrhage control of trauma patients**
Recombinant activated factor VIIa has been shown to significantly improve abnormal clotting assays and control, or slow down within minutes massive bleeding in trauma patients resistant to conventional surgical and medical hemostasis [6,9,10]. This was supported by a pig model of massive trauma [19]. Data from the Israeli Trauma Registry of 36 critically ill, massively-bleeding trauma patients with hypothermia, acidosis and profound coagulopathy, showed that administration of rFVIIa resulted in cessation of bleeding in 75% of patients, with a 61% survival rate (Martinowitz U.,unpublished data).

These results are encouraging compared to published reports on survival of critically ill multi-transfused trauma patients [15, 39-42]. In most of these patients rFVIIa was administered as an adjunct treatment to surgical hemostasis. In a few of these cases the bleeding ceased after administration of rFVIIa alone, which may suggest its potential benefit in the prehospital settings.

An important observation from our pig study and registry was that rFVIIa overcomes the hypothermic and complex coagulopathy in trauma. This finding is further supported by in vitro data demonstrating that hypothermia inhibits thrombin generation via the TF-dependent pathway, but enhances thrombin generation via the TF-independent pathway [8,]. Another important observation was the impact of pH on the response to rFVIIa. We observed that the response of patients with acidosis was significantly worse compared to patients with higher pH. Correction of pH with HCO3 resulted in immediate improvement of the hemostatic response to rFVIIa. The effect of acidosis on the response to rFVIIa was further supported by an in vitro study demonstrating marked inhibition of thrombin generation with the decrease of pH in both the TF-dependent and TF-independent pathways[8].

**The role of rFVIIa in the management of Traumatic Brain Injury (TBI)**

Traumatic brain injury is a major cause of morbidity and mortality in combat casualties. Hemorrhagic lesions frequently increase in size after the initial impact [43] and it had been shown that the progression is a major cause of morbidity and mortality among these patients [44-46]. Theoretically, the hemostatic effect of rFVIIa observed in trauma patients should occur also in the brain, resulting in decreased morbidity and mortality. However, it may increase thromboembolic complications to an extent that will abolish the effect of the improved hemostasis.

Preliminary results from a large multicenter phase II trial in spontaneous intracranial hemorrhage (ICH) revealed reduced hemorrhage growth and significant improvement in neurological outcome (47). There was a minor nonsignificant increase in thromboembolic complications. This is encouraging, since patients with TBI are younger, healthier and have less risk factors for thromboembolic complications compared to ICH patients.
In the last 2 years six patients with pure severe TBI (five with penetrating and one with blunt trauma) have been treated with rFVIIa in Israel. In all six patients rFVIIa changed the expected devastating course of the brain insults and abruptly stopped progression of brain contusion and bleeding. Five of these six patients recovered, but one expired from severe brain injury and vasospasm [48]. If the drug is found safe and efficacious in TBI, it will undoubtedly be of added value to those patients who frequently have devastating complications, especially in the combat setting where treatment modalities are poor.

**rFVIIa in Blast-Induced Lung Injury (BILI)**

BILI is a common finding among victims of explosion, ranging between 38-47% of those surviving the initial injury [49]. The mechanism of blast injury is complex: Primary injury due to the sudden increase of air pressure caused by the explosion, affecting gas-containing organs, namely lungs, ears and gut. Secondary injuries are the result of flying objects (shrapnel, among others) causing penetrating injuries. Tertiary blunt injuries are the effect of acceleration–deceleration shear forces. Other mechanisms of blast-related injuries involve smoke inhalation, burns and biochemical reactions, namely free radical-mediated oxidative stress that may contribute to the lung injury [50]. Damage to the lungs may range from minimal hemorrhage to hemothorax and massive pulmonary hemorrhage. Acute respiratory distress may develop, often requiring mechanical ventilation which carries the risk of tension pneumothorax, or air emboli [50].

Theoretically, administration of rFVIIa may rapidly control the pulmonary hemorrhage resulting in reduction of acute respiratory distress and the need for mechanical ventilation with all its consequences. In the past year, three patients suffering from severe BILI with massive uncontrolled life-threatening pulmonary hemorrhage were treated in Israel. In all cases the hemorrhage ceased abruptly following administration of rFVIIa. Two of the patients enjoyed a full recovery, one died of septic shock 5 days later.

**Combat Prehospital use of rFVIIa**

No clinical data is available on the use of rFVIIa in combat or prehospital settings and it is unlikely that such studies will be performed in the combat situation. However, the results of two recent animal studies support a potential benefit for early prehospital treatment with rFVIIa. A pig model of severe liver injury demonstrated that early administration of rFVIIa alone resulted in a significant reduction of first hour mortality and marked prolongation of survival from a few minutes to 2 hours [11]. Another pig model of aortic laceration showed that rebleeding after resuscitation occurred at significantly higher mean arterial pressure (MAP) and was less severe in the rFVIIa-treated group vs. controls [33].

The rationale for combat prehospital use of rFVIIa is therefore based on:

- The high early mortality from exsanguinations on the battlefield.
- The role of coagulopathy in development of massive bleeding
- The limited diagnostic and therapeutic options in this setting,
- The capacity of rFVIIa to bypass the complex trauma-related coagulopathy, and control or slow down massive bleeding as demonstrated by limited clinical experience and animal models in both in hospital and prehospital settings, 
- The possible beneficial effect of rFVIIa in TBI and blast injury
- The encouraging safety profile as evident by the accumulating clinical experience (studies and series) which may be explained by its compartmentalized mechanism of action at the site of injury.

All these raise the possibility that early use of rFVIIa in the prehospital settings may improve the prognosis of combat casualties. The indications, patients selection, dosing and timing will have to be defined by extrapolation from the in hospital experience.
REFERENCES


Hemostatic Damage Control Surgery Combined with Strategic Evacuation in an Intensive Care Airborne Unit Saved Life in a Critical Gun Shot Injury

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DAMAGE CONTROL AND STRATEGIC EVACUATION

A Norwegian officer was shot and critically wounded in Afghanistan in 2003. His life was primarily saved by damage control surgery in a Dutch military hospital in Kabul. He was evacuated with an intensive care air transport from Kabul, via Termez to Oslo, Norway, where final surgery was performed. He has recovered. The case demonstrates how advanced damage control surgery in the area of operation combined with an organization equipped and staffed for strategic evacuation of intensive care patients can offer treatment to combat casualties on the level of western urban trauma care.

1.0 INTRODUCTION

The first priority in trauma care is to maintain oxygen delivery by stopping hemorrhage, if necessary by early emergency surgery and damage control. Damage control is a three step procedure: 1) Transport without delay to a surgical facility where the hemorrhage is stopped by operative packing without final repair before the patient becomes cold and acidic and develops bleeding disorders; 2) Intensive care with normalization of the hemostasis; 3) Definitive, reparative surgery [1]. This procedure for handling critical bleeding has been seen to improve survival [2, 3]. The present case story from the International Security and Assistance Force (ISAF) in Afghanistan demonstrates the concept.

2.0 PATIENT

On the 13th May 2003 a Norwegian major, a 44 year old male, on CIMIC duty 20 km north of Kabul in Afghanistan was hit by a shot fired from a Kalashnikov AK47 at a distance of 60 meters.

The entry wound was in the back of the chest, just to the right of the spine. The lower lobe of the right lung was lacerated. The exit wound low anterolateral in the right chest was half the size of a palm. Two ribs were crushed. The missile had touched the top of the diaphragm and left a 7 cm long laceration. There were multiple lacerations in the liver going from dome all through the parenchyma to the hilum (Organ Injury Scale grade IV [4] (figure 1).

![Figure 1: The crushed liver with hemostatic packing as it was demonstrated by CT on arrival in the trauma center three days post trauma. Multiple lacerations from the dome of the liver down to the hilum can be seen.](image)

3.0 CLINICAL COURSE

The patient was evacuated by a German helicopter manned by a flight surgeon, medic, ICU nurse and anesthesiologist to a role 2+ facility run by the Dutch at the international airport in Kabul. The victim was hit 1145 and moved out of the area of danger. Medevac was requested 1155. The helicopter was alerted 1210, was on scene 1230, left the scene 1250. On site the patient was responding. He was pale, in pain and with an increased pulse rate. The patient arrived in ER 1310. BP was then 150/80, HR 85, SaO2 95% with 10 liters of Oxygen. A chest drain produced 300 ml. He was brought to operation 1340. Laparotomy was performed by surgeons LCCDR Idenburg, MAJ Massagè, MAJ Bille and with COL van Aggelen in charge of anesthesia. Control of profuse bleeding from the liver was achieved by compression and packing. The exit wound was left open. Next day the rupture of the diaphragm was sutured to facilitate more effective repacking. Sharp edges of the ribs were resected.

He was kept on a ventilator. Hemoglobin dropped to 5,5 mmol/l and he was transfused with two units of erythrocytes.
The third day post trauma the patient was evacuated from Kabul. He was first taken to Termez in Uzbekistan by a C160 Transall transport plane with intensive care capacity. In Termez the patient was transferred to a German Airbus A310 Multi Role Transporter and flown to Oslo, Norway (figure 2).

Figure 2: The distance from Kabul to Oslo is more than 6000 km, in part over mountainous and deserted areas.

The Airbus 310 has the capacity of lifting 44 patients in a whole, 6 of them under intensive care ventilator therapy and the rest under intensive care monitoring and treatment. The plane is staffed with 25 nurses and doctors (figure 3 and 4). From Oslo Airport to Ullevål University Hospital the patient was transported in an intensive care ambulance staffed and equipped for all types of intensive care including extracorporeal circulatory support.

Figure 3: The German Air Force Airbus A310 Multi Role Transporter has a range of 9540 km and can evacuate patients to virtually any destination in the world. It is available at any time.
Figure 4: The Air Force Airbus A310 Multi Role Transporter equipped for intensive care treatment can carry 44 patients, 6 on ventilator treatment.

On the night of arrival in Oslo angiography revealed no ongoing hemorrhage. At relaparotomy the packing was removed, the wounds revised. Repeated wound revisions were necessary. The entry end exit wounds were left to granulate. He suffered infectious complications and pulmonary insufficiency. There was biliary leakage. He spent three weeks under intensive care and was discharged after two months. He is now rehabilitated (figure 5).

4.0 DISCUSSION

Damage control surgery in a forward area is an option when hemorrhage is too severe to permit evacuation to a larger surgical installation further back where intensive care facilities exist. The surgical problems of damage control surgery may be overcome in a forward field hospital. However, by introducing damage control surgery in forward areas intensive care patients are created. The bleeding may be arrested, but the problem of getting an intensive care patient out remains [5].

To succeed in damage control lifesaving surgery, all links in the chain of the treatment must be understood, from the first responder through to the surgeons doing the final repair. Time must not be wasted before surgery. Low-pressure fluid resuscitation is recommended. When the hemorrhage has been controlled, time is available to stabilize the patient and organize the evacuation with expert support. A trained organization must be in place.

Ideally we would prefer to do final repair as soon as the patient is hemodynamically stable, usually after 24 to 36 hours. In this case it took some time to arrange the evacuation. The patient was Norwegian. The organization that undertook the evacuation is German. There are no automatic routines for one nation to undertake strategic patient evacuation for another. All administrative problems were however smoothly solved. For a small nation it is unrealistic to maintain a complete organization for strategic evacuation. In areas were there are no security risks the Norwegian military rely on the civilian “Norwegian Air Ambulance” for intensive care transports. This firm cannot be used in areas with ongoing hostilities. It seems rational to leave strategic evacuation to one lead nation. But in future international operations a binding, detailed agreement on this should be in place and the military command must have detailed knowledge on how to activate the service.

In our courses in war surgery training on an animal model is practiced with the primary goal to disseminate knowledge about damage control surgery. We are encouraged by this case story and see it as an argument in support of the concept. It is important to consider the surgical and intensive care and the
transportation as complementary parts. A system for strategic evacuation on permanent standby is expensive. The decision to implement the training and maintain the organization will be a political one.

5.0 CONCLUSION

With training and an understanding of the importance of early hemostatic damage control surgery and backed by an organization equipped and staffed for strategic evacuation of intensive care patients, critical combat casualties may be given treatment on the level of western urban trauma care.

Figure 5: In a newspaper interview six months post trauma the patient expressed the wish to volunteer for a new mission, although discouraged by his spouse and daughter.

6.0 REFERENCES


Hemostatic Damage Control Surgery Combined with Strategic Evacuation in an Intensive Care Airborne Unit Saved Life in a Critical Gun Shot Injury
Lessons Learned from Bravo Surgical Company
(Part of I MEF) in Operation Iraqi Freedom

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NOTE: The opinions expressed herein are those of the authors and not necessarily the view of their commands, Naval Medicine, the Department of Defense, or the United States. Some of the recommendations in this report are already being put into place.

ABSTRACT

Problem: The combat medical delivery system for the US Marines was redundant and seriously hampered by marginal leadership.

Background: There are multiple demands on a combat medical delivery system, which often are in direct opposition of one another. Compromise of these demands without careful forethought will significantly weaken a system and even lead to its ineffectiveness. One must remember that an advanced medical delivery system is a large logistical liability to the combat support commander. The closer to the front lines, the more difficult it is to support. Concrete evidence to show real benefit is required to justify supporting this liability. To state the simple yet often overlooked obvious, if the medical system cannot deliver average level of care for a severe mass casualty, then it should be dismantled.

One of the difficulties in building a modern system is the extraordinary success seen since the Vietnam conflict. In modern conflicts since, we have been confronted with an inept foe without airpower matched against our highly trained, high tech (offensive and defensive gear) war fighter. Our casualties have been extremely low and therefore never taxing our medical system. Medical personnel dedicated to make the system work covered the deficiencies. In essence, one could erroneously conclude that medical delivery requires no overhaul. One could accept this do-nothing attitude if the assumption can be made that we will...
always have air superiority and overwhelming force with minimal US casualties in any future conflict. I propose the opposite and that we prepare for a future formidable foe in which we will confront high casualty rates and difficulty with air superiority limiting patient evacuation.

The purpose of this paper is to cite these discrepancies and offer practical solutions. Overcoming the inertia of “if it ain’t broke, don’t fix it” mentality is crucial to transforming a medical system that had not been updated in one or two decades. An analogy is that the coalition forces could have still had a military victory utilizing Sherman tanks and M-1 rifles. This would have generated a complacency that concluded no changes were needed. This conclusion would be disastrous. Our medical shortcomings must continue to be recognized and fixed now.

I will break the problems down to headings that will be listed as annexes. They will consist of three paragraphs: Issue/Discussion/Solution.

The common denominator for each of these medical units is a central Shock Stabilization Unit (SST) and prompt transport to definitive care. The vital ink is medical evacuation availability. Its degree of nonavailability defines what type of holding capability is needed as well as basic surgical capabilities for life threatening conditions that will not tolerate any delay and cannot be stabilized in the SST.

1.0 PART I – ADMINISTRATIVE ISSUES

The following lessons learned are put in a three-paragraph format. The first paragraph is the issue, the second is a brief discussion of the issue and the third paragraph outlines a solution. They are not listed in any particular order.

1. The wrong professional mix reported to the AOA.

THCSRR’s\(^1\) approach to “find a home” for every TRICARE friendly specialty by giving them a wartime mission was not a good idea. It delivered podiatrists instead of orthopaedic surgeons, OB/GYN instead of trauma surgeons, pediatricians instead of surgical intensivists and labor nurses instead of ICU nurses. Fortunately, there were few Marine casualties. The surgical company could only utilize one-third to one-half of its Main OR beds due to lack of surgeons. One can only look back in horror if a dozen critically injured Marines had been evacuated to the surgical company.

Modify THCSRR. From the ground up, look at how many critical combat skilled professionals are needed – Ortho, general, Neurosurgeons, Anesthesiologists, Intensivists, ER physicians and nurses (ER, ICU, ward) Send the birth product line out into the network; it costs more than we take in, gobbles resources and is high liability.

\(^1\) THCSRR is the Total Health Care Support Resource Requirements Allocation Plan. It identifies those personnel required to meet the day-to-day operational support to the Navy and Marine Corps mission, the wartime mission and those personnel required for sustainment.
2. Not enough emergent resuscitative, perioperative and intensive care physicians assigned to the unit.

There were not enough physicians to respond to airway and vascular access concerns in the ER and still be able to supervise the perioperative care of the combat casualty. There were no intensivists assigned to the ICU.

Change the ratio of CRNA and Anesthesiologist. I recommend five to six anesthesiologists and three CRNAs to allow full coverage as intensivist for the ICU, supervision for the three OR’s as well as assist in the SST for resuscitative emergencies.

3. Unsatisfactory staffing

There is a poor mix of nurses and physicians (see above). For higher patient flows, Bravo surgical company would not have met its mission.

Recommend the following manning:
SST 4-6 ER physicians with 1 FP and 1 PA
Anesthesia 5 anesthesiologist one to assist SST/OR, 2 for intensivist/OR and one for each OR, 3 CRNAs
Surgery 4 general surgeons and 3 orthopaedic surgeons
(if medical company becomes modularized then one each of the specialties ent, uro, neuro) if humanitarian peds, ob/gyn, for women one ob/gyn would be good
ICU- 3 intensivist and 6 icu nurses
Ward mixture of Internal med, FP and peds

4. Critically skilled physicians were allowed to remain in positions that could have been filled by GMOs.

There were several specialty-trained physicians such as neurologist, cardiologist, pulmonologist, ICU, and even anesthesiologist that were filling STP or GMO positions. They were critically needed in the surgical company. There were multiple trauma trained general surgeons; orthopaedic surgeons and anesthesiologists in Echo and Foxtrot companies that did not have a mission but could have been used.

A coordinated medical leadership that is supposed to know what physician assets are in theater. Identify them and switch them to best configure the entire medical function in the AOA.
5. Establish a robust officer and enlisted FMF qualification

The medical company lacks a cadre of long-term medical professionals that stay with the company. Current policy is to treat them like a temporary group that comes and goes. A lack of community exists secondary to a lack of interest and concern to recruit good people. Poor techniques, outdated equipment and confused medical leadership results.

Institute training, encourage TAD course attendance, allow one to stay with surgical company throughout their career to maintain a professional core similar to flight surgery and undersea medicine. Grade unit and MTF leaders by their ability to recruit and maintain such a group. Establish FMF qualifications to distinguish those that stay with the program.

6. Poor interaction between MTF and Medical Battalion.

A blue side / green side division exists that is unacceptable. The MTF is skewed to a managed care philosophy that is driving away great physicians and nurses and displeasing our line commanders. The green side is more concerned about non-medical operational issues than even line commanders themselves at the expense of quality medical care. A blending of the two sides would fix the problem.

Roll the Med battalion under the MTF with its CO in charge of Med battalion. The MTF rolls under the MEF surgeon. The MEF surgeon must have served as a previous MTF CO or XO.

7. Modularize the company in functional areas

The complaint for medical company is that it is too big and too slow and hard to move. It is thought to have become irrelevant in today’s fast moving Marine combat operations. Yet the little FRSS with its meager ability to handle large number of casualties is clearly inadequate in major battles where casualties are expected.

The component parts of the each platoon need to be broken down into its smallest independently functioning pieces and loaded into containers appropriately. When a mission arises, then the appropriate number of ORs and ward can be sent with the SST to the assigned location. Remaining assets, including a hotel services platoon that holds the laundry, berthing tents, showers etc., can follow this movement. With this doctrine, medical company can be ready to receive patients in hours instead of days with only a moderate lift requirement.
8. Medical battalion was unable to accept all the missions requested by the commanding general

The surgical company was directed to perform emergent combat related trauma surgical intervention. Its supply and somewhat its personnel attempted to reflect this very narrow medical assignment. Consequently, it could not perform sick call, emergent medical treatments, i.e. myocardial infarction and humanitarian assistance, despite having personnel that were more than qualified.

Form platoons to various missions. A purely combat surgical platoon should have general and orthopaedic surgeons with a robust intensive care staff with multiple intensivists. A platoon of primary care physicians that can handle ward as well as humanitarian missions. An additional list of augmentees such as OB GYN and pediatrics personnel should humanitarian mission be a part of the operation.

9. Communications were unacceptable

At no point upon landing into the area of operations was I ever afforded a means to communicate other than a mail. I was unable to obtain information on equipment, supplies and most importantly medical information. In this era of Internet and cellular communication in addition to convention military communications systems, our capabilities were unacceptable.

Identify a minimum level of communications and then double that need. Verify and re-verify that communication gear and personnel are onboard to operate and maintain them.

10. Poor medical Intel

On a number of occasions, poor medical information/Intel resulted in surgeons guessing what is available out there since information of the availability of services at other facilities and the amount of time/wait anticipated to get them their was unknown

Assign an aggressive medical Intel team that constantly updates its knowledge of all available theater assets and the time required to get there.

11. Medevac was troubled on occasion

Difficulty in communicating with medevac operations resulted in time delay or unnecessary holding of the asset on the flight pad.
Consider an embedded USAF unit to handle the coordination of the medevacs. This will require USAF personnel to learn more about USMC operations and the sometimes-tortuous command chain to obtain medevac.

12. Medevac could improve

Need to identify lifts of opportunity would expedite care. Physicians identified to accompany patients.

Utilize flight surgeons for medevac transport. They have the skill and the situational awareness for the job. In addition, when they are not needed they are working with their squadrons. This cadre can also be enlisted and educated about surgical company so they return to it when they become specialty trained later thus forming our next generation of surgical company leadership.

13. Eliminate rank inversion

The assignment of junior officer in charge of senior more experienced officers is a flawed policy. Junior/senior relationships throughout the command were adversely affected. Working with officers outside the command was a confused process, as commanders outside the command sought the unempowered senior officers for assistance.

Identify, train and place senior medical officers as CO. Train junior medical officers in their early tours by placing them in charge of divisions with organic senior enlisted.

14. Lacked a pediatric capability

Children will always arrive in a conflict, face up to the fact and be prepared

Bring a pediatric amal for preop, operative and post op care

15. Infectious disease risk

Staff suffered a gastroenteritis outbreak. Flies landed on surgical field

Assigned PMT to company. Have bug lites and traps in OR
16. Initial consumable and equipment allotments were inadequate

The AMAL lists for consumables were inconsistent. For a double operating room that was to handle 25 serious casualties there were shortages of both medications and consumables. In addition, the Propac did not have SaO2 and CO2 monitoring capability, two devices that are affordable, durable and increases the quality of care. These instruments had to be taken from the enroute care boxes (someone had the foresight to give enroute care personnel these essentials but neglected the OR and ICU

Go through a major mock OR case. Lay out the equipment and consumables used. Multiply those items by 25 to obtain what should be in the AMAL. Use the same consumable numbers to make resupply cans. Purchase one propaq with SAO2 and CO2 monitoring capability for each or bed, each ICU and each SST.

17. Equipment familiarization and checkout was poor

There was an ongoing problem with familiarization and maintaining the operability of the company’s equipment.

Transfer equipment to CO MTF (deployed company commander) for daily use in the MTF. This equipment is cycled in and out of the MTF. It is to be maintained in a high state of readiness and repaired/replaced immediately when broken. It is marked medical company and shipped out when needed.

18. Problems with sterility procedures

There are problems with both sterilizing and maintaining things as sterile. Sterilizers failed under combat conditions.

Recommend obtaining Genesis pans, cidex, boiler tech/biomed tech check out sterilizer prior to deployment to include a full boiler hydro check, cssr tent with shelves and two tables, double ply instrument wrap, dust cover for sets

19. Consumables were lacking

There were consumables that were needed in greater supply

Wider and greater supply of suture material
Irrigation fluid with a warmer for fluids
Tape
Non-sterile gloves
Arm boards
Foley catheter
Pediatric ET tubes and IV catheters

20. Advanced monitoring equipment

A shortage of monitoring equipment (pulse oximetry, end tidal CO2 and temperature) existed on the ward, ICU and to the operating room to a lesser extent.

End tidal CO2 must be available for each MOR beds (six) with one spare. The ICU should have two of these monitors to help manage ventilator patients. Specifically a dozen more dependable pulse oximeters with additional probes for attachment. Propac has YSI temp probes that work well for continuous monitoring of body temp. Seven for the OR, 3-4 for the ICU. Tympanic temp probes for the wards.

21. Equipment difficulties

The following problems were encountered by periop nursing:
- Different litter did not fit well, need straps
- Need arm boards to perform orth cases
- Brushes to clean crevices of the or table well
- Poor ortho support- not enough external fixation devices
- Not enough xeroform or bulb syringes
- Preprinted pt log book for the or
- More trauma scissors so everyone has one set to cut away dressings, clothing etc without injuring pt
- Fluid warmer for iv fluids and irrigation fluid
- Step ups for the various heights of the surgeons
- Holders for garbage bag liners
- Attachment to hold IV bags to the table
- Cots that allow head up and head down position
- Large number of plastic buckets and detergent to encourage personnel to wash clothing
- Ample supply of deet and premethrin
22. Medical leadership in theater was overlapping, often weak, misinformed and without a chain of command

There were multiple medical leaders in the AOA that quickly asserted "they were in charge" but failed to deliver on action routinely. A fragmented chain of command prevented medical leadership from forming a comprehensive plan. It was very difficult to find a leader who had the large picture and medical contingencies for possible emergencies. The humanitarian assistance football was disgraceful. The problem was evident a year ago yet it was being addressed as line commanders brought child casualties to medical units that were intentionally not given the AMAL to address humanitarian issues.

Identify the medical chain of command starting from the MEF. If a unit commander disagrees with a medical plan or policy, have him take it up with the CG.

1.1 PART II- CLINICAL MATERIAL

Rectal Injuries

Classic teaching of of treatment of rectal injuries includes four Ds

- Diverting colostomy
- Drainage
- Debridement
- Distal wash out

The last part of the treatment protocol is questioned in the civilian trauma cases and only first three are instituted in many centers. It was pointed out that only high velocity military injuries warrant distal rectal wash out. We had two cases of rectal injuries. Rectal exam was grossly positive for blood. Urine was clear. Patient underwent exploratory laparotomy, diverting colostomy and drainage. Skin was left open.

Patients were cared post operatively at rear echelon facility. No distal rectal wash out was performed. JP drains were removed between 5-7 days. Patients were subsequently transferred to Iraqi civilian hospital 14 days later in stable condition.
Based on the limited data, the following conclusions are drawn.

- No diagnostic tools available to detect rectal injuries. High index of suspicion is required.
  - We did not have tables to place patients in lithotomy position. It makes proctoscopic exam very difficult and placement of presacral drains almost impossible. Our patients had drains placed anteriorly.
  - Distal rectal wash may not be necessary for healing of rectal wounds even in military setting. More number of cases are required to validate the point.

Vascular injuries:

We attempted vascular repair with placement of prosthetic shunts. One was in upper extremity and it clotted off almost immediately. Patient required amputation. 2nd repair was in lower extremity. Shunt remained open in the immediate post op period. However, it clotted off during transport and patient had amputation. Attempting repair with native vein is very time consuming and not practical at the front lines. Better alternatives need to be explored for vascular injuries.
Clinical Record of Emergency Vascular Access Using Adult Intraosseous (IO) Devices

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SUMMARY

Introduction/relevance to the Symposium

Accessing the medullary space of the bone, offers a rapid, effective, and reasonably foolproof alternative to IV access. Newly introduced adult intraosseous (IO) technologies have been suggested as one means to improve success rate of emergency vascular access. The National Academy of Sciences’ Institute of Medicine Report recommends IO infusion as the preferred initial vascular route for combat casualty care.

Rationale

This review compares IO access devices, with a focus on the clinical record of the five FDA approved adult IO devices in use today and of one in advanced development.

Description of methods employed and results obtained

Published adult IO case reports, studies and clinical trials were identified and suggest utility and effectiveness as well as documenting complication rates. A user survey of American and British military medics and corpsmen and US paramedics is underway.

Adult IO Devices: The Jamshidi IO access needle is similar to the sturdy trocarred needles first used in WWII, and commercially available since the late 1960’s. Two sternal access devices were developed by the Dutch military and the US Army. The adult Sur-Fast (Cook Medical) IO needle is a one-piece 12-gauge screw/cutting tipped needle with a side port for infusion and a handle allowing insertion. More recently a device to safely insert a 16-gauge IO port into the sternum (FAST-1, Pyng Medical) and a device to inject a trocarred needle into the tibia (BIG, Wais Med, Ltd) achieved FDA approval. New IO devices being developed
include the EZ-IO (Vidacare), which is the first battery powered IO device that has received FDA approval in 2004.

**Case Studies and Controlled Trials:** Several positive case reports and trials of adult IO have recently been reported, but most have had support and participation from IO manufactures. The few independent reports suggest effectiveness, and interestingly, seem to establish equivalence of different devices. Notably the Army conducted a special operations medic user study comparison of IO access using FAST-1, BIG, Sur-Fast and Jamshidi in human cadavers. No device was a clear favorite, all had similar success rates (94-97%) and access times (70-114 seconds) suggesting that training and experience may be a larger factor in success than the device. The first 50 uses of FAST-1 by practicing paramedics and emergency physicians reported an 84% success rate, mean access time of 77 seconds and maximum flow rates of 80 –150 ml/min via gravity or pressurized bag; most failures were attributed to obesity. US regulatory approval of IO devices is based on the 510k process, which means that the FDA has ruled that the different IO devices are substantially equivalent. Complications of IO access have been extensively reviewed and are similar to those of IV access with a few exceptions to be presented. An ongoing survey of adult IO use will report on the operational experiences of both civilian and military medics with currently available adult IO devices.

**Conclusions**

IO vascular access devices appear to fill a special need for combat casualty care, but independently funded cohort analyses and randomized controlled trials are needed to fully evaluate efficacy and safety.

**PURPOSE**

The purpose of this communication is to review the availability, acceptance and effectiveness of intraosseous (IO) infusion devices to provide rapid vascular access for the administration of resuscitation fluids and drugs in time-critical emergency scenarios, in both institutional (hospital) and pre-hospital settings and to suggest future directions for such technology. Emphasis in this communication will be on IO use in adults.

**INTRODUCTION**

**History of IO vascular access and IO needles:** The scientific background on the use of the intraosseous vascular access route dates to at least the 1920’s when Cecil Drinker reported that fluids, dyes and blood administered into bone marrow rapidly entered the circulation (Drinker et al., 1922). Prior to World War II, in a series of elegant experiments in England, Tocantins and O’Neill (1940, 1941) demonstrated, first in animals and then in human patients, the effective use of intraosseous vascular access and infusion for treatment. Henning (1940) used the sternal IO route to transfuse a patient with granulocytopenia. During WW II, IO access was used to deliver fluid and blood in a number of case reports and reviews, most often in a hospital environment (Bailey, 1944; Quilligan and Turkel, 1946). The culmination of this work was one of the earliest reports of resuscitation by a ‘first responder’ and the use of IO for prehospital combat casualty care. A B-29 bomber crew revived a seriously injured and bleeding crewmember with collapsed veins which prevented starting an IV. During the long mission over Japan, the crew established IO vascular access and successfully infused sufficient plasma into the sternal circulation to allow subsequent venous cannulation and recovery from shock and eventual long term survival. (Detroit News, March 13 1944). While it was not stated in the article, a variation of a Turkel needle was probably used, as Dr. Turkel had published several reports of his new trocared IO needle with adjustable depth guard for sternal access. (Turkel, 1944) Such early reports in hospitals and this dramatic story of prehospital delivery of life saving resuscitation emphatically underscored the potential for the IO route to provide prompt vascular access in time-critical situations under less than ideal conditions.
After WW II, in the 1950’s through the 1970’s, the use the IO route greatly diminished and there were few reports in the literature. Peace seemed to remove the stimulus for its use. In 1950’s, there were no paramedics, no prehospital care, no emergency physicians and few emergency protocols that required IV drug therapy. IV vascular access was mainly used for infusion of blood and plasma for treatment of hemorrhage or anemia. The wide acceptance of crystalloid therapy to treat hemorrhage was just beginning to be developed. Additionally, plastic catheters were just beginning to be used and with their widespread acceptance, IV therapy became the standard of care for in hospital use for a growing number of indications. IVs were mostly started by doctors and almost exclusively used in hospitals.

Table 1 lists IO needles use for adult vascular access and briefly described in the text.

<table>
<thead>
<tr>
<th>Device, Reference</th>
<th>Source</th>
<th>Design &amp; comments</th>
<th>Insertion Site</th>
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<tbody>
<tr>
<td>Trocored needle Tocantins, 1940</td>
<td>George Pilling &amp; Sons;</td>
<td>4 part trocared needles with wing handle, ball guard to adjust length</td>
<td>sternum</td>
</tr>
<tr>
<td>Trocored needles Paper 1942</td>
<td>Becton Dickerson</td>
<td>2 part trocared needle. Used by military</td>
<td>sternum</td>
</tr>
<tr>
<td>Trocored needle, Bailey, 1944</td>
<td>Whilen Brothers, England</td>
<td>Trocared needle with wing handle apparently used in London Blitz</td>
<td>sternum</td>
</tr>
<tr>
<td>Turkel Needles Quilligan, 1946</td>
<td>Unknown</td>
<td>Stylet-trephened needle for tibia, femur or sternum. Steel reusable.</td>
<td>Tibia, &amp; Sternum</td>
</tr>
<tr>
<td>IO Device Dankmeijer, 1975</td>
<td>Dutch Navy</td>
<td>Screw-threaded needle. Used for cold water rescue by Dutch Navy</td>
<td>Sternum, Sternum</td>
</tr>
<tr>
<td>Jamshidi needle*, Glaeser, 1993</td>
<td>Baxter Health Care</td>
<td>Trocared needle adjustable length, disposable</td>
<td>Tibia, Ilium Sternum</td>
</tr>
<tr>
<td>SAVE, Halvorsen, 1990</td>
<td>US Army &amp; University of California</td>
<td>Screw threaded needle, not commercialized</td>
<td>Sternum</td>
</tr>
<tr>
<td>SurFast*, LaSpada, 1995</td>
<td>Cook Critical Care</td>
<td>Screw-threaded needle with chisel point &amp; removable handle</td>
<td>Tibia</td>
</tr>
<tr>
<td>FAST-1*, Macnab, 2002</td>
<td>Pyng Medical, Vancouver, British Columbia</td>
<td>Rapid manual insertion, corrects for skin thickness</td>
<td>Sternum</td>
</tr>
<tr>
<td>B.I.G.* Waisman, 1997</td>
<td>Waismed Ltd, Israel</td>
<td>Spring loaded injectable needle, rapid insertion</td>
<td>Tibia</td>
</tr>
<tr>
<td>EZ IO, FDA approval 2004</td>
<td>VidaCare San Antonio, Texas</td>
<td>Battery-powered drill; semi-Permanent, controlled insertion</td>
<td>Tibia</td>
</tr>
<tr>
<td>FirstMed in development</td>
<td>Resuscitation Solutions, Inc. Galveston</td>
<td>Spring-loaded auto-injector; single 2–ml dose, disposable</td>
<td>Sternum or Tibia</td>
</tr>
</tbody>
</table>
It was in the 1970’s that the new specialty of emergency medicine was emerging and physicians like William Spivey rediscovered the IO route (Spivey, 1985) and applied it to a specific ER problem – delays and failure rates for emergency fluid and drug delivery for pediatric emergencies. (McNamara et al., 1986) The late 1970’s through the early 1990’s saw a flurry of animal research articles showing pharmacokinetic equivalence between IO and IV with several drugs (Shoor et al., 1979; Cameron et al, 1989; Orlowski et al, 1990; Dubick et al, 1992; Neufeld et al, 1993; Kentner et al., 1999) and several reviews emerged on the use of IO. During this time period, the IO technique was introduced to paramedics who used the Pediatric IO needles made by Cook Critical Care as well as the Jamshidi needles, which were disposable and similar in design to the reusable trocared needles of Paper and Bailey needles (Papper, 1942; Bailey 1944; Glaeser, 1993). Reviews frequently mentioned that IO access could be used in adults and adult IO use was sometimes reported in a few cases (Iserson, 1989), but the focus remained on pediatric emergencies. The Dutch Navy was the first armed service since World War II to adopt and use an IO needle and they used a unique self starting screw tip hollow needle for sternal access in the late 1970’s (Dankmeijer, 1970). This device was employed as part of the emergency equipment on rescue helicopters for starting vascular access in victims of hypothermia. The US Army and University of California collaborated on another independently designed sternal access vascular entry (SAVE) device which also had a self tapping screw and a stabilization plate and unique design to stop advancement once the marrow was reached (Halvorsen, 1990; Runyon, 1993).

The SAVE technology (cf. above) was licensed to Pyng Medical (Vancouver, British Columbia, Canada) who studied the limitations of sternal access with standard needles. Pyng eventually adopted and commercialized another design and introduced the first of the new breed of sternal needles, the FAST-1 vascular access system. The FAST-1 was designed to deliver more rapid and safer sternal infusion based on an ingenious design that allowed rapid manual insertion as a series/circle of outer needles that stop on the bone surface, thus allowing the placement of the central IO needle a fixed distance beyond the depth of the outer needles. The short IO needle, with attached tubing, would be placed at the correct depth regardless of skin thickness. The FAST-1 system also provides a patch to mark the insertion site and a hard plastic dome to protect the inserted catheter.

Another novel technology to rapidly gain vascular access, focused on tibial access using a spring loaded injector, is the Bone Injection Gun (B.I.G., Waismed, Israel) that has been used in several studies and trials (see Table 3). And recently, a new battery powered IO vascular access device has been described (EZ IO, VidaCare, San Antonio, TX). This unit is a hand-held, battery-powered drill for quickly placing IO devices. The EZ IO has recently received 510k approval from the U.S. Food and Drug Administration and should be appearing commercially in the near future. Such an approach may provide greater success at access and also provide a needle more tightly attached to the bone than manually inserted devices. For the military the weight and size of a battery powered driver is a discouragement for deployment with field medics.

Intraosseous infusion is also entering the autoinjectors arena. One such device, designed to put automated IO drug delivery in the hands of a wide variety of first responders, is FirstMed (Resuscitation Solutions, Inc., Galveston, TX). FirstMed is a single use, spring-loaded auto-injector capable of administering 2-ml of emergency drugs in time-critical situations. This device is still in development.

Surveys of IO use

Several surveys have explored the familiarity with or acceptance of IO device use within the medical community. Overwhelmingly, the emphasis has been on pediatric use. Therefore, the information obtained from such surveys cannot be extrapolated easily to the use of IO devices in an adult population. Major surveys devoted to the IO application are presented in Table 2.
Table 2 Surveys of IO vascular access

<table>
<thead>
<tr>
<th>Authors</th>
<th>Devices</th>
<th>Respondents</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spivey, 1988</td>
<td>Tibia, sternum</td>
<td>303 Emergency &amp; Pediatric Depts., Medical Schools</td>
<td>808 IO cases cardiac arrest, dehydration, shock &amp; seizures – 82% success</td>
</tr>
<tr>
<td>Zimmerman 1989</td>
<td>Pediatric tibia</td>
<td>133 aeromedical programs</td>
<td>~80% success; 69% knew of, but did not consider IO</td>
</tr>
<tr>
<td>Lavis, 2000</td>
<td>Jamshidi</td>
<td>157 Emergency Consultants, Gr. Britain</td>
<td>Adult IO use is not taught (11%) or widely used (7%)</td>
</tr>
<tr>
<td>Kramer, Bruttig, 2004</td>
<td>All adult</td>
<td>EMTs, military medics, physicians</td>
<td>On-going survey</td>
</tr>
</tbody>
</table>

William Spivey conducted an extensive postal survey sent to over 1,193 physicians of 898 medical schools in 76 countries and presented the results of the survey at the 2nd International Conference on Emergency Medicine. He had 303 respondents who reported on 808 cases over a two-year period of the mid-1980’s. In the US and Canada, IO access was most often used to treat cardiac arrest with drug delivery, while in the Middle East and South America, IO access was used mostly to deliver fluids for dehydration and shock. Overall success rates were calculated to be 82%. The major complications were cellulitis and osteomyelitis and were reported to have an incidence rate of 1.3%.

In a survey of aeromedical evacuation programs conducted by personnel of the University of Wisconsin (Zimmerman et al., 1989), regarding adoption of intraosseous infusion as part of pre-hospital care, 69.2% (of 133 programs) had not implemented IO access nor were they considering implementation, 13.5% had used IO access and 15.8% had not used it but had implemented an in-service training program for its use. Of the programs using or considering use of IO access (39 programs), seven programs restricted IO insertion to physicians while thirty-two programs permitted insertion of IO devices by nurses or paramedics. Of the programs using or considering IO access, half of the programs would use it after 5 minutes of attempting to obtain an IV access. Of the programs that were using IO access, 80% of the attempted insertions were successful, with few and minor complications.

Lavis et al. (2000) conducted a postal survey to determine acceptance and use of IO for rapid vascular access in adults. The survey was sent to 559 emergency departments, with an apparent response of 332 departments. “Seventy four per cent of respondents were aware that intraosseous infusion could be used in adult resuscitation, while only seven per cent used the technique. All (100%) were involved with training their medical staff and 11% said they taught the technique for use in adults. The majority of respondents were accredited in at least one of the adult resuscitation training courses.” The authors concluded that IO use was infrequently taught and made a plea for greater teaching effort.

In a recent literature survey (Kahn & Kissoon, 2000) regarding IO use in pediatric patients, the authors categorize their findings for complications into 5 areas (extravasation and compartment syndrome; bone marrow and fat embolism; localized cellulitis, subcutaneous abscess and osteomyelitis; iatrogenic bone
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fracture; injury to epiphysis) with the two complications of any concern being extravasation and compartment syndrome. Infection was considered less of a concern after 1980 due to better aseptic technique. The authors emphasize that many complications are the result of poor technique and recommended more and better training.

With the advent of renewed interest for IO devices as a viable option for rapid access to the vascular space, and increasing concerns about terrorism, mass casualty situations, combat casualty care, and other forms of emergency medicine directed at adult populations, a survey focused on IO use in adult populations is warranted. Also, due to the recent comments voiced by in the ACLS textbook, namely that IO access has limited evidence based publications, a more up-to-date and comprehensive survey of IO use, emphasizing benefit and the documentation of complications is warranted (ACLS reference textbook, 2003). Such information may provide the preliminary data to justify a call for clinical trials to more rigorously evaluate any benefits of IO access versus IV access or one IO regimen versus another. Therefore, our research group (Kramer, Bruttig & Wu) is currently launching a web-based survey of IO use in adults, by civilian and military emergency medical personnel, in order to address some of these issues.

Clinical Trials and Case Reports

There have been several small clinical trials for IO use in adults, and the scientific literature also contains several case reports, especially from 1965 forward to today. IO use has been employed in fits and starts, in the adult community, for over 60 years, yet there is reluctance to take full advantage of the benefits of IO access as a first choice treatment modality. Most often, IO access is attempted only after repeated attempts at the more traditional forms of IV access (venipuncture and venous cutdown). Reviews usually suggested the utility of IO access in adults, but until the development of special adult needles, adult IO use was rare. Table 3 summarizes some of the literature documenting studies and use of IO access in adults.

Iserson (1989) conducted a study to determine the utility of IO in adults. They studied 22 patients between 36 and 84 years (mean = 65.1 years) that arrived in the emergency room in cardiac arrest from nonhypovolemic causes (i.e., no hemorrhage), and for whom no functioning IV existed. They used Jamshidi needles to establish IO access above the medial malleolus of the tibia in less than one minute. Fluid was infused under a pressure of 300 torr. Flow rates were described as 5-12 mL/min which seems very low for a well established IO access port under pressure. The IO access port was used to administer emergency drugs such as sodium bicarbonate, lidocaine, atropine, and vasopressors. They concluded that IO administration of drugs “appears to hold promise as another useful modality for adults and older children during nontraumatic resuscitations.”

Chavez-Negrete (1994) used sternal IO to administer hypertonic/hyperoncotic resuscitation using hypertonic saline/dextran (HSD) in 10 patients with hemorrhagic hypotension; other patients were treated with IV HSD or IV isotonic crystalloid. This was one arm of a larger study investigating the benefit of IV administration of hypertonic/hyperoncotic resuscitation solutions. The results were the same (beneficial) as those for 16 patients receiving HSD via peripheral vein, where all HSD treated patients had an effective normalization of blood pressure. Several animal studies have reported safe and effective resuscitation via the intraosseous route (Halvorsen, 1990; Dubick, 1992), but a recent report of treatment of dehydrated hemorrhaged swine with multiple IO infusions of HSD resulted in muscle necroses some days after treatment (Alam, 2002). In light of the recent regulatory approval of HSD in most NATO countries, further research is warranted and until then caution on IO delivery of hypertonic fluids is in order.
### Table 3 Case Reports and Trials of Adult IO vascular access

<table>
<thead>
<tr>
<th>Authors</th>
<th>Devices</th>
<th>Subjects/Study</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocantins &amp; O’Neill, 1940 &amp; 1941</td>
<td>Stylet-trephen needle, sternum</td>
<td>14 &amp; 52 patients</td>
<td>Effective</td>
</tr>
<tr>
<td>Quilligan &amp; Turkel, 1946</td>
<td>Trocared needle, sternum</td>
<td>Referenced more than 800 adult and pediatric cases</td>
<td>Effective, osteomyelitis was number one complication, &lt;2%</td>
</tr>
<tr>
<td>Iserson, 1989</td>
<td>Jamshidi, Medial Malleolar IO</td>
<td>22 nonhypovolemic ER patients - cardiac arrest. Fluids -drugs infused under pressure (300 torr).</td>
<td>&gt;80% success in &lt;1 min; few complications</td>
</tr>
<tr>
<td>Chavez-Negrete., 1991</td>
<td>14 gauge needle, Sternal IO</td>
<td>10 patients GI bleeding 250 ml HSD plus 2.3 + L crystalloid;</td>
<td>Effective initial treatment of hemorrhagic shock.</td>
</tr>
<tr>
<td>Glaeser et al., 1993</td>
<td>Jamshidi, tibia</td>
<td>142 children &amp; 10 Adults IO placement by EMT-Ps</td>
<td>Success rate = 76%; Evidence of clinical response =24%</td>
</tr>
<tr>
<td>Waisman &amp; Waisman, 1997</td>
<td>Bone Injection Gun (B.I.G.), tibia</td>
<td>50 adults 27 -78 years, 12 w/ multiple injuries, &amp; 7 underwent emergency resuscitation.</td>
<td>Success rate = 100%</td>
</tr>
<tr>
<td>Lavis, 1999</td>
<td>14 gauge IO trocared needle, iliac crest</td>
<td>4 adults trauma &amp; cardiac arrest</td>
<td>Successful &amp; recommended</td>
</tr>
<tr>
<td>Calkins et al., 2000</td>
<td>FAST-1, B.I.G., Sur-Fast, Jamshidi, Sternum &amp; tibia</td>
<td>Human Cadavers</td>
<td>All IO devices easy to learn &amp; place; 94+% success</td>
</tr>
<tr>
<td>McNab et al, 2000; Susak et al., 2000</td>
<td>FAST 1, sternum</td>
<td>50 patients Pilot study of FAST-1 insertion times, and complications.</td>
<td>Success = 74% - first-time users, 95% - experienced users; Failure in &quot;very obese”</td>
</tr>
<tr>
<td>Frascone, 2003</td>
<td>FAST-1, sternum</td>
<td>severely burned adult patient</td>
<td>“underwent a successful cardiac resuscitation”</td>
</tr>
</tbody>
</table>

Glaeser et al. (1993) conducted a 5-year, nonrandomized trial of patients (all ages from newborn to 102 years) to “evaluate the ability of emergency medical technician-paramedic (EMT-P) units to become and remain proficient in the performance of the intraosseous infusion procedure”. The study enrolled 152 patients with EMT-P placed IO lines using the Jamshidi sternal IO needle placed in the proximal tibia. EMT-Ps made 165 attempts to place the IO needles, with a success rate of 76%. There was slightly greater success in newborns and infants than in older children. The group noted the proficiency of the EMT-Ps as high and that proficiency was maintained over the 5-year period. They also noted that the most common errors in establishing IO access were errors in landmark identification and bending of needles. They noted evidence of clinical response (to treatment through the IO port) in 28 patients.

Waismann and Waismann (1997) conducted a prospective study to determine the feasibility of using IO infusions in adults using the newly developed Bone Injection Gun (BIG) for an industry-sponsored study. This
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The study was done in Israel where the device was used for a number of years before regulatory approval in the US. The study had two groups, one composed of adult patients with recent, closed long-bone fractures needing surgery with regional anesthesia, and another group that required “emergency or semi-emergency vascular access” where standard IV access could not be established in reasonable time. The study enrolled 50 patients ranging in age from 27-78 years. Seven patients required emergency resuscitation. IO placement was reported to be 100% successful and there were no complications. The authors emphasize the need to consider the intraosseous route for rapid vascular access.

Lavis (1999) reported on use of adult IO access in 4 patients suffering from either cardiac arrest or traumatic injuries. Notably they used a 14 gauge trocared needle to enter the iliac crest and reported success with access and delivery of fluid and drugs.

Macnab et al. (2000) studied the first 50 uses of the FAST 1 IO device (Pyng Medical) in the sternum of adults. Six emergency departments and 5 prehospital sites, in both Canada and the U.S., provided data for this industry-sponsored study. Data included success rates, insertion times, and complications. “The overall success rate for achieving vascular access with the system was 84%. Success rates were 74% for first-time users, and 95% for experienced users.” Failure to achieve placement occurred most often due to obesity and the thick tissue layer overlying the IO placement site – the sternum. Maximum flow rates for gravity drip infusions were 80 ml/min and 150 ml/min for bolus injections by syringe. Syringes can generate pressures in the several hundred torr range. No complications were reported in a follow-up study 2 months later. The group concluded that the FAST 1 IO system “may provide rapid, safe vascular access and may be a useful technique for reducing unacceptable delays in the provision of emergency treatment.”

Calkins et al. (2000) conducted one of the few independent studies comparing different adult IO devices. They determined the ability of 31 military Special Operations medical personnel (Army, Navy and Air Force) to learn and use IO as a means of rapid vascular access in cadavers. They also compared four commercially available IO devices, the Jamshidi, the Sur-Fast, the BIG and the Fast-1 (see Table 3). Training was comprised of lecture and videotape. Each study subject used all four IO devices. A post-study questionnaire revealed that each device was easy to learn. Placement success ranged from 94-97% and though all of the devices were acceptable, the study did not recommend one device over the others. The Bone Injection Gun was a favorite of the medics (65%) with the Jamshidi coming in second at 52%.

Finally, Frascone et al. (2003) provide a case report describing the successful placement of a sternal IO device in a severely burned patient, through burned skin. Since burns have been considered a contraindication, this single patient case report is significant. The authors state that the patient was “in full arrest” and successfully underwent cardiac resuscitation with the aid of the device. No complications were described.

Overall then it appears that placement and use of a variety of IO devices is associated with a high likelihood of success, and few and minor complications are encountered. Studies addressing the teaching of IO use describe the ease with which students of various training levels learn to become proficient in the placement and use of IO devices, and the retention of that proficiency over several years.

The extensive literature and use of IO vascular access resulted in a change of the standard of care for treating pediatric emergencies. The approach was validated by adoption into the American Heart Association’s Pediatric Advance Life Support guidelines. The American military had a special need for a better means of adult vascular access as medics and corpsmen were trained in starting IV, but did not get the continual clinical practice required to maintain competency. When faulty competence is coupled with the
difficult environments faced in combat casualty care, the need for a faster, more reliable and foolproof system of vascular access was apparent. The National Academy of Science’s Institute of Medicine organized a select panel to make recommendations for fluid resuscitation and they advocated the adoption of IO use for field resuscitation (Pope et al., 1999). Additionally, several recent reviews have discussed the special needs of the military for IO vascular access (Calkins, 2000; Dubick & Holcomb, 2000; Holcomb, 2001; Dubick and Atkins, 2003). Whether the use is military or civilian, it appears clear that IO use in adults is well justified in the prehospital environment. It also appears that emergency departments worldwide are beginning to consider, if not embrace such technology for use in adults.

Future Trends in Intraosseous Use

For all of the promise for IO use indicated above, there remain reasonable concerns regarding the efficacy and safety of IO use as an alternate route for vascular access. In the critically injured or ill, time matters and therapy must be prompt and sustained until definitive care is provided. Future work on how well the IO route holds up to continuous and intermittent use over several hours is needed. The relative safety and efficacy of the tibial IO sites versus sternal sites need to be examined. Direct comparison of all the currently FDA approved adult IO access devices needs comparison in randomized trials if possible and balanced cohort trials if not. The range of infusion rates possible in the tibia and sternum need to be determined and how this can be increased with pressurized fluids when large volume therapy is needed.

In the special case of exposure to chemical weapons agents (nerve toxins, mustard agents, etc.), IO use provides a very rapid vascular access. Moreover, the ease of use and the ease of learning the techniques of IO use will make IO use available to a wider variety and greater numbers of “first responders” – a true force multiplier. In the response to exposure to chemical weapons agents, it takes several minutes after IM autoinjector delivery of nerve gas antidotes to reach pharmacologically effective levels with an intact circulation. This contrasts with the rapid onset of symptoms following exposure. Treatment of chemical weapon casualties may need direct vascular entry of drugs such as can be accomplished via the IO route. A recent simulation was designed to test the impact of using IO delivery of nerve gas antidotes to mass casualties with medical personnel in full protective gear (Vardi, 2004). IO and IV delivery of antidotes can be life saving compared to IM delivery, as it takes 10-20 minutes longer for pharmacologically effective concentrations to be reached in a normal or healthy circulation (Sidell, 1971). The simulated survival rate with physicians equipped with BIG IO needle injectors was 73.4%, while with standard treatment modalities, IM injectors, the survival rate was 3.3%. One wonders if the use of a self contained IO drug injector would be most efficacious for that scenario.

A recent chapter in the American Heart Associations 2003 ACLS textbook (pp. 214-218) emphasizes the potential for adult IO access and infusion for treatment of prehospital cardiac emergencies in conjunction with AEDs for earlier treatment of cardiac arrest. However, the authors are critical because the peer literature is mostly industry driven with an emphasis on “competitive data” such as procedure or access time and time to infusion. Formal guideline recommendations by the American Heart Association await higher levels of evidence. There are virtually no randomized trials or reports or even a priori designed studies of clinical outcomes. Questions addressing the impact of any speed advantage on outcomes need to be addressed. Can new IO technologies put more advanced medical capability in the hands of First Responders? We agree that more evidence-based data is needed. The AHA, the NIH and US Army need to encourage such efforts through specific requests for proposals as was done in the recent PULSE initiative by the National Heart, Lung and Blood Institute in collaboration with the US Army (Becker et al, 2002).

One might look at the use of IO access in adults as a future trend, but as the historical literature points out, this in itself is not new. A recent article exploring other intraosseous sites (clavicle, iliac crest, etc.;
Iwama et al., 1994) in adults indicates that alternate or more effective IO sites are still the subject of investigation. Even the use of bony sites without traditional medullary cavities are being explored as potential IO sites (McCarthy et al., 2003) in the critically ill adult. Greater use of IO devices for the initial administration of time-critical drugs or biologics will likely be the subject of future investigation. IO technology is being used to validate IO sites as reasonable for blood chemistry analyses (Johnson et al., 1999; Hurren, 2000), and one group, so far, is giving anesthesia through IO sites (Waisman et al., 1995). Moreover, the use of the IO device as a stable platform for incorporation of physiologic or pharmacologic sensors represents another possible new consideration IO technology. Another new effort that will likely refine IO technology will be greater emphasis on clinical, outcomes-based research. This will represent a move away from the “classic” speed-of-procedure investigations characteristic of much of the scientific literature. But the biggest effort for technical investigation will likely be using the mechanically stable IO platform as a portal of choice for closed-loop fluid- and drug-based resuscitative therapy.

CONCLUSIONS – POSSIBLE BENEFITS FOR NATO ALLIES

There has been renewed interest in military applications for IO vascular access, especially within the U.S Army and Special Operations Forces (Calkins, 2000; Dubick & Holcomb, 2000; Holcomb, 2001; Dubick and Atkins, 2003). Such interest is due in part to the recognition of the threats caused by delays in establishing effective vascular access, particularly under difficult field conditions of combat. For the casualties of military trauma, the IO route is particularly attractive for many reasons: battlefield visibility and lighting can be impaired; medics may be wearing heavy globes and MOPP suits, making IV access more difficult; medics and corpsmen may have less than ideal IV skills, since they have less ongoing clinical trauma experience than civilian paramedics.

The available literature suggests that IO device insertion is generally more rapid than establishing traditional IV access, and considerably faster than establishing a venous cutdown. These times should only get faster as technologies and teaching, practice and use policies are refined. This means more casualties will benefit more quickly from resuscitative fluids or drugs, and potentially, more lives will be saved. Greater use of the technology and greater confidence in its efficacy may eventually remove some of the restrictive barriers now faced by IO use, namely, initial and varying delays while often futile attempts at IV insertion fail, or restriction of use to certain classes of healthcare workers. It is encouraging to note that the rate for various possible complications is extremely low and often mitigated with proper training and technique. While the data indicate greater current acceptance of this technique for pediatric than adult use, adults are at equal or greater risk for time-critical events necessitating intraosseous vascular access. The literature further teaches us that training in the actual use of intraosseous devices is often modest and that this may be the largest factor limiting its use and its success when used. The lack of overall awareness of the technique, lack of proper training tools, a lack of acceptance by training teams or an institutional policy unfriendly to widespread use of IO access all contribute to lethargic adoption of IO use for adult populations. However, adult IO access training will expand due to the greater emphasis of authors of papers on IO use in adults and the support now emerging from institutional groups such as the Institute of Medicine, the American Heart Association and the military. Continued refinement of the technology and greater acceptance of its role in pre-hospital care will lead to reduced logistic burdens for IO devices, compared with the technology in use today. All of these changes will benefit pre-hospital, emergency care and the logistic concerns associated with both, several of the forward medical treatment issues about which NATO has great concern. Finally, Since the use of IO technologies is taught in the U.S., Canada, the U.K., Germany, France, the Netherlands and elsewhere, the countries of the NATO alliance already have a firm foundation for greater adoption and wider use of IO and IOI applications.
LITERATURE CITATION


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Strategies for Small Volume Resuscitation

Hyperosmotic-Hyperoncotic Solutions,
Hemoglobin Based Oxygen Carriers and
Closed-Loop Resuscitation

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ABSTRACT

Introduction: Logistic constraints on combat casualty care preclude traditional resuscitation strategies which can require volumes and weights 3 fold or greater than hemorrhaged volume. We present a review of quantitative analyses of clinical and animal data on small volume strategies using 1) hypertonic-hyperosmotic solutions (HHS); 2) hemoglobin based oxygen carriers (HBOCs) and 3) closed-loop infusion regimens.

Methods and Results: Literature searches and recent queries to industry and academic researchers have allowed us to evaluate the record of 81 human HHS studies (12 trauma trials), 19 human HBOCs studies (3 trauma trials) and two clinical studies of closed-loop resuscitation.

There are several hundreds animal studies and at least 82 clinical trials and reports evaluating small volume 7.2%-7.5% hypertonic saline (HS) most often combined with colloids, e.g., dextran (HSD) or hetastarch (HSS). HSD and HSS data has been published for 1,108 and 392 patients, respectively. Human studies have documented volume sparing and hemodynamic improvements. Meta-analyses suggest improved survival for hypotensive trauma patients treated with HSD with significant reductions in mortality found for patients with blood pressure < 70 mmHg, head trauma, and penetrating injury requiring surgery. HSD and HSS have received regulatory approval in 14 and 3 countries, respectively, with 81,000+ units sold. The primary reported use was head injury and trauma resuscitation. Complications and reported adverse events are surprisingly rare and not significantly different from other solutions.

HBOCs are potent volume expanders in addition to oxygen carriers with volume expansion greater than standard colloids. Several investigators have evaluated small volume hyperoncotic HBOCs or HS-HBOC formulations for hypotensive and normotensive resuscitation in animals. A consistent finding in resuscitation with HBOCs is depressed cardiac output. There is some evidence that HBOCs more efficiently unload oxygen from plasma hemoglobin as well as facilitate RBC unloading. We analyzed one volunteer study, 15 intraoperative trials, and 3 trauma studies using HBOCs. Perioperative studies generally suggest ability to deliver oxygen, but one trauma trial using HBOCs (HemAssist™) for treatment of trauma resulted in a dramatic increase in mortality, while an intraoperative trauma study using Polyheme™ demonstrated reductions in blood use and lower mortality compared to historic controls of patients refusing blood. Transfusion reductions with HBOC use have been modest. Two HBOCs (Hemopure and Polyheme) are now in new or planned large-scale multicenter prehospital trials of trauma treatment.

A new implementation of small volume resuscitation is closed-loop resuscitation (CLR), which employs microprocessors to titrate just enough fluid to reach a physiologic “target”. Animal studies suggest less risk of rebleeding in uncontrolled hemorrhage and a reduction in fluid needs with CLR. The first clinical application of CLR was treatment of burn shock and the US Army.

Conclusions: Independently sponsored civilian trauma trials and clinical evaluations in operational combat conditions of different small volume strategies are warranted.

1.0 INTRODUCTION

Most of the modern clinical perspective of trauma care is from reports of urban trauma centers where prompt arrival of paramedics lends itself to rapid transport of patients to trauma centers for definitive care. [1, 2] Prehospital care for rural trauma, mass casualty and combat casualty are different than urban trauma for several reasons. 1) Patient transport times can be lengthy and the initiation of transport may be greatly delayed
2) Logistic constraints can result in a limited amount of volume being available for initial care of mass casualties and combat casualties. 3) Further, a high ratio of victims to caregivers can occur such that focused care is unavailable for most patients. A better and more efficient means to treat trauma patients in these scenarios is needed. Small volume resuscitation can be considered a concept to improve the efficiency of fluid therapy such that there is physiological equivalence in a smaller volume. This can be approached by changing the composition of the fluids by changing the infusion regimens. For the military application small volume resuscitation does not have to be superior to standard of care therapy, rather it could simply be equivalent or the best possible choice after considering logistical constraints. On the other hand, all of the clinical work on these approaches has been for civilian care and thus in general researchers have attempted to determine if small volume resuscitation is better than conventional care. We will review three approaches that may allow resuscitation to be limited in volume by increasing the physiological efficiency. We present some background and physiology based on animal studies but the focus will be on the clinical trial data. These three approaches are: 1) the use of concentrated hyperosmotic-hyperoncotic small volume formulations; 2) the use of synthetic hemoglobin based oxygen carriers; and 3) the use of automated systems that titrate fluid therapy to endpoints.

2.0 HYPEROSMOTIC-HYPERONCOTIC SOLUTIONS

2.1 Historic development of hypertonic saline

There has been substantial interest and extensive preclinical and clinical experience in evaluating the use of hypertonic saline solutions for volume support. These effects have universally been shown to reduce volume needs [5-10]. Hypertonic solutions mobilize an amount of cellular water proportional to osmotic load and tends to reduce overall volume needs in perioperative patients [11, 12]. Because cells become edematous during shock and surgical stresses [13-15], hypertonic resuscitation of shock will often normalize cell volume rather than reduce it below normal [16, 17]. Mildly hyperosmotic saline solutions (1.5-2.0%) are well described in studies of intraoperative volume replacement [7, 8] and for the resuscitation of major burns [5, 6]. In general, these mildly hypertonic solutions are reported to reduce fluid volume requirements; however, to date such formulations have not received widespread usage.

In the last 20 years, extensive research efforts have focused on a more concentrated hyperosmotic 7.2-7.5% NaCl solutions alone or mixed with a hyperoncotic colloid for small-volume resuscitation. The calculated osmolality of such solutions is 2464-2567 mOsm, but the measured osmolality is slightly less and they have been collectively referred to as 2400 mOsm formulations, since the first reported study by Velasco et al [18]. Because hyperosmotic crystalloid solutions provided profound, but often only transient hemodynamic improvement, consideration was given to mixing a hyperoncotic colloid with the hyperosmotic NaCl [19]. The rationale was that while the hyperosmotic sodium chloride would expand the vascular space by mobilizing extravascular water, adding a hyperoncotic colloid might selectively retain more of this water in the vascular space. Several independent groups confirmed the better hemodynamics, survival and higher cardiac outputs with HSD compared to HS alone in different models using hemorrhaged pigs, dogs and sheep [20-26]. These beneficial effects were attributed to a slightly better initial and, particularly, a more sustained plasma volume expansion.

Of particular note, Maningas et al, and Wade et al showed that treatment of severe hemorrhage in conscious pigs using small volumes of HSD caused a 100% survival, while similar volumes of HS alone, dextran alone or normal saline resulted in significantly less survival [26, 27] with survival benefit confirmed by others [20]. Hypertonic saline mixed with hetastarch (HSS) produced similar cardiovascular responses [28-31]. The confirmation of the sustained effectiveness of HSD suggested an ideal small-volume formulation for the
military [32, 33]. The Maningas studies are historically important because they were the stimulus that launched the clinical trauma trials of HSD.

2.2 Physiological Mechanisms

Intravenous infusion of a small-volume hyperosmotic-hyperoncotic solution in hemorrhaged animals rapidly initiate major physiological responses affecting vascular volume, heart and peripheral blood vessels that work synergistically together to increase cardiac output. These mechanisms along with their clinical correlations are schematically illustrated in Figure 1. Associated physiological and clinical responses include reduced peripheral vascular resistance, reduced pulmonary vascular resistance, diuresis/natriuresis, restoration of membrane potentials, correction of cellular edema, and lower subsequent volume requirements [16, 17, 34-36].

![Figure 1.](image-url)
Strategies for Small Volume Resuscitation

Figure 2.

Such powerful and rapid physiological effects could be deleterious, particularly if not used with an understanding of their effects. Figure 2 illustrates the physiological equivalence of HSD with lactated Ringer’s and this simple picture is perhaps the single best guide to understanding the acute benefit as well as the potential dangers of hypertonic resuscitation. Most of the initial cardiovascular changes can be explained by the very rapid volume expansion, which occurs as soon as the fluids are infused [37]. Very aggressive resuscitation with rapid increases in blood pressure were believed to be an advantage when HSD was first studied in the mid 1980’s [35]. Subsequent animal studies showed that early application of aggressive resuscitation deleteriously affected outcomes in animal models of uncontrolled hemorrhage as rapid bolus infusions of HSD caused rapid increases in blood pressure, internal bleeding and higher mortality [38-43]. Such increased bleeding is not due to the nature of the volume expander, but rather the rate at which the fluid is administered and the volume expansion and hemodynamics elicited. Since HSD has the hemodynamic impact of close to 3 liters of crystalloid, it should perhaps be infused as 3 liters of lactated Ringer’s would be infused for trauma resuscitation. Limited resuscitation to intermediate levels of blood pressure and with slower infusions has been shown to lower mortality in anesthetized swine and rat models of uncontrolled hemorrhage [40-42] when compared with resuscitation designed to normalize blood pressure. Most notable is the enhanced survival benefit demonstrated by Stern et al when HSD was infused slowly versus rapidly [44]. Small volume resuscitation with slower infusion may improve clinical outcomes, while simultaneously accommodating special needs of the military by reducing the cube and weight of fluid needed in the field. There is also evidence that slower infusions may increase relative volume sparing. Greater volume sparing has been reported for both HSD and HSS in clinical intraoperative trials than in acute hemorrhagic shock trials where HSD has been bolused [12]. When HSD or LR was infused slower and titrated to physiological effect the ratio of isotonic to hypertonic volume needs were increased to 15-19 or greater than the 10 to one difference often referenced [45, 46].

It should be noted that rapid bolus infusion in anesthetized animals and humans can cause vasodilation and can transiently reduce blood pressure before increasing it [47-49]. This hypotension is due to an effect on the peripheral circulation, rather than the heart, as blood flow, both coronary blood flow and cardiac output are increased during the hypotension [48]. Another situation when infusions can be too rapid to be safe is with deep anesthesia and a preexisting compromised circulation [50, 51]. It has been suggested that in the operating room the use of hyperosmotic solutions should be titrated to physiological effect with respect to both dose and infusion rate [52]. Such data and rationale suggest that a slower infusion may be safer than a more rapid infusion in all conditions.
Recently, studies have provided conflicting conclusions about the effects of hypertonic saline and HSD infusion on cardiac function showing that infusion of hypertonic saline solution into the circulation or directly into coronary vessels causes increased contractility [53, 54], little effect on contractility [55-58], or decreased contractility [59, 60]. Some of the negative reports may be the result of studying very high doses or very fast infusion rates. Rapid infusions or inappropriately high doses cannot only cause fluid overload, but also arrhythmias [50, 61, 62]. Such doses or infusion rates can transiently cause very high concentrations of extracellular sodium or osmotic pressures and this is further rationale for slower infusions.

2.3 Rate of Infusion

More efficient volume expansion should not be a contraindication for trauma care, particularly for the combat casualty care, but rather optimal use of hypertonic fluids may require different infusion guidelines as to infusion rate. If the physician or medic appreciates the volume equivalency illustrated and estimated in Figure 2 and considers administering 250 mL of HSD in a regimen similar to 3 liters of LR the likelihood of misuse may be reduced. On the other hand, most of the clinical trauma trials of HSD were performed in the early 1990’s when aggressive resuscitation was the standard of care. When HSD was infused rapidly per prehospital resuscitation protocols of the day, there was a survival benefit in these civilian trauma patients most representative of combat injuries and penetrating trauma requiring surgery [1, 63].

2.4 Peripheral Circulatory Effects

The effects of infusing hyperosmotic-hyperoncotic solutions on the peripheral vasculature and the microcirculation are generally to induce changes that augment flow. These are a reduction in peripheral vascular resistance, which is primarily due to arteriolar vasodilation [64]. Capillary perfusion may be further augmented by the ability of HSD to reverse specific cellular effects of ischemia and ischemia-reperfusion. HSD infusion shrinks endothelial cells that are swollen by hemorrhagic shock [16].

2.5 Immune Modulation of Hypertonicity

In the last 10 years there has been a growing body of evidence on the immune modulation of hypertonic resuscitation. *In vitro* and *in vivo* effects of hypertonicity on white cells suggest that a hyperosmolarity above 330 mOsm can down-regulate the initial inflammatory activation of neutrophils and upregulate immunological protection provided by lymphocytes [65-67]. Most recently, the down regulation of inflammatory cytokines and neutrophil activity along with proliferation of lymphocytes counts have been demonstrated in trauma patients treated with HSD [68]. Such data have resulted in one NIH sponsored injury trials of blunt trauma focusing on immune function as well as clinical outcome [69].

The strong and elegant science behind hypertonic immune modulation has suggested to some that HS and HSD be considered primarily as an anti-inflammatory drug and not a volume expander. From this consideration, HS alone is likely to be as efficacious as HSD. This may be a shortsighted viewpoint in that it negates the proven physiologic value to restoring vascular volume, perfusion and oxygen delivery in trauma patients. Physicians and medics administer fluids to trauma patients with an immediate need for augmentation of volume expansion and tissue oxygen delivery. The extensive animal work on HS versus HSD and the outcomes from clinical trials support the rationale for providing better volume expansion and associated hemodynamics of HSD compared to HS.
Table 1: All HS.

**Human Trials/Experiences with 7.5% NaCl**

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<tr>
<th>Author</th>
<th>Sol.</th>
<th>Dose</th>
<th>Site</th>
<th>Patients</th>
<th>HS</th>
<th>HSD</th>
<th>HSS</th>
<th>Iso</th>
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<td>ICU</td>
<td>refractory shock</td>
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<td>aneurysm</td>
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<td></td>
<td>13</td>
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<tr>
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<td>ER</td>
<td>trauma</td>
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# Strategies for Small Volume Resuscitation

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<td>Acta Neuro-Chir (Wien) 50(S): 126-129</td>
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<td>Krenn, 00</td>
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<td>Wall, 00</td>
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<td>effectively treated raised ICP or low BP</td>
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<td>Jarvela, 01</td>
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## Strategies for Small Volume Resuscitation

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<th>Site</th>
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<td>Clin volunteers</td>
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<td>ICU sepsis</td>
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<td>Clin volunteers</td>
<td>8</td>
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<td>Kollmar, 04</td>
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<td>14</td>
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<td>25</td>
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<td>Kolsen-Peterson, 04</td>
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<td></td>
<td></td>
<td>42</td>
<td>Anesthesiology. 100(5):1108-18, 2004 May</td>
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### Ongoing trials, marketing & pharmacovigilance data

1. **Bulger, ongoing 2004**  
   - HSD 250 mL  
   - PH Blunt trauma  
   - U Washington news 6-16-03, personal communication

2. **Tripartite (US, Canada & Great Britain) HSD trauma trial, to be started, 2004**  
   - HSD 250 mL  
   - PH Blunt trauma  
   - personal communication

3. **Schimetta, 02**  
   - HSS 250 mL  
   - trauma, head injury  
   - 56,000  
   - Wien Klin Wochenschr 114:89-95, 2002

4. **Buckley, 04**  
   - HSD 250 mL  
   - trauma  
   - 25,000  
   - personal communication

**Trial Totals** = 610 1,130 392 1,355

**All Hypertonic** = 2,232
2.6 Clinical Record of Hyperosmotic/Hyperoncotic Solutions

The clinical use of ~2400 mOsm solutions had been studied for both perioperative use and most extensively in randomized blinded trials in which 250 mL of HSD was infused as first treatment for hypotensive trauma in the field or emergency room. Complete references are found in recent reviews [12, 37, 70-72] and are listed in Table 1 which lists all clinical reports and trials with ~2400 mOsm solutions that we are aware of. The reported number of patients treated with hypertonic saline continues to grow. The concentrated ~2400 mOsm hypertonic saline solutions have a remarkable record for safety and also suggest significant efficacy for the following indications: intra-operatively for volume expansion, to attenuate hypotension after aortic cross clamping and during renal dialysis, treat hypotension due to bleeding gastric ulcers, fluid maintenance of patients with burn injuries or sepsis, to reduce intracranial pressure and improve cerebral blood flow, and in the resuscitation of patients with hypotension and injuries due to trauma and hemorrhage (Table 2). Of all studies there is only one report of a negative outcome in which a HS-hetastarch formulation caused acute volume overload and cardiac instability in patients with cardiac failure [50]. Subsequent clinical studies examined hypertonic saline hetastarch in cardiac patients and found that poor outcomes are a result of not anticipating the large volume and potent volume expansion of hyperosmotic-hyperoncotic small volume formulations. The proper clinical perspective is that 250 mL of HSD or HS-hetastarch is equivalent to a ~3 liter infusion of isotonic crystalloid and hyperosmotic/hyperoncotic solutions should NOT be infused over a set time course where 3 liters of crystalloid is unwarranted. This message can perhaps be applied to certain young prehospital trauma patients with penetrating injury and ongoing bleeding as well as to older cardiac patients getting perioperative care. Hypertonic solutions for patients at risk for fluid overload or cardiac disease should be used cautiously and not in fixed doses, but rather titrated to effect [52]. Several studies report benefit from using HSD and HSS appropriately in patients during cardiac surgery [12, 73, 74], or after heart failure [75].
Table 2: 30 Day Mortality Outcomes of Hypertonic Resuscitation Trials for Trauma & Hemorrhage.

<table>
<thead>
<tr>
<th>Hypertonic Saline (HS) alone, total n=948</th>
<th>Reference</th>
<th>HS, n=454</th>
<th>SOC, n=494</th>
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<td>20.0%</td>
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<tr>
<td>Vassar, 90[11]</td>
<td>53.1%</td>
<td>37.0%</td>
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<td>Vassar, 93[77]</td>
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</tr>
<tr>
<td>Fabian, 94[79]</td>
<td>35.8%</td>
<td>37.3%</td>
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<td>Fabian, 94[79]</td>
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<tr>
<td>Cooper, 04[80]</td>
<td>44.7%</td>
<td>50.4%</td>
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<tr>
<td><strong>all HS trials</strong></td>
<td>35.4%</td>
<td>34.4%</td>
<td></td>
</tr>
<tr>
<td><strong>Δ HS vs SOC</strong></td>
<td>-0.7%</td>
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<table>
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<td>22.5%</td>
<td>16.7%</td>
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<td>44.0%</td>
<td>51.1%</td>
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<td><strong>Δ HSD vs SOC</strong></td>
<td>-4.2%</td>
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</table>

2.7 HS and HSD Trauma Trials

Table 2 shows the 30 day mortality data of all trauma and hemorrhagic shock trials in which 7.5% NaCl (HS) alone or 7.5%NaCl-6% dextran (HSD) have been used to treat trauma. The trails were blinded and randomized with one exception [83] as to treatment with HS or HSD compared to an equal volume of the standard of care solutions (SOC; normal saline, Ringer’s solution or Plasmalyte A). All solutions have been evaluated at 250 mL dose with additional fluids and medical care given as deemed clinically necessary. It should be made clear that these solutions were given in addition to all of the normal and subsequent care the patient required per trauma center protocol. No treatment was withheld.

Trauma trials with 7.5% HS without a colloid have overall shown less efficacy than trials with HSD as reviewed in a meta-analysis [86]. There were no statistically significant differences with the overall mortality being 0.7% less with the HS treatment, Table 2. A recent randomized study of the use of HS to treat traumatic head injury showed a 5.5% difference favoring HS, but this was not statistically significant [80].

On the other hand, the outcomes in the trauma trials with HSD more strongly support efficacy as shown in Table 2 and by an extensive individual patient data meta-analysis [63, 87, 88]. Most all of these trials documented an improvement in blood pressure, and several documented reduction in total volume needs.
Only one trial was statistically significant alone [85] and also suggested that the greatest survival benefit of HSD was in the patients with the lowest entry blood pressures. The view that HSD is more beneficial in severely injured has been borne out in several subgroup analyses of the more severely injured patients who often did show statistically significant increased survival in trauma patients with head injury [87], dehydration [89] and penetrating injury [1, 63]. Taken as a whole the HS and HSD studies suggest safety, volume sparing and improved outcome.

Based on the randomized controlled trauma trials, Table 2, HSD appears to reduce mortality of hypotensive trauma. Over all the difference can be considered small, a 4.2% patient weighted mean change, or a 15% reduction of mortality. The largest subset of hypotensive trauma patients would survive without treatment and a smaller subset would die regardless of treatment. Only a small subset of perhaps 10-20% can benefit or be harmed by fluid therapy. Taken in light of this argument the benefit seems profound. However, treatment effects in randomized control trials can be greater or less than in standard clinical usage. Perhaps more important than a new round of controlled trials is to encourage post regulatory approval monitoring in those countries where HSD is approved for use. If civilian trauma centers can be matched as to general patient population, and a form of standardized outcome data collection can be generated, such data might be more valuable than a clinical trial because it could provide real world outcome effects. New trauma trials sponsored by the NIH and with military funding from the US, Canada and Great Britain has recently started or in final planning. Such trials may lead to US regulatory approval and/or use by US Armed forces. On the other hand, HSD has regulatory approval in most of the NATO countries and hypertonic saline hetastarch (HSS) in a growing number of them. Thus, many NATO military units could evaluate hypertonic resuscitation. Military surgeons and anesthesiologists in countries for which HSD or HSS is approved should be encouraged to become familiar with the extensive backgrounds of such products and use them electively in their homeland practices. Product placement with selective combat medical units along with a post regulatory monitoring program would provide the first real combat experience of small volume resuscitation and should be encouraged. The outcomes from case reports of units deployed with and without hypertonic formulations could be compared by an expert panel.

2.8 HSD versus HSS

Early studies comparing HSD versus HSS formulations suggested equivalent physiologic effects. HSD has had more extensive US exposure and use in trauma trials, while HSS has more European exposure and is most often used in intraoperative trials, particularly for cardiac surgery. In the small volume formulations the particular benefit or any side effect of the type of colloid is likely to be negligible. HSD had been show to be devoid of any apparent effect on coagulation or blood typing or inflammation in the trials to date. An extensive record of clinical safety has been established for HSS in Austria where it has been approved since 1991 and used in over 56,000 patients [90]. The primary indications for its use have been head injury, trauma, and intraoperative volume sparing.

2.9 Hypertonicity, Inflammation and Organ Failure

The renewed interest in HSD or HS alone has resulted from the pioneering studies of Junger and Hoyt who first established profound anti-inflammatory properties of a hypertonic bolus [91, 92]. Studies in cell culture and rodent models have suggested efficacy as survival is improved and organ failure (histology) greatly attenuated by hypertonic resuscitation [93, 94]. Thus, the concept of hypertonic therapy as a drug is intriguing. Indeed, incidents of organ failure (ARDS, renal failure, etc) were reduced in the USA multi-center trial 5/211 with HSD vs 20/211 with SOC as well as in incidents of MOF in the individual patient meta-analysis [88].
2.10 Combat Casualty Care

Despite all of the new hypertonic publications on inflammation and the older publications on the physiology of resuscitation, the most straightforward rationale for its use of any fluid combat casualty care can be summarized in Figure 2. Even if HS alone is as effective at reducing inflammation as HSD, the better volume expansion with HSD or HSS versus HS alone is sufficient rationale for choosing HSD or HSS over HS. Better volume expansion also equates with better cardiac output and blood pressure. Thus, periods of hypotension are less likely with head injury. The only rationale for choosing HS alone over HSS or HSD would be cost or untoward clinical results with HSD. However, taken as a whole the extensive clinical record of HSD and HS in trauma suggests, but does not prove, that HSD and probably HSS may be superior with respect to outcomes.

The early volume expansion properties of HSD are about 10-fold greater than that of standard crystalloids [95, 96]. Figure 2 provides the main rationale for use of HSD for combat casualty care volume sparing. More efficient volume expansion provides the rationale for its use in situations where hypovolemia impairs oxygen delivery. In situations where over resuscitation is a concern due to uncontrolled hemorrhage and/or cardiac insufficiency, the experimental record suggests that the solutions should be infused slowly and/or titrated to effect. If the medic appreciates the 10:1 volume equivalency and considers administering 250-mL dose in a regimen similar to how they would administer 2.5 liters, the potential for misuse could be lessened. Three special patient populations to consider for combat casualty care are the safety and efficacy of hypertonic resuscitation with pre-existing dehydration, traumatic brain injury or penetrating injury.

2.11 Safety and Efficacy of 7.5% NaCl with Pre-existing Dehydration

A special problem of combat casualty care is that wounded combatants are almost always dehydrated. The anticipation is that at some level preexisting dehydration negates the safety and clinical effectiveness of hypertonic infusions. This concern motivated several studies that analyzed the safety and effectiveness of HSD in dehydrated animals and patients. Hemorrhaged and dehydrated rats infused with hypertonic saline after occlusion of the renal artery showed an increase in incidence of renal failure and a high mortality rate compared to groups treated with isotonic fluid [97]. However, these results were not confirmed in more realistic long-term studies of renal function in large-animal models with a 4 mL/kg dose of 7.5% NaCl dextran [98-101]. The beneficial volume expansion and cardiovascular effects of HSD were still apparent after water restriction over 2 to 4 days and increased preinfusion osmolalities of 325-340 mOsm/L in dehydrated sheep and swine [98, 99, 101] subjected to moderate to severe hemorrhage.

Of relevance to dehydration is the effectiveness of HSD’s ability to increase survival in trauma patients with high preinfusion serum sodium [100]. Presumably, this patient population has pre-existing dehydration. Survival rates were low in this group when they were administered standard of care solutions, but survival was greatly and significantly improved in the HSD group. Counter intuitively, HSD has been used to effectively treat experimental dehydration in US Army sponsored studies [102, 103].

2.12 HSD and HS for Treatment of Head Injury

There is a strong physiological rationale for the use of hypertonic fluids to treat head injury particularly in the presence of hypotensive hypovolemia. Increased plasma hyperosmolality can translocate CSF and cellular water out of the brain and reduce the intracranial pressure associated with head injury. This edema lessening effect occurs in the regions of brain less traumatized, but a global reduction in ICP increased perfusion throughout the brain [104]. In animals with experimental mass lesions hypertonic resuscitation reduced ICP...
and improved blood flow [105]. Again, at first this would suggest that HS would be expected to be as beneficial as HSD for these patients and this is likely true for the effects on ICP. But a key component of mortality in patients with traumatic brain injury is the prevention of hypotension. Chesnut et al showed that episodes of hypotension were significant predictors of outcome in head injured patients [106]. A single episode of systolic pressure below 90 mmHg doubled the mortality. The ability of HSD to restore and sustain volume expansion, cardiac output and blood pressure better than HS alone has been well demonstrated in animal trials and clinical trials and is the rationale for why HSD may be particularly effective in patients with head trauma. Wade et al performed a cohort analysis of individual patient data on patients with traumatic brain injury [87]. Treatment with HSD resulted in a survival until discharge of 37.9% (39 of 103) compared with 26.9% (32 of 119) with standard of care (p = 0.080). Using logistic regression, adjusting for trial and potential confounding variables, the treatment effect can be summarized by the odds ratio of 2.12 (p = 0.048) for survival until discharge. Practically, this means that patients who have traumatic brain injuries in the presence of hypotension and receive HSD are about twice as likely to survive as those who receive standard of care. A recent prehospital trial of HS alone for treatment of head injury showed a small, but statistically insignificant 5% difference in outcomes favoring HS [80]. It is likely that the clinical benefit of HSD shown in trauma trials results from both the direct affect on lowering ICP as well as the indirect affect of improving arterial pressure and cerebral perfusion.

2.13 Risk of Increased Bleeding in Penetrating Trauma

Increasing blood pressure will logically increase bleeding from injuries in which hemostasis is not established. Animal models of uncontrolled hemorrhage typically demonstrate worse outcomes with aggressive resuscitation. This has suggested the concepts of limited or hypotensive resuscitation. The risk of how HSD might induce bleeding and affect mortality can be addressed by evaluation of the clinical trauma trials. In general, nearly half the patients had penetrating injuries. These data have recently been reviewed [63]. In brief, HSD was more efficacious in penetrating trauma than in blunt trauma. Lower mortality was significant in the first USA multicenter trial for patients with penetrating injury that required surgery [1] and the conclusion was further supported by an individual patient data meta-analysis with data from the US multicenter study [63]. It would appear that the overall benefit of HSD on early hemodynamics and immune function outweighs any deleterious effect on bleeding. This also suggests that most penetrating trauma patients do not have lesions similar to those induced by a fixed size aortotomy or tail transection. Animal models of uncontrolled hemorrhage may have limited value for predicting responses of most trauma patients with penetrating injury.

3.0 HEMOGLOBIN BASED OXYGEN CARRIERS

3.1 Introduction

Immense scientific and commercial efforts continue towards the development of a safe and effective synthetic oxygen carrying solution that could be used in place of blood or packed red blood cells (RBCs). The greatest progress has been in the development of modified hemoglobin solutions, commonly called hemoglobin based oxygen carriers (HBOCs). The goal has been to produce a safe and effective HBOC with the functionality of packed RBCs and without the significant limitations of blood, i.e. immune suppression, loss of efficacy with storage and risk of viral contaminants. Such a product would have a huge market for preoperative and critical care medicine as a replacement for the current blood supply. Further, the hope is that an easily storable product could be used effectively for prehospital and battlefield trauma where current fluid resuscitation strategies are lacking in efficacy.
Strategies for Small Volume Resuscitation

The complex challenge of developing an oxygen carrier and the relative availability and familiarity with plasma expanders has focused the development of RBC substitutes almost exclusively on their ability to load and unload oxygen. This is unfortunate because HBOCs have unique pharmacologic and physiologic properties in solution, which can impart unexpected effects on colloid osmotic pressure (COP), volume expansion as well as associated hemodynamic responses. Several recent reviews have focused on the oxygen carrier properties of RBC substitutes or on their clinical utility [107-109].

3.2 Utility of HBOCs

The clinical need and physical characteristic of HBOCs suggests two different roles: 1) correction of anemia and 2) resuscitation of hypovolemic blood loss. Formulations of free hemoglobin tetramers made-up to the concentration of blood (12-18-g/dl) or to packed-RBCs (20-25-g/dl) would be excessively hyperoncotic. Polymerization is a strategy used to increase Hb concentration, while minimizing increases in COP and the two HBOCs that have advanced the farthest in clinical trials are both glutaraldehyde polymerized hemoglobins made from human (Polyheme) and bovine blood (Hemopure) with COPs similar to healthy humans. While normal COP for humans is 28-mmHg, most surgical and anemic patients have some level of hemodilution and substantially lower COPs.

Hyperoncotic solutions can be effective for correction of hypovolemia as they are efficient volume expanders. However, packed RBCs are rarely administered to correct volume, but rather are used to correct anemia. Fresh whole blood is logically the ideal product for blood loss, but it is rarely used for resuscitation. Anemic patients are typically normovolemic or even hypervolemic, and thus, in order to deliver an effective load of Hb, a concentration higher than normal blood is needed. Packed red blood cells have a hemoglobin concentration of ~25 g/dl, normal whole blood is ~15 g/dl and all of the HBOCs under development are more dilute, 10-13 g/dl. Use of HBOCs to correct anemia has the potential to induce hypervolemia. Hypovolemia is often not well tolerated in patients with cardiac dysfunction attributable to heart disease or acute traumatic insult. To the extent that the HBOCs have a colloid osmotic pressure higher than patients plasma the in vivo concentration of hemoglobin after infusion can be further reduced.

The other potential role for a hyperoncotic HBOC is as a resuscitative fluid in patients with hemorrhagic shock in which hypovolemia and not anemia is the primary deficit. Standard of care treatment of hemorrhage and trauma is to administer asanguineous fluids, crystalloid or colloids. Resuscitation with asanguineous fluids can restore lost volume, increase cardiac output and oxygen delivery. However, the improvement in oxygen delivery is limited by the hemodilution. HBOCs would at first seem to be an ideal solution, as colloids they should be excellent volume expanders and they also can maintain or even correct hemodilution. For this review we will analyze clinical trial data and animal studies to assess the record and potential of HBOCs as a RBC substitute and as a resuscitative fluid. While traditional volume expanders cause some level of anemia, Hct levels as low as 25-30 are tolerated in most patients.
Table 3: Hemoglobin and Perfluorocarbon Based RBC Substitutes with Advanced Clinical Testing.

<table>
<thead>
<tr>
<th>Company</th>
<th>HBOC name</th>
<th>Source</th>
<th>Clinical Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northfield Labs</td>
<td>Polyheme</td>
<td>Human RBC</td>
<td>Phase II-III, intraop and prehospital trauma</td>
</tr>
<tr>
<td>Biopure</td>
<td>Hemopure</td>
<td>Bovine Hb</td>
<td>Phase II-III, orthopedic &amp; general surgery</td>
</tr>
<tr>
<td>Hemosol</td>
<td>Hemolink</td>
<td>Human RBC</td>
<td>Phase II, cardiothoracic surgery-trials</td>
</tr>
<tr>
<td>Curacyte, Inc</td>
<td>PHP-Hb</td>
<td>Human RBC</td>
<td>Phase II, sepsis, cancer</td>
</tr>
<tr>
<td>Sangart</td>
<td>Hemospan</td>
<td>Human RBC</td>
<td>Phase I completed, Phase II, elective surgery</td>
</tr>
</tbody>
</table>

**Failed Products**

<table>
<thead>
<tr>
<th>Company</th>
<th>HBOC name</th>
<th>Source</th>
<th>Clinical Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter</td>
<td>HemAssist†</td>
<td>Human RBC</td>
<td>Phase III, adverse outcomes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ mortality in trauma</td>
</tr>
<tr>
<td>Somatogen</td>
<td>Optro†</td>
<td>Recombinant Hb</td>
<td>Phase II, cardiac surgery excessive vasoconstriction, poor clinical results</td>
</tr>
</tbody>
</table>

† Development cancelled or trials stopped due to adverse outcomes.

Physical and chemical properties of RBC substitutes

<table>
<thead>
<tr>
<th>HBOC</th>
<th>Chemistry</th>
<th>conc. g/dl</th>
<th>COP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyheme</td>
<td>Pyridoxylated tetramers and glutaraldehyde-polymerized human</td>
<td>10</td>
<td>~28</td>
</tr>
<tr>
<td>Hemopure</td>
<td>Glutaraldehyde- polymerized bovine</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Hemolink</td>
<td>α-Raffinose-polymerized human</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>PHP-Hb</td>
<td>Pyridoxylated tetramers conjugated with polyoxyethylene</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>Hemospan</td>
<td>Tetramers conjugated with PEG</td>
<td>4.4</td>
<td>46</td>
</tr>
<tr>
<td>HemAssist</td>
<td>Diaspirin Cross-linked tetramer</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>Optro</td>
<td>Cross-linked by generic mutation</td>
<td>5</td>
<td>≈ 15</td>
</tr>
</tbody>
</table>

3.3 Products in development

Table 3 lists most of the HBOCs that are or have been in clinical trials as part of the US Food and Drug Administration’s (FDA) regulatory process. Perhaps the most extensively studied and financed, HBOC was HemAssist™ or diaspirin cross-linked hemoglobin (DCLHb), which dramatically failed in trauma trials. Over one hundred animal studies and several trials in volunteers and elective surgery patients suggested that DCLHb had acceptable safety and efficacy. However, when used as early emergency room treatment of severely traumatized patients a significantly increased mortality was observed [110, 111]. Subsequent animal studies which mimicked severe trauma and hemorrhage also showed an increase in mortality with DCLHb vs packed RBCs, particularly when DCLHb was infused along with large volume crystalloid infusions [112-114]. The take home message may be that most animal models and even clinical trials do not have the sensitivity to fully evaluate safety or efficacy of HBOCs in severely injured patients. Prehospital or emergency room use of HBOC may be more challenging than intraoperative use where skilled anesthesiologists pharmacologically titrate infusion rate and administer drugs to prevent extreme hemodynamic alterations.
### Table 4: Selected HBOC Trials.

<table>
<thead>
<tr>
<th>Trial subjects</th>
<th>Transfusions patients (n=)</th>
<th>Physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBOC</td>
<td>Cont.</td>
</tr>
<tr>
<td><strong>Hemopure (Biopure)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>orthoped. Surgery[115]</td>
<td>350 RBC (338)</td>
<td>1.4u</td>
</tr>
<tr>
<td>cardiac surgery[116]</td>
<td>49 RBC, (49)</td>
<td>1.7 u</td>
</tr>
<tr>
<td>surgery patients[117]</td>
<td>42 LR, (26)</td>
<td>3.3 u</td>
</tr>
<tr>
<td>aortic repair[118]</td>
<td>48 RBC, (24)</td>
<td></td>
</tr>
<tr>
<td>preop hemodilut. [119]</td>
<td>12 hespan (12)</td>
<td></td>
</tr>
<tr>
<td>preop hemodilut. [120]</td>
<td>6 hespan (6)</td>
<td></td>
</tr>
<tr>
<td>exercise volunteers[121]</td>
<td>6 RBC (6)</td>
<td></td>
</tr>
<tr>
<td><strong>Hemolink (Hemosol)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>autologous donation[122]</td>
<td>149 pstarch (150)</td>
<td>0.3u</td>
</tr>
<tr>
<td>cardiac surgery[123]</td>
<td>30 pstarch (30)</td>
<td></td>
</tr>
<tr>
<td>cardiac surgery[124]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polyheme (Northfield)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trauma surgery</td>
<td>171 historical</td>
<td></td>
</tr>
<tr>
<td>trauma surgery[125]</td>
<td>21 RBC (23)</td>
<td>7.8u</td>
</tr>
<tr>
<td><strong>Hemassist (Baxter)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>major surgery[126]</td>
<td>92 RBC (89)</td>
<td></td>
</tr>
<tr>
<td>prehospital trauma[127]</td>
<td>58 RBC (63)</td>
<td>3.1L</td>
</tr>
<tr>
<td>major surgery[128]</td>
<td>12 RBC (12)</td>
<td>similar</td>
</tr>
<tr>
<td>cardiac surgery[129]</td>
<td>104 RBC (105)</td>
<td></td>
</tr>
<tr>
<td>aortic surgery[130]</td>
<td>34 RBC (105)</td>
<td></td>
</tr>
<tr>
<td>ER trauma[111]</td>
<td>52 RBC (46)</td>
<td></td>
</tr>
<tr>
<td>stroke[131]</td>
<td>40 saline (46)</td>
<td></td>
</tr>
</tbody>
</table>

### 3.4 Clinical Trials

Table 4 lists selected clinical trials of Polyheme, Hemopure, Hemolink and HemAssist (DCLHb). The Hb substrate used by the different pharmaceutical companies comes from outdated human and bovine blood.
Northfield Laboratories’ Polyheme™ and Biopure’s Hemopure™, and Hemosol’s Hemolink have advanced to large scale FDA Phase 3 trials [132, 133]. However, research setbacks and disappointing trials have occurred more often than not. Diaspirin cross-linked hemoglobin (DCLHb) developed by the US Army and Baxter’s Hemoglobin Therapeutics is the best-studied RBC substitute. DCLHb’s development was cancelled after it exhibited a high mortality rate in trauma trials [110]. Somatogen cancelled development of Optro after cardiac surgery trials of its product produced adverse events. Hemosol’s phase III trial of Hemolink has been halted to evaluate an imbalance in adverse events. Northfield and Biopure continue with their phase III clinical research. Prehospital trauma trials have just started for Polyheme and are planned for Hemorpure. The FDA halted Biopure’s US clinical trials in 2003 pending some key animal experiments to address specific questions. Because of the dramatic failures in safety issues the FDA is likely to be cautious and conservative before granting marketing approval to a RBC substitute. A commercially available blood substitute will likely not be available in the next two years.

3.5 Plasma Volume Expansion

Fischer et al compared plasma volume expansion (ΔPV) after a 30-min infusion of 20 mL/kg 6% DCLHb, iso-oncotic 7.8% human albumin versus 60 mL/kg of LR in conscious sheep under conditions of normovolemia and hemorrhagic hypovolemia [134]. The ΔPV for DCLHb calculated from Evans blue indicator dilution and Hct dilution was nearly 2x greater than for albumin. The relatively increased expansion of 10% DCLHb versus 7.8% human albumin is quite surprising as the albumin was made-up to be an iso-oncotic control to the DCLHb. The explanation for the enhanced volume expansion of DCLHb is unknown, but several mechanisms can be hypothesized. PV enhancement could be due to a reduction in capillary pressure due to arteriolar vasoconstriction. Alternatively, increased lymphatic pumping could return interstitial protein into the circulation and augment the plasma colloid osmotic pressure and expansion. Indeed, Fischer et al. did report an increased plasma protein concentration, increased total vascular plasma protein and increased COP in the DCLHb group despite the albumin and DCLHb being matched for volume infused and colloid osmotic pressure [134].

Oxyglobin is a FDA approved veterinary product made from bovine hemoglobin (Biopure) but has a higher colloid osmotic pressure (~40 mmHg) than the human product, Hemopure. Oxyglobin was also found to be a potent volume expander increasing blood volume more than hespan in hemorrhaged rabbits. There is little data in the literature that we are aware of on the volume expansion effects of Hemopure, Polyheme or Hemolink. No direct comparisons have been made with the products under clinical evaluation of volume expansion, Table 3.

3.6 Relationships between HBOC Volume Expansion and Cardiac Output

The goal of volume expansion is almost always to increase venous return and cardiac output (CO). Reports of HBOC infusion are shown to have no effect or cause only a modest increase or an actual decrease in CO [134, 135]. Cardiac output could be reduced by the increase in left and right heart afterload known to occur due to vasoconstriction. Binding or scavenging of nitric oxide (NO) by interstitial hemoglobin blocks the normal basal level of vascular dilation due to NO diffusion from the endothelial cell to smooth muscle. It is hypothesized that polymerized HBOCs cause less vasoconstriction than the tetramer HBOC due to reduced vascular leakage into the interstitium [136]. Figure 3 shows ΔCO plotted versus right arterial pressure for LR, albumin and DCLHb as calculated from data of Fischer et al. and Brauer et al [134, 137]. Data suggest an altered starling filling pressure cardiac output curve with DCLHb. A suggested hypothesis is that the HBOCs do not increase CO because of the greater O₂ delivery. However, this is not satisfying, because all other volume expanders increase O₂ delivery and O₂ therapy alone does not reduce CO. Vane et al. found some
deaths in animals treated with DCLHb after large volume LR treatment of hemorrhage in an anesthetized model of a major abdominal surgical procedure [138]. These authors concluded that the combination of vasoconstriction, hypervolemia and cardiac depression likely contributed to the poor outcomes. These data suggest that some level of cardiac dysfunction or impairment can occur with some HBOCs. Human volunteer and patient data comparing how infusion of HBOCs and traditional plasma expanders alter CO, right atrial pressure and blood volume are not available. However, depressed CO has been reported in several clinical trials of both tetramer HBOCs and polymerized HBOCs [116, 119-121, 130].

3.7 HBOCs as RBC Substitutes

Animal models demonstrating effectiveness of HBOCs often focus on its ability to deliver oxygen in exchange transfusion or with infusion in normovolemia “top loading.” There are few clinically relevant animal models in which the HBOCs have been compared with RBC transfusion. On the other hand, several clinical trials have evaluated this. In general, such trials suggest a moderate reduction in blood needs in the first 24-hrs that diminish, approaching insignificance over 7-days. Table 4 shows that HBOC typically reduced transfusion volume per patient by about 20-50% and they only eliminate transfusion in 15-30% of patients compared with control groups. The apparent reason for this modest sparing of transfusion requirements is the short half-life of the HBOCs, typically 10-20 hrs versus the long half-life of several days for RBCs. None of the present HBOCs are likely to provide a more than a partial solution to blood replacement. Notthfield sponsored a single group study showing that Polyheme was tolerated in 171 patients with clinical outcomes better than historical controls of patients who refused blood [139]. Interpretation of
results versus such a control group is difficult, particularly in light of an earlier two-armed Polyheme versus RBC trial where the HBOC only reduced RBC transfusion volume needs by 31%. It is most likely that HBOCs may provide a bridge or early treatment, but do not appear to replace transfusion. The disappointing intraoperative trial results is perhaps the main reason that the two leading HBOC companies now focus on prehospital resuscitation of trauma.

3.8 HBOCs as Resuscitative Fluids

HBOCs or oxygen carrying plasma expanders have the potential to be an effective resuscitation solution. Their long shelf life and lack of cross-typing requirements make them attractive as a prehospital fluid. Additionally, substantial oncotic pressure and volume expansion of the HBOCs makes them attractive for the treatment of hypovolemia. Asanguineous fluids expand vascular volume, increase cardiac output, but dilute RBCs and oxygen content. However, increased cardiac output may effectively increase oxygen delivery several fold from depressed levels associated with shock to normal or even supranormal levels. Augmentation of supranormal levels of CO with fluid resuscitation often occurs without full restoration of blood pressure presumably due to lowered viscosity and widespread vasodilation from local autoregulatory mechanisms. Unfortunately, many, if not all HBOCs appear to impair CO enough such that DO$_2$ is not increased above that reported for conventional volume expanders. Recent comparison of the use of Polyheme compared to Hextend in hemorrhaged anesthetized swine and conscious rats when both solutions were infused to maintain a systolic blood pressure to $\approx 70$ mmHg for limited ‘hypotensive’ resuscitation showed no oxygenation or hemodynamic advantage (rat, pig) and an increased mortality (rat) [140, 141]. It may be that the oxygen carrying plasma expanders offer minimal advantage in limited resuscitation regimens due to the small dose of Hb administered.

Similar conclusions on ineffectiveness of HBOCs in small volumes can be reached studying the combination of hypertonic 7.5% saline plus HBOC (HSHb). Such mixtures were suggested as an improvement over a small increase in oxygen delivery attributable to replacing dextran with an HBOC and assuming cardiac output is increased equally. However, an analysis of experimental data suggests that HSD [142] is almost as effective as HSHb [143] assuming equivalent CO augmentation. Results would be worse for HS-Hb if plasma hemoglobin induced depression of CO.

3.9 New Formulations

A novel approach to HBOC development has been the development of a counterintuitive formulation of polyethylene glycol-modified human hemoglobin (MalPEG-Hb). MalPEG-Hb is an anemic (4-g/dl), viscous, hyperoncotic formulation with a P50 of 5.5 mmHg. Data suggests that the free Hb in plasma unloads oxygen more efficiently compared to RBC Hb due to the removal of the microcirculatory spatial heterogeneity imposed by cellular Hb [144-146]. Enhanced O$_2$ unloading might increase arteriolar O$_2$ tension and induce arteriolar vasoconstriction [147-149]. This opposes the conventional well-researched view that Hb’s affinity for nitric oxide (NO) is responsible for the vasoconstriction. [150] In theory the elevated O$_2$ affinity (low P50) of MalPEG-Hb delays the early release and prevents vasoconstriction. Further, vasodilation may be induced by MalPEG-Hb’s high viscosity increasing blood-endothelial sheer forces and thus enhancing NO release. [151]

The vision is that MalPEG-Hb’s other unique features might increase its effectiveness enough to compensate for its diluted concentration. Data of microcirculatory function in a skin window suggests enhanced O$_2$ delivery [152], but such enhancement may not take place in more critical tissue with higher O$_2$ demands and life sustaining function. Still the concept that a hemoglobin solution with high oncotic pressures and high viscosity has enhanced efficacy is intriguing and deserves evaluation in clinically relevant models.
3.10 HBOCs as Small Volume Formulations

A series of experiments sponsored by the US Air Force evaluated Hemopure as a small volume formulation for hypotensive resuscitation [153-155]. When infused to a hypotensive target pressure (60mmHg) the volume sparing of an HBOC can be profound due to induced vasoconstriction, as these investigators demonstrated. Outcomes measured in an anesthetized swine model were better or equal to small volume Hemopure compared with large volume lactated Ringer’s or HSD [155]. However, acute doses of LR and HSD studied were exceedingly high (19+ liters of LR and 1500 mL of HSD). Thus, control animals were over resuscitated and 1500 mL of HSD is 6x the recommended dose and probably toxic. Hemopure caused a notable reduction in cardiac output and venous oxygenation. One interpretation is that enhanced HBOC oxygen unloading and reduced venous oxygenation are evidence of enhanced tissue delivery of oxygen. However, the traditional view is that cardiac output and oxygen delivery (DO₂) were insufficient and lower tissue oxygenation occurs in at least some tissues. Recently, Polyheme was provided to the US Army for independent animal testing using hypotensive resuscitation models in three laboratories. Polyheme did not increase DO2 more than Hextend in hemorrhaged swine [156], nor did it improve mortality versus Hextend in swine and rats [157, 158]. It may be that small volume or limited resuscitation with HBOCs is ineffective due to the limited dose. Resuscitation to hypotensive targets can reduce HBOC volume needs compared to Hextend due to HBOC vasoconstrictor activity, but there was no apparent advantage in survival or physiology when compared with Hextend formulations. Most HBOCs appear to cause some level of vasoconstriction and depression of cardiac output. Polyheme and Hemospan (MalPEG-Hb) may be the least vasoconstrictive agents. Until recently there was almost no preclinical data on Polyheme in the literature. Recently, the US Army sponsored studies in swine models demonstrating that Polyheme also causes systemic vasoconstriction and depressed CO [156, 157].

3.11 HBOC Conclusion and Recommendations

Hemoglobin based oxygen carriers are potent plasma expanders with a modest vascular half-life. Both properties may be a limitation for use as a blood substitute, but may have utility and advantages as an acute resuscitative fluid. The limited amount of independent experience with the HBOC solutions currently under development makes conclusions difficult.

Infusion regimens for HBOCs will likely be different than for packed RBCs or asanguineous fluids due to the unique physical properties and physiological effects of HBOCs. At present it is not clear if such solutions will offer an improvement in standard of care. Safe and effective oxygen carrying plasma expander remains an attractive goal. It is likely that effective Hb molecular structure, optimal concentrations, and carrier solutions will be developed. Such development and clinical utility will take substantial preclinical and clinical study to define the safety and efficacy and the optimal therapeutic regimens of such formulations.

4.0 TITRATED CLOSED-LOOP RESUSCITATION

One approach to reducing volume needs may be to provide automated computer controlled fluid resuscitation, which can be tailored to individual patient needs and frees up clinical personnel. Severe hemorrhagic hypotension must be quickly addressed and corrected to prevent cardiac arrest, ischemic injury and organ dysfunction. On the other hand, the ideal system would eliminate wasteful and excessively rapid resuscitation that could be deleterious. The rationale is that rapid increases in blood pressure can lead to additional bleeding. A method to accurately guide and control fluid resuscitation of hemorrhage could improve outcomes by reducing incidences of both excessive and inadequate resuscitation.
Endpoint resuscitation occurs when fluid therapy is titrated proportional to the measured values of a specific physiological variable or endpoint. The use of endpoint resuscitation has largely been restricted to the intensive care unit and operating room environments where continuous monitoring and staffing allow careful titration of therapy to a target variable level or range. With the development of new portable monitoring technologies and computer-controlled infusion pumps, automated “closed-loop” titrated endpoint resuscitation may be feasible for prehospital and emergency room use. Fred Pearce of Walter Reed has historically been an advocate of early closed-loop control for combat casualty care. An effective "Resuscitation System" would need to fulfill several requirements. We have been using automated resuscitation systems both to facilitate our research, but also as a means to test the concept of closed-loop fluid therapy in hemorrhage and burns [159-162]. The first report of closed-control of fluid therapy we are aware of is that of Bowman and Westenskow who built and tested, in dogs and patients, a system that provided microprocessor-controlled fluid resuscitation of burn shock using urinary output as an endpoint [163]. Kramer et al. have designed and tested a similar system in sheep [162] and have begun evaluating a fluid balance monitor in burn-injured patients as a first step in doing closed-loop clinical trials. Burn injury is one scenario where excessive fluid therapy has become common [164] and a system of tightly controlling fluid therapy to achieve, but not exceeding urinary output targets may ultimately reduce morbidity of fluid overload. Such a fluid therapy system lends itself to initial care through enroute care and the first 24 – 28 hours. Burn resuscitation is a relatively slow process that occurs over many hours to days.

Hemorrhagic shock typically provides a more acute life threatening challenge than burn injury. In hemorrhage, fluid therapy is needed in a manner of minutes and stabilization must occur in a manner of a few hours or less. Urinary output is not a useful endpoint for acute resuscitation of hemorrhage. In order to perform initial closed-loop resuscitation of hemorrhage, measurement of rapidly responsive endpoints (arterial pressure, cardiac output or skeletal muscle oxygenation) have been evaluated [159-161].

Resuscitation System prototypes have used a LabView controller with preprogrammed algorithms that convert the value of an endpoint variable into a specific infusion rate. We suggest that such algorithms, which define infusion rate as a function of an endpoint variable, may not be optimized by a linear relationship. Thus, we designed non-linear decision table algorithms that infuse fluid quickly when the endpoint variable is low near an a priori defined ‘critical level’, but then greatly reduce infusion rate as the defined “stable level” was approached [159]. Such a system can be designed to provide different algorithms for different clinical scenarios. For example, with penetrating injury hypotensive resuscitation might be optimal to reduce risk of rebleeding, while with head injury normotensive resuscitation would likely be needed since periods of hypotension increase morbidity and mortality with head trauma. Further, different endpoints and different targets might be used to provide initial care, e.g., blood pressure versus sustained care in which lactate and urinary output might be more useful indices.

A secondary goal of such an approach is to reduce fluid volumes required for combat casualty care. This approach did appear to reduce the extent of rebleeding when compared against aggressive fluid therapy such as has been shown to increase bleeding and death in sheep and swine models of uncontrolled aortic bleeding.[161, 165]

However, much research and development remains to determine if such closed-loop resuscitation has real clinical applicability or if it will remain a laboratory tool.
5.0 CONCLUSION

5.1 Different Strategies for Small Volume Resuscitation

Different strategies for small volume resuscitation include making volume expansion more efficient (hyperosmotic-hyperoncotic formulations) adding oxygen carrying capacity (HBOCs) or using titrated resuscitation regimens. Small volume resuscitation regimens could be particularly useful to address the logistic limitations of combat casualty care. At present the only approach that has a proven clinical record and product approval is hypertonic saline mixed with dextran or hetastarch. Military medial use and evaluation in NATO countries with product approval is encouraged.

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Strategies for Small Volume Resuscitation


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Strategies for Small Volume Resuscitation


Strategies for Small Volume Resuscitation


Strategies for Small Volume Resuscitation


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Potential Resuscitation Strategies for Treatment of Hemorrhagic Shock

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ABSTRACT

Exsanguination is the major cause of death on the battlefield. Of those who die on the battlefield, it is estimated that 20% could be salvaged before exsanguination if provided with immediate care. Upon arrival at the scene, a First Responder must immediately control bleeding. If the injury is on the body surface or extremity and compressible, direct pressure or a tourniquet is current standard treatment for attempting adequate hemostasis. Ideally, a hemostatic dressing would circumvent the tourniquet by staunching severe bleeding, and require no further attention by the medic. For suspected non-compressible bleeding, for which there is currently no adequate treatment, the ideal would be an intravenous resuscitation solution containing a substance that enhances clotting or clot stability only at the bleeding sites. Once bleeding is controlled, the next step is to resuscitate the patient. In the battlefield, if hemostasis is not assured, aggressive resuscitation may dislodge the clot and exacerbate bleeding; aggressive resuscitation also requires large volumes of fluid, presenting a logistical difficulty. An improved strategy would resuscitate only to the point at which survival was assured and would not cause further bleeding even during the predicted prolonged evacuations that may occur in an urban warfare environment. This article gives an overview of recent work using a severe hemorrhagic shock animal model with an arterial injury on 1) the point at which blood pressure dislodges the thrombus (the “pop-clot” pressure); 2) an injectable clot stabilizer (“fix-a-leak”) that is a naturally occurring factor in the clotting cascade (human recombinant Factor VIIa); and 3) the maximum time up to 24 hours for hypotensive resuscitation below the “pop-the-clot” pressure (“how low for how long”).

1.0 INTRODUCTION

The concept that early, aggressive high volume resuscitation is critical to the optimal treatment of hemorrhagic shock was widely accepted and practiced during the Vietnam War [1]. Subsequently, the practice of large volume crystalloid resuscitation became the standard of care for civilian trauma patients [2]. The foundation of this practice rests on the controlled hemorrhage studies conducted in the late 1960s and 1970s by Shires et al [3,4]. The metabolic benefit of fluid resuscitation was definitively demonstrated in controlled hemorrhage animal models and then implemented in patients suffering uncontrolled hemorrhagic shock [3].

1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

However, numerous animal studies of uncontrolled hemorrhage have shown that there is increased blood loss following resuscitation induced by injury to blood vessels or organs [5-18]. Recent randomized clinical studies [19-21] and a review of data collected during WWI [22] and WWII [23] similarly question the prudence of aggressive resuscitation in patients prior to hemorrhage control. While the metabolic benefits of fluid resuscitation have long been recognized [24-26], these benefits must be balanced against the deleterious effects of rebleeding. It is therefore essential to determine if there is a reproducible point at which rebleeding occurs. The optimal endpoint of resuscitation in patients with truncal injury without definitive hemorrhage control might then be just below this rebleeding point.

Our laboratory has developed an animal uncontrolled hemorrhage model that uses an injury in the aorta that closely approximates a severe arterial hemorrhage potentially encountered in the military setting. A round hole is made in the aorta with a skin biopsy punch that creates a wound profile of a ballistic or shrapnel fragment injury with actual loss of a piece of arterial wall. Using small punches, we can create an injury that spontaneously clots but, if rebleeding occurs, the additional hemorrhage will likely be fatal. Using large punches, we can create an injury that causes the animal to exsanguinate unless the hemostatic agent that we are testing is effective. Using large punches, we can create an injury that causes the animal to exsanguinate unless the hemostatic agent that we are testing is effective. In another paper in this issue, Kheirabadi et al. describe how this model has been modified to test dressings that would be effective with accessible compressible injuries. For this paper, we will focus on reducing bleeding in non-compressible injuries.

2.0 POP THE CLOT PRESSURE

The first study explored the possibility of a reproducible point of rebleeding, or a “pop-the-clot” pressure. In catheterized 40 kg anesthetized pigs in the supine position, through a midline incision in the abdomen, an initial aortotomy made with a 2 mm skin biopsy punch (Fig. 1), simulated an injury to the aorta, and was allowed to clot. The unique feature of this experimental design is that all bleeding can be quantified because we have suction tubes in the abdomen. The aorta bleeds as it would in a real injury with the intestines over the aorta and injury. When the blood drains to the sides, the suction tubes take the blood into canisters that are on a balance and the weight of blood in the canister is recorded on a computer instantaneously. Figure 2 shows the blood pressure tracing in a representative experiment for the two-hour experimental period with simultaneous measurement of resuscitation volume and hemorrhage volume.

To simulate varying times of arrival at the scene, we delayed aggressive resuscitation to 5, 15, or 30 minutes after the initial bleeding. We used two rates of resuscitation, 100 and 300 ml/min, with warmed lactated Ringer’s solution (6 or 7 animals per group). The 300 ml/min rate is approximately that which can be delivered on the battlefield through a large-bore (≤ 16 ga) catheter using a manually inflated pressure bag. The resuscitation was continued until the clot was dislodged and the aortotomy rebled. The blood pressure at the point when blood appeared in the suction canister is designated as the rebleed pressure. We thought that, if the clot gained strength as it matured, we would find that the rebleed pressure would increase with time, or that the higher rate of resuscitation would cause rebleeding sooner. Instead, as can be seen in Table 1, there were no systematic significant changes, regardless of delay or rate of infusion. The rebleed blood pressure, averaged over all the groups, proved to be a reproducible mean arterial pressure (MAP) of 64 ± 2, a systolic pressure of 94 ± 3, and a diastolic pressure of 45 ± 2 mmHg [27].
Figure 1: Abdominal aortotomy. A large central clot (white arrow) forms over the aorta to stop the initial bleeding. The majority of the blood has been suctioned into canisters and is not important in the initial hemostasis at the site of the clot (A). Postmortem, aortotomy size (arrow) is verified by exposing the site by clot removal (B). Interestingly, the gel clot (A) appeared to be the same even after rebleeding, so apparently, the clot was loosened with resuscitation, but not lost.

Figure 2. Blood pressure tracing over two-hours measuring resuscitation volume and hemorrhage volume. Resuscitation was discontinued at rebleed. Baseline MAP was taken. Intestines were retracted and aorta exposed. This caused variable changes in the MAP reflected by a transient drop and recovery of the MAP prior to the aortotomy at time 0. The first red line denotes the aortotomy hemorrhage volume and spontaneous clotting at 5 minutes. Blood pressure spontaneously recovered to near stable value below baseline. Resuscitation at 30 minutes, resuscitation began with warmed LR (blue line). Rebleed MAP was determined by appearance of blood in canister after resuscitation (second red line). Mean arterial pressure=MAP; lactated Ringer’s solution = LR. Red line=instantaneous hemorrhage volume; blue line=resuscitation volume; black line=MAP
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Table 1: Mean arterial pressure at which rebleeding occurred in response to resuscitation with warmed lactated Ringer’s solution at a rate of either 100 or 300 ml/min. The resuscitation was delayed 5, 15, or 30 minutes from the end of the initial hemorrhage.

<table>
<thead>
<tr>
<th>Delay (minutes)</th>
<th>Rate (ml/min)</th>
<th>100 (mmHg)</th>
<th>300 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>100</td>
<td>70 ± 5</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>57 ± 6</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>63 ± 6</td>
<td>72 ± 4</td>
</tr>
</tbody>
</table>

3.0 CONTINUE OR STOP RESUSCITATION AFTER REBLEED POINT

Although the primary question in the rebleed study was to determine whether there is a reproducible rebleed pressure, another question that could be asked is what happens if resuscitation is either continued to return blood pressure to baseline levels, or if the resuscitation is stopped to minimize rebleeding. The animals were re-randomized into “continue” or “stop” resuscitation groups once they rebled. At that point, resuscitation (either at 100 or 300 ml/min rate) was either continued until the MAP returned to pre-hemorrhage baseline levels or was discontinued. In the continue group, the resuscitation pump was turned off when the pressure was at baseline, or was turned back on until baseline pressure was obtained. In the stop group, no further resuscitation was given once rebleeding occurred and the animals were observed until death or 2 hours. There was also a group of animals that received the aortotomy, but no resuscitation (negative control group).

As can be seen in Table 2, all three groups bled a similar volume from the initial aortotomy. In the continue resuscitation group, the hemorrhage continued and the rebleed hemorrhage volume was four times higher than the rebleed hemorrhage volume in animals in which blood pressure was not returned to baseline levels after rebleeding occurred. In addition, 5 times the volume of lactated Ringer’s was used in the continue group compared with the stop group. Despite the large amount of additional fluid received by the continue group, survival time was not significantly affected (Table 2).

Table 2: Initial hemorrhage volume, rebleed hemorrhage volume, volume of lactated Ringer’s (LR) administered, and survival times in the No resuscitation, continue and stop resuscitation groups. ** different from all other groups. * different from No resuscitation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Resuscitation (n=10)</td>
</tr>
<tr>
<td>Initial hemorrhage volume (ml/kg)</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Rebleed Hemorrhage Volume (ml/kg)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Volume of LR administered (ml/kg)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Survival time (minutes)</td>
<td>95 ± 13</td>
</tr>
</tbody>
</table>
Figure 3 shows the pattern of bleeding in representative experiments in the continue group. In some animals, although not all of them, rebleeding continued as long as resuscitation was continued. Contrast this with Figure 2 in which rebleeding stopped as soon as the resuscitation was discontinued. However, the blood pressure remained well below baseline values. Despite the fact that the continue group received far more fluid than the stop group, survival was not improved (Table 2). Part of the reduced survival was a result of the design of the study in which aggressive resuscitation was used throughout and resulted in a very low hematocrit that in and of itself resulted in death. To prevent this blood products are given as soon as possible in the emergency department. Only crystalloids and colloids are currently available on the battlefield, however, so we limited the choice of fluids to make the study relevant to the far-forward scenario.

![Graph showing bleeding pattern](image)

Figure 3. Three representative experiments of continued resuscitation after rebleed point. All three animals spontaneously recovered blood pressure following initial hemorrhage. After rebleeding, blood pressure fell and resuscitation continued for as long as blood pressure remained below baseline. Rebleeding continued as resuscitation continued if blood pressure did not return to baseline (top 2 panels). This amount of resuscitation caused hematocrit to fall, and all resuscitation was stopped when the hematocrit reached 10 percent. Animal #61 spontaneously recovered to point of spontaneous rebleeding (bottom panel). Resuscitation was begun and the baseline blood pressure obtained. Resuscitation caused a third incidence of rebleeding, that did not continue despite continued resuscitation. The animal survived the entire 2 hours, hematocrit > 10 percent. Mean arterial pressure=MAP; lactated Ringer’s solution = LR. Red line=instantaneous hemorrhage volume; blue line=resuscitation volume; black line=MAP

The interesting result was that there was no worse or even slightly improved survival in the animals that received no resuscitation at all – and that had no additional loss of blood. This suggests that even a small amount of rebleeding was associated with decreased survival. However, the lack of any resuscitation also resulted in less than a two-hour survival for the majority of the animals. These results agree with those from
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studies that investigated an arbitrary partial resuscitation with either a given volume [7] or to an arbitrary blood pressure [12]; these demonstrated improved survival with limited resuscitation.

The finding of a reproducible rebleeding point suggests two strategies. One is to administer something that will stabilize the clot so that resuscitation to the baseline can be achieved while preventing further rebleeding. The other is to resuscitate to blood pressures below the rebleeding point. In the modern battlefield scenario, prolonged evacuation may occur due to wide dispersal of troops. Since others have demonstrated short term (up to 4 hours) benefits of hypotensive resuscitation, the remaining question is whether hypotensive resuscitation with fluids currently used by the combat medic can sustain a subject for as long as 24h at a blood pressure less than the rebleeding point. If hypotensive resuscitation is not beneficial for as long as 24h, then it becomes even more important to: 1) stabilize the clot so that a higher pressure can be obtained safely, or, 2) develop better resuscitation solutions that will increase survival in a prolonged hypotensive state.

4.0 STABILIZATION OF THE CLOT

Recombinant activated Factor VII (rFVIIa) is an FDA approved drug commonly utilized for the treatment of patients with hemophilia [28,29]. Attention has turned to its potential effectiveness in reducing bleeding in traumatic hemorrhage. Recent trauma studies have demonstrated its effectiveness in decreasing blood loss in models of hypothermic coagulopathic swine with Grade V liver injuries [30,31]. A growing body of literature documents its successful use in surgical and trauma patients with the acquired coagulopathy of trauma [32-34]. The purpose of this study was therefore to determine whether administration of rFVIIa to a pig—with normal coagulation and an uncontrolled hemorrhage—would enhance clot stability and increase rebleeding MAP in response to resuscitation.

In these experiments, the animal was prepared in a similar manner to the “pop-the-clot” study described above [27]. Five minutes before the aortotomy was made, an intravenous injection of either vehicle control, low dose (180 µg/kg) or high dose (720 µg/kg) rFVIIa was given. Five minutes after the injection was completed, the intestines were retracted and a 2.0-mm hole was made in the infra-renal aorta with a disposable skin biopsy punch. Ten minutes after the hole was made, resuscitation at 100 ml/min with lactated Ringer’s solution (LR) at 37°C was begun. Rebleed pressure was determined by noting the blood pressure at the time blood appeared in the suction canister. If the MAP reached a plateau after 4 L of fluid were given without causing rebleeding, the LR pump was stopped and an infusion of epinephrine at 1.0 µg/kg/min, as needed, was given to raise MAP to as high as 200 mmHg. If no rebleeding occurred with this treatment, the animal was recorded as a non-rebleeder. The total volume of LR administered and the rebleed hemorrhage volume were recorded. Survival time up to two hours post aortotomy was recorded.

Pre-treatment with rFVIIa significantly increased the MAP at which rebleeding occurred during resuscitation of an uncontrolled hemorrhage from 53 ± 7 mmHg in the control group, to 71 ± 6 mmHg in the low dose group, and to 88 ± 17 mmHg in the high dose (p=0.05 between high dose and control). More resuscitation fluid volume (55 ± 12 ml/kg at the high dose) was given compared with the control (20 ± 9 ml/kg, p≤ 0.005) before rebleeding occurred. Resuscitation was given for a longer time (21 ± 5 min at the high dose) before rebleeding was induced compared with the control (8 ± 4 minutes, p≤0.005). There was a trend toward a reduced rebleed hemorrhage volume with rFVIIa, from 39 ± 9 ml/kg in control to 21 ± 7 ml/kg at the high dose, but it did not reach statistical significance (p=0.055). There was no reduction in the initial hemorrhage volume among the groups (22 ± 2, 20 ± 3, and 19 ± 2 ml/kg in the control, low, and high dose groups, respectively), despite high levels of circulating rFVIIa.
Although the reduction of rebleed hemorrhage volume with rFVIIa treatment did not reach statistical significance, there was a significant metabolic consequence from the increased blood loss in the control group leading to an elevated plasma lactate concentration compared with the 180 and 720 µg/kg groups and a trend toward a more negative base excess in the control group compared with the 180 and 720 µg/kg groups. The change in the arterial base excess was not due to changes in the ventilation since the animals were on a ventilator. Although not significant, the control group showed a trend toward a shorter survival time than the low and high dose FVII groups (73 ± 11, 87 ± 11, and 95 ± 11 min, respectively, p=0.238).

A very interesting pattern emerged among the groups and this pattern is depicted in the representative experiments shown in Figure 4 above. In this model, the usual finding was that, once the thrombus has been disrupted, bleeding continued for as long as resuscitation was administered, as occurred in 70% of the animals.

Figure 4. Control, low and high dose rFVIIa experiments: intermittent rebleeding with rFVIIa despite continuing resuscitation. Mean arterial pressure=MAP; lactated Ringer’s solution = LR. Red line= instantaneous hemorrhage volume; blue line= resuscitation volume; black line= MAP
in the control group (Top panel, Fig. 4 and in the pop-the-clot animals, Fig. 3). Although there was rebleeding in the groups that received rFVIIa, this bleeding stopped, at least for a short time, in 100% and 88% of the low and high dose animals, respectively, despite continued resuscitation (middle and bottom panels, Fig. 4).

Interestingly, rFVIIa pretreatment in the current study provided no hemostatic benefit in reducing the initial hemorrhage volume. This may indicate that the presence of rFVIIa has no measurable effect when the blood flows are high as they are in the pigs at baseline. Similar results were obtained by Schreiber et al, who treated pigs with rFVIIa 30 seconds after the induction of the liver injury [31] and began resuscitation 15 minutes after the injury. At high blood flows in the normotensive subject, the shear forces may therefore prevent platelets and other factors from concentrating at the site of injury. During the hypotension following hemorrhage, platelets may be able to collect at the injured site and a rapid, full thrombin burst may help to form a more stable clot with a firm fibrin structure that can better resist dislodging when normal rates of flow are reestablished following resuscitation.

Promising preliminary results from a group in England suggest that rFVIIa has a significant effect on survival time and hemorrhage volume in their model of combined controlled-uncontrolled hemorrhage. The rFVIIa was given just before an aortotomy was made and resuscitation begun, during a short period between the end of the controlled hemorrhage and the start of the uncontrolled phase. The animals were then given either full resuscitation to Advanced Trauma Life Support (ATLS) standards or hypotensive resuscitation to a systolic pressure of 80 mmHg. The different resuscitation methods (complete vs. hypotensive) showed a tendency towards a beneficial effect for hypotensive resuscitation that was most pronounced when the rFVIIa was combined with hypotensive resuscitation (Wayne Sapsford, Defense Science and Technical Laboratories, Porton Down, UK, personal communication).

5.0 HYPOTENSIVE RESUSCITATION

As mentioned previously, hypotensive resuscitation to arbitrary endpoints has been shown to reduce bleeding in uncontrolled hemorrhage models, at least in the short-term. We are currently conducting experiments to determine if resuscitation to a systolic blood pressure of 80 mmHg can be sustained for 24 hours. We chose a systolic pressure of 80 mmHg because it is below the “pop-the-clot” systolic pressure of 94 mmHg; additionally, it is the pressure at which a radial pulse can be detected and is therefore an appropriate target achievable on the battlefield. We are comparing various fluids that are either FDA-approved, or are undergoing application to the FDA for approval, for their efficacy under conditions of this hypotensive resuscitation. The fluids are lactated Ringer’s solution, 6% hetastarch in a lactated Ringer’s base (Hextend™), and a hemoglobin-based oxygen carrier (Polyheme™). The questions we are asking are 1) whether there is rebleeding during the hypotensive period; 2) whether the animals can tolerate prolonged hypotension; and 3) which fluid provides the best metabolic support with the least volume. It is possible that the prolonged hypotension might cause some tissues to be relatively ischemic, so we are also taking samples to assess tissue function, oxidative, and nitritative states, and coagulation status. To see if there are changes in the synthesis of heretofore unknown metabolites, we are also performing genetic microarray analysis of the white blood cell response over the 24 h experimental course as is described in other papers found in this issue (Dubick and Bowman). At the end of the 24 h, we repair the aortotomy and then let the animal recover for an additional 2 days to ensure that multiple organ dysfunction does not develop. Based on acute studies and short-term clinical trials [19,20,35], the recommendations for hypotensive resuscitation has been promulgated for the special operations medics [36].
6.0 SUMMARY

The large animal, severe hemorrhagic shock models that we have been studying also allow us to investigate endpoints of resuscitation that are more sensitive than blood pressure. For example, in a series of non-resuscitated animals that bled different volumes in response to injury, three survival patterns emerged: those who survived for less than one hour, those who survived between 1 and 2 hours, and those who lived for the entire 2 hours (unpublished observations). Noninvasive and metabolic data from these experiments may yield new endpoints of resuscitation that may be early warning signs of impending circulatory collapse.

![Graph showing MAP in three groups of non-resuscitated pigs. Death < 1 hour = black line; Death at 1-2 hours = red line; Survival > 2 hours = green line. Early changes in arterial lactate and base excess may differentiate between survivors and non-survivors although blood pressures are similar.](image)

Figure 5. MAP in three groups of non-resuscitated pigs. Death < 1 hour = black line; Death at 1-2 hours = red line; Survival > 2 hours = green line. Early changes in arterial lactate and base excess may differentiate between survivors and non-survivors although blood pressures are similar.
Potential Resuscitation Strategies for Treatment of Hemorrhagic Shock

The normal response to severe hemorrhage when bleeding stops is for the blood pressure to spontaneously increase as the animal’s intrinsic compensatory mechanisms start to operate. As can be seen in Figure 5 above, those animals that did not start to increase their blood pressure within 10 minutes did not survive a full hour. The other two groups did compensate with similar increases in blood pressure, yet one group succumbed earlier than the other. By looking at other endpoints, we found that large changes in lactate and arterial base excess as early as 15 minutes after injury distinguish between those who die early and those who survive. Because of studies and results like these, we feel that strategies to develop rugged instruments and realistic decision assist algorithms can be developed to better help the medic perform triage in the far-forward environment.

7.0 REFERENCES


Hemodynamic Variables and Tissue Energetics during Resuscitation of Porcine Hemorrhagic Shock with Hextend® or Lactated Ringer’s Solution

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SUMMARY

There has been recent interest in resuscitation of hemorrhagic shock using new colloid solutions such as Hextend® (6% hydroxyethyl starch in balanced salt solution). We examined the effects of resuscitation of porcine hemorrhagic shock with Hextend® or lactated Ringer’s (LR) solution on hemodynamic parameters and tissue energetics. Anesthetized, instrumented pigs underwent hemorrhagic shock (35% total blood volume, 90 minutes) and were randomized to resuscitation with Hextend® 10 cc/kg (n=5), Hextend® 20 cc/kg (n=8), or LR 20 cc/kg (n=10) per step in four steps. Endpoints measured included invasive hemodynamics, near infrared (NIR) spectroscopic measures of tissue hemoglobin saturation (StO2) in stomach, liver, and hind limb, and in vivo nuclear magnetic resonance (NMR) measures of tissue phosphoenergetics of liver and hind limb.

limb. Both groups receiving Hextend® resuscitation demonstrated increased cardiac output and oxygen delivery (DO₂) compared to animals resuscitated with LR. Phosphoenergetics (hind limb phosphocreatine) improved more rapidly in Hextend® groups compared to animals receiving LR. There were no significant differences between groups with respect to StO₂ in hind limb or stomach. Hextend® resuscitation resulted in improved hemodynamics and tissue energetics secondary to improved filling pressures in this porcine model of controlled hemorrhagic shock. Equivalent hemodynamic improvement was achieved with Hextend® at 1/3 the volume of LR. This product may have significant application in austere environments where the volume of resuscitative fluid is limited due to mission constraints.

1.0 INTRODUCTION

The ideal fluid for volume expansion in both civilian and military trauma has been the topic of debate for several decades. In terms of outcome, the ideal fluid has not yet been identified. A number of recent expert panels have suggested various fluids in the setting of a paucity of randomized, prospective trials. Without an obvious optimal fluid, lactated Ringer’s (LR) has been the suggested resuscitation fluid for acute treatment of victims of trauma. However, this strategy has the potential disadvantages of immunosuppression and the need for administration of at least three times the volume of blood lost to provide intravascular volume expansion. For the military in a combat/field situation, the “cube” (or weight and volume) of the fluid is a significant consideration. For this reason, recent military planners have included colloid (Hextend®) as the resuscitation fluid of choice in far forward units. Hextend® is a preparation of hydroxyethylstarch in a physiologically balanced electrolyte solution. However, its effects on coagulation and effectiveness in restoration of intravascular volume are still controversial. Additionally, recent research into low-volume resuscitation has raised questions regarding the appropriate volume of fluid and whether vigorous restoration of intravascular volume actually risks furthering hemorrhage. As a result, low-volume fluid resuscitation has more recently been considered to avoid “popping the clot.”

Arguably, an endpoint for resuscitation after hemorrhage is restoration and normalization of tissue energetic levels, as prompt restoration of these levels would be optimal for survival of cells and tissues. Although direct measurement of mitochondrial energy production is not clinically available, measurements of tissue energetics can be assessed in the experimental setting. With use of nuclear magnetic resonance (NMR) spectroscopy, tissue energetics in the form of high-energy phosphates can be evaluated in real-time during experimental shock for a dynamic perspective during shock and resuscitation. It has been demonstrated that energy levels are highly conserved during ischemia, likely through use of alternate ATP-producing pathways and down-regulation of non-essential processes. Animal studies have suggested that organ failure from shock is coincident with energetic failure. However, a direct comparison of energetic levels in vivo between colloid and crystalloid resuscitation after hemorrhagic shock has not previously been reported.

This study was performed to evaluate tissue energetics during resuscitation from hemorrhagic shock with use of Hextend® as compared to LR in vivo and in real time with use of nuclear magnetic resonance (NMR) and near-infrared (NIR) spectroscopy. We hypothesized that the two fluids would be similar in their ability to restore post-shock tissue energetics.

2.0 METHODS

2.1 Animal Protocol:
This experimental protocol was approved by the University of Minnesota Animal Use Committee and was conducted in accordance with established guidelines of the treatment of laboratory animals. Thirty-two male Yorkshire-Landrace pigs (Fanning Farms, Howe, Indiana) weighing 13-20 kg were used for experimentation.
Each animal was maintained without food and with free access to water for 12 hours prior to the experiment. Animals were anesthetized with althesin and inhaled nitrous oxide as previously described.\textsuperscript{12-13}

The following devices were placed: Pulmonary artery catheter via the right internal jugular vein, 12 Fr venous bypass catheter in the inferior vena cava (IVC), cystostomy catheter in the bladder, and an arterial catheter in the right carotid artery. Near-infrared spectroscopy probes (Hutchinson Technology, Inc, Hutchinson, Minnesota) were placed directly on the liver at laparotomy, on the surface of the hind limb, and into the stomach via a modified nasogastric tube. Specially-constructed NMR surface coils were placed on the liver surface as well as on the hind limb.

Splenectomized and instrumented animals were randomized to receive either Hextend\textsuperscript{®} at 10 cc/kg, Hextend\textsuperscript{®} at 20 cc/kg, or LR at 20 cc/kg for resuscitation. The shock/resuscitation protocol is illustrated in Figure 1. Hemorrhagic shock was induced by a 35% bleed (estimated by weight) into a heparinized blood collection bag via IVC cannula. The animals remained in shock for 90 minutes at which time, following measurements, they received resuscitation with either Hextend\textsuperscript{®} or LR. Resuscitation was divided into four boluses using either Hextend\textsuperscript{®} at 10 cc/kg/bolus, Hextend\textsuperscript{®} at 20 cc/kg/bolus, or LR at 20 cc/kg/bolus, depending on pre-shock randomization. Hemodynamic, NMR, and NIR measurements were taken at baseline, during shock (every 30 minutes for 90 minutes), and after each fluid bolus. Surviving animals were euthanized at the end of the fourth resuscitative measurement.

\textbf{Figure 1:} Shock and resuscitation protocol

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1}
\end{figure}

2.2 Measurements:
An NIR reflectance probe was placed in the stomach, liver, and hind limb. Percent StO\textsubscript{2} is a measure of hemoglobin oxygen saturation of blood contained in the volume of tissue illuminated by near-infrared light. For each StO\textsubscript{2} measurement the multiple optical absorbance values were processed as previously described\textsuperscript{17}. In vivo \textsuperscript{31}P NMR spectra were recorded at 25.80 MHz in a 1.5T whole-body superconducting magnet (Magnex Scientific, Abingdon, UK) interfaced to an Apollo spectrometer (Tecmag Inc., Houston, USA) as described.\textsuperscript{12-13} Pulmonary artery catheter measurements of cardiac output (CO) were made via thermodilution and obtained at baseline and in synchrony with NMR measurements. These measurements were used to calculate oxygen delivery (DO\textsubscript{2}) and oxygen consumption (VO\textsubscript{2}) and indexed by animal weight in kilograms. Arterial and mixed venous blood gases as well as lactate, hemoglobin (Instrument Laboratories, Lexington, MA) and hemodynamic parameters were measured at baseline and in synchrony with the NMR measurements.
2.3 Analysis of NIR/NMR data:
NIRS measurements were expressed as percent oxyhemoglobin saturation (StO₂). NMR spectra were imported into ACD/Spec Manager Software (Advanced Chemistry Development Inc., Toronto, Ontario, Canada) to determine peak height and area. Tissue pH (pHi) was determined using the chemical shift of the inorganic phosphate (Pi) peak relative to phosphocreatine (PCr) peak as described. NMR measurements were expressed as area under the curve relative to total phosphorus and were normalized to baseline measurements.

2.4 Analysis of data:
Animals that survived the hemorrhagic shock protocol to receive resuscitation were used for analysis. No intent-to-treat analysis was performed. Three groups were compared: 1) animals resuscitated with LR at 20cc/kg/bolus 2) animals resuscitated with Hextend® at 10cc/kg/bolus (Hextend-10) 3) animals resuscitated with Hextend® at 20cc/kg/bolus (Hextend-20). Comparisons of hemodynamics, NIRS, and NMRS measurements were made between groups at baseline, after 30, 60, and 90 minutes of shock, and after each of four resuscitative steps. A one-way analysis of variance (ANOVA) with least squared post-hoc testing was performed to determine statistically significant differences between groups at each time point. A p-value of <0.05 defined significance.

3.0 RESULTS

Thirty-two animals were randomized to one of three resuscitative strategies (Figure 2).

Animals in the three groups were similar with respect to body weight, hemorrhage volume, baseline mean arterial pressure (MAP) and other hemodynamic parameters (Table 1a and b).

Table 1a: Baseline weights (in kilograms) and hemorrhage volumes (in cc/kg) for each group. Mean ± standard deviation. LR=lactated Ringer's group; H-10=Hextend-10 group; H-20=Hextend-20 group.

<table>
<thead>
<tr>
<th>Weight in kilograms</th>
<th>Volume hemorrhaged</th>
</tr>
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<tbody>
<tr>
<td>LR 15.8 (2.3)</td>
<td>H-10 17.1 (2.0)</td>
</tr>
<tr>
<td></td>
<td>H-20 17.8 (1.7)</td>
</tr>
<tr>
<td></td>
<td>LR 28.0 (5.1)</td>
</tr>
<tr>
<td></td>
<td>H-10 29.6 (2.8)</td>
</tr>
<tr>
<td></td>
<td>H-20 28.7 (6.6)</td>
</tr>
</tbody>
</table>

Figure 2: Total animals enrolled in protocol, surviving shock, and surviving to resuscitation
Table 1b: Hemodynamic variables between the three resuscitation groups. Mean ± standard deviation. (LR=lactated Ringer’s group resuscitated at 20 cc/kg; H-10=Hextend-10 group resuscitated at 10 cc/kg; H-20=Hextend-20 group resuscitated at 20 cc/kg; MAP = mean arterial pressure in mmHg; Hgb = hemoglobin in mg/dL; PCWP = pulmonary capillary wedge pressure in mmHg; DO₂ = oxygen delivery in cc/kg/minute; VO₂ = oxygen consumption in cc/kg/minute; lactate expressed in mmol/L).

<table>
<thead>
<tr>
<th></th>
<th>MAP</th>
<th>Hgb</th>
<th>PCWP</th>
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<tbody>
<tr>
<td></td>
<td>LR</td>
<td>H-10</td>
<td>H-20</td>
</tr>
<tr>
<td>Baseline</td>
<td>82.2±13.2</td>
<td>92.7±9.7</td>
<td>81.8±15.2</td>
</tr>
<tr>
<td>Shock-90</td>
<td>62.5±12.6</td>
<td>36.2±4.5</td>
<td>54.8±14.4</td>
</tr>
<tr>
<td>Resus-1</td>
<td>68.0±12.7</td>
<td>48.1±13.7</td>
<td>75.9±17.7</td>
</tr>
<tr>
<td>Resus-4</td>
<td>78.9±16.7</td>
<td>82.7±19.8</td>
<td>89.3±25.4</td>
</tr>
</tbody>
</table>

With hemorrhage, the cardiac output of all animals predictably dropped, and the Hextend-10 group dropped significantly lower than the other two groups at 60 minutes of shock (Figure 3). This drop was also reflected in a significantly lower hind limb tissue pH (pHi) for the Hextend-10 group at 60 minutes of shock (Figure 4). With resuscitation, significant differences in cardiac output developed between groups. After the first and second resuscitative steps, the Hextend-20 group had a significantly greater cardiac output than either the LR or the Hextend-10 group. However, by the fourth resuscitative step, both Hextend® groups averaged a significantly greater cardiac output than the LR group, with no significant difference between Hextend® groups (Figure 3).

Figure 3: Cardiac output during shock and resuscitation. Hextend-10 group significantly lower than either LR or Hextend-20 group at 60 minutes of shock (*p=0.044). Hextend-20 group significantly greater than LR or Hextend-10 groups at Resus-1 and Resus-2 (*p<0.012). At Resus-3, Hextend-20 group significantly greater than LR group alone (*p<0.012). At Resus-4, LR group significantly lower than either Hextend group (**p=0.001).
Hemodynamic Variables and Tissue Energetics during Resuscitation of Porcine Hemorrhagic Shock with Hextend® or Lactated Ringer’s Solution

Hextend (10cc/kg) Hextend (20cc/kg) Lactated Ringer’s (20cc/kg)
Resus-4 Resus-3 Resus-2 Resus-1

Figure 4: Hind limb tissue pH (pHi) as determined by chemical shift of in vivo NMR spectroscopy during shock and resuscitation. Hextend-10 group significantly lower than Hextend-20 or LR group (*p<0.05).

The amount of change in cardiac output between the end of shock (Shock-90) to the end of resuscitation (Resus-4) was significantly greater for the Hextend® groups than the LR group. These differences were reflected in oxygen delivery (DO₂) values which were significantly higher for both Hextend® groups compared to the LR group by Resus-4 (Table 1b; p<0.05). There was no statistical difference in DO₂ between Hextend® groups (Table 1b; p=0.157) at Resus-4. With respect to increased cardiac output and DO₂ with resuscitative measures, LR given at 60 cc/kg in 2 doses was equivalent to Hextend® at 20 cc/kg given as either a single dose or two doses (Figure 5).

Figure 5: Cardiac output and oxygen delivery after resuscitation with 60 cc/kg (3 boluses) of LR, 20 cc/kg Hextend in one bolus (1 dose), or 20 cc/kg Hextend in two boluses (2 doses).
Changes in tissue phosphoenergetics were also observed between the three groups during resuscitation. After the second and third resuscitative steps, hind limb (skeletal muscle) levels of phosphocreatine (PCr) were greater in the Hextend-10 group than the Hextend-20 or LR groups. However, by the end of resuscitation (Resus-4), both Hextend® groups were significantly greater than the LR group, and there was no difference between Hextend® groups (Figure 6a). No significant differences between tissue ATP levels in the hind limb (Figure 6b) or liver (not shown) were observed between groups during resuscitation, except exclusively after the third resuscitative step. As previously reported, hind limb phosphomonoester (PME) levels uniformly increased during shock in all groups and then decreased with resuscitation.12 Consistent with previous observations, the animals that were more severely affected by the shock protocol had a significant elevation in PME levels during shock (Hextend-10 group) (Figure 6c).12,13

Figure 6a: Hindlimb phosphocreatine/inorganic phosphate (Pi) ratio in vivo during hemorrhagic shock and resuscitation. *p<0.05 when Hextend-10 group compared to other groups. **p<0.01 when LR group compared to both Hextend® groups.

Figure 6b: Adenosine triphosphate (ATP)/ inorganic phosphate (Pi) ratio in hind limb (skeletal muscle) during shock and resuscitation as determined by in vivo NMR spectroscopy. *p<0.02 when compared to Hextend-10 group.
Predictably, StO₂ of the liver, stomach and hind limb dropped in all groups with shock and returned towards baseline levels with resuscitation. Interestingly, we noted a significant decrease in liver StO₂ during the later phases of resuscitation in the animals receiving 20 cc/kg/step of Hextend® as compared to animals resuscitated with LR (Resus-3, p=0.03). There were no other significant differences in StO₂ between groups.

4.0 DISCUSSION

Hextend® resuscitation resulted in increased systemic oxygen delivery (DO₂) and improved tissue energetics as measured by NMR spectroscopy. We believe that this increase is secondary to the early increased filling pressure in the heart from colloid administration as demonstrated by increased pulmonary capillary wedge pressure (PCWP) in animals receiving Hextend®. This increase contributed to an earlier improvement in cardiac output and the resulting improvement in DO₂ and phosphoenergetics. Interestingly, this increase was not reflected in an increase in StO₂ in any of the tissue beds monitored. The volume expansion achieved with Hextend® was similar to that classically described, 4,19 with a similar increase in global hemodynamic parameters achieved with 1/3 the volume of crystalloid resuscitative fluid.

One issue not addressed in our controlled hemorrhage model was the level of blood pressure safe to prevent “popping the clot”, resulting in increased hemorrhage. Human studies have demonstrated the safety and efficacy of hypotensive resuscitation in short term care of trauma patients. 8 Animal studies have placed the “safe” pressure limit at approximately 60 mm Hg. 20 The US military has adapted the previous ATLS protocol for care of injured patients in the field to limit intravenous fluids to those patients with abnormal pulses and altered mental status. 21 Hextend®, with its more effective volume expansion, may be more apt to cause loss of the primary protective clot due to its more rapid restoration of cardiac output.

One of the weaknesses of this study was the randomization scheme, which randomized animals prior to shock. This resulted in fewer animals available for analysis in the Hextend-10 group than the other two groups due to death of several more animals during the shock period. Additionally, it appears from the hemodynamic profile and elevated lactate levels of the analyzed animals in this group that these animals were more severely stressed by the shock protocol than animals in the other groups. Interestingly, despite this issue, animals receiving Hextend® appeared to improve more rapidly with resuscitation compared to animals treated with LR as demonstrated by hemodynamic parameters and tissue energetic indices (Figure 3). NMR data from the
liver, which would have aided in interpretation of changes in StO₂ signal in this organ, was not interpretable during major portions of this protocol due to poor signal/noise ratio. Finally, one must be careful in application of findings derived from a controlled animal shock model to a clinical scenario.

In conclusion, we have demonstrated that Hextend® resuscitation results in improved hemodynamics and tissue energetics secondary to improved filling pressures in a porcine model of controlled hemorrhagic shock. This product may have significant application in austere environments where the volume of resuscitative fluid is limited due to mission constraints. Further study of this product is needed in clinical settings to validate these results.

REFERENCES:


Trans Sodium Crocetinate: Novel Treatment for Hemorrhagic Shock

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SUMMARY

Whole-body oxygen consumption is decreased after hemorrhage. Typical methods for increasing oxygen consumption have involved increasing the blood oxygen concentration using enriched oxygen gases, hemoglobins and fluorocarbon compounds; however, clinical trials involving these have not been totally successful. Increasing the oxygen concentration increases its diffusion rate through blood plasma; however, an alternative method would be to increase the diffusion coefficient of oxygen itself. This has been shown to be possible using a novel compound, trans sodium crocetinate (TSC). TSC also increases oxygen consumption in hemorrhaged rats and results in an increased survival rate. TSC has also been shown to increase blood pressure and to reduce the acidosis that forms with hypoxia and to reduce damage to liver and kidney.

INTRODUCTION

There is a decrease in whole-body oxygen consumption after hemorrhage, and it has been suggested that this is linked to mortality (1). Recovery from hemorrhagic shock has long been suggested to depend on restoration of oxygen to the tissues (2, 3), and a recent report (4) suggests that even small enhancements in oxygen consumption could reduce rates of morbidity and mortality.

Typical methods for increasing oxygen consumption are usually designed to enhance its delivery to the tissues. These include the use of synthetic hemoglobins or fluorocarbons, or the breathing of concentrated and/or hyperbaric oxygen gases. Acting via a different mechanism to increase the delivery of oxygen, a new drug, trans sodium crocetinate (TSC), has been found to result in increased whole-body oxygen consumption (and survival) during hemorrhagic shock in rats (5). TSC offers the possible advantage of being more quickly and easily utilized in a traumatic situation.

In addition to oxygen consumption, a number of physiological parameters are altered after hemorrhage. During the early stages of shock, patients suffer from decreased blood pressure, tachycardia, decreased blood pH and increased lactate levels (6, 7). Although similar changes are not always found with the various animal models used to study hemorrhagic shock, we have used a rat model which shows similar responses to hemorrhage as do humans. These animals were allowed to recover for about 30 minutes after surgery, until the mean arterial blood pressure stabilized at around 100 mm Hg and a heart rate of 300 to 400 beats/min before the hemorrhage occurs.

Thus, we have used this severely-bled rat model in order to learn more about the effect of TSC after hemorrhage. TSC has been used for the treatment of hemorrhagic shock as a single bolus followed 30
minutes later by an infusion of normal saline. TSC has also been dissolved in normal saline and infused in that manner. However, all of the studies mentioned here will involve small-volume injections of TSC with no infusion of any fluid. Thus, the effects seen should be due to the action of the drug alone, although this is not to say that fluid replacement is not important in any real therapy.

**BLOOD PRESSURE**

Male, Sprague-Dawley rats were used with a protocol approved by the Animal Care and Use Committee of the University of Virginia. The rats weighed between 290 and 350 grams each, and were fed *ad libitum* until the day of the experiment. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital, 47.5-50 mg/kg. The right carotid artery was exposed and cannulated with PE-50 polyethylene tubing filled with normal saline, which was passed subdermally to the back of the neck and withdrawn through the skin. The incision was closed using Surgalloy CV-23 taper silk sutures, and 2% lidocaine applied to the wound. The rat was then allowed to recover as described previously and 6 to 9 minutes of baseline values were obtained before the animal was bled. At the end of the experiment, the animals were sacrificed using an overdose of pentobarbital.

A constant-volume protocol was used for all hemorrhages. This model has been suggested to replicate hemorrhagic shock scenarios more closely than a constant-pressure protocol (10). Studies were done which involved removing 60% of the estimated blood volume, assuming that the normal rat blood volume is 60 ml/kg of body weight (11, 12). To hemorrhage the animals, a saline-filled cannula leading to the carotid artery was attached to a syringe pump, and blood was removed in a period of about 9 to 10 minutes.

All hemorrhaged animals in this study were treated with a single bolus injection of either normal saline or TSC given immediately after the hemorrhage ended (the saline-dosages were given to the control group). The volume of saline or TSC injected into the animal ranged from 0.2 to 0.3 ml, depending on the dosage of TSC. Normal saline was given to the control group, with an injection volume of 0.25 ml per animal. A Digi-Med Blood Pressure Analyzer (Micro-Med, Louisville, KY) was used to simultaneously determine instantaneous values of arterial blood pressure (mean, systolic and diastolic). The carotid cannula was attached to this device once the surgery was completed, and pre-hemorrhaged values were recorded for 10 minutes after the 30-minute recovery time. These values were usually a MAP of 100 mm Hg and a HR between 300 and 400 beats/minute. After the hemorrhage ended and the injection given, blood pressure was recorded at 3-minute intervals for 50 minutes.

The mean blood pressure in all groups decreased to a value around 35 mm Hg immediately after the hemorrhage ended. It continued to decline in the control group, and the majority of those animals died. However, the blood pressure began to increase soon after the TSC was given, and rose until the mean blood pressure was about 80% of the baseline value. The final value of blood pressure attained was slightly higher when the higher TSC dosage was used, and all animals survived that had received either dosage of TSC. These results are presented in graphical form in Reference 13. Systolic, diastolic and mean arterial blood pressures were recorded every 3 minutes, and all three parameters appeared to change proportionately to each other throughout the study.

So, in summary, the mean blood pressures of the TSC-treated animals rose to about 80% of the pre-hemorrhaged values, depending on the dosage used. A slight decline in the average blood pressures after time was noted, at around 25 minutes for the lower TSC dosage and at around 45 minutes for the higher one. After these slight declines, the blood pressures again stabilized at around 70% of the pre-hemorrhage value.
times at which the blood pressure stopped increasing closely correspond to the clearance times for TSC. We also examined the effect of TSC on blood pressure in normovolemic animals. TSC also resulted in an increase in the blood pressure of non-hemorrhaged animals. That increase, although statistically-significant, lasted only a short time and the mean blood pressure soon returned to a normal value. Thus, although TSC caused an increase in blood pressure of 45 mm Hg during shock that persisted while the drug was present in the blood stream, it did not elevate the blood pressure as much nor did the increase persist as long in the normovolemic animals.

Since catecholamines are known to increase after hemorrhage, their levels were determined in order to see if they were the mediator for the blood pressure effect of TSC. This study was done slightly differently, in that the animals were not treated until 20 minutes after the hemorrhage ended, and, at that time, repeat injections of either TSC or saline were given every 10 minutes. The catecholamine levels were determined from a plasma sample collected 90 minutes after the hemorrhage ended. The levels of both epinephrine and norepinephrine were determined for plasma samples taken before hemorrhage and 90 minutes after hemorrhage. Base line epinephrine levels (± standard deviation) were similar in both groups, about 167 ± 10 pg/ml for the control group and 167 ± 11 pg/ml for the TSC group. The control group experienced a 361% increase in circulating epinephrine while the TSC-treated group underwent a 175% increase. The difference between the control group and the TSC group are statistically significant (p<0.05). The increase in the control levels with hemorrhage are the same magnitude as those reported by others. In addition, TSC also decreased the norepinephrine response to hemorrhage, with the levels of the controls rising about 400% with hemorrhage as compared to a 220% rise for the TSC-treated animals. Thus, these results indicate that treatment with TSC reduces sympathetic activation, as represented by the reduced circulating levels of epinephrine and norepinephrine. This reduction in sympathetic activation may be the result of a lesser degree of stress on the body due to an increased oxygen delivery with the administration of TSC.

HEART RATE

Rats have much higher heart rates than humans, with pre-hemorrhage values being similar for all of our animals: 348 ± 34 beats/minute for the TSC-treated animals and 352 ± 30 beats/minute for the controls. These values were determined using the same DigiMed Analyzer which was used to record blood pressure. Our controls experienced about a 50% increase in heart rate after hemorrhage (see Reference 13), which is the same percentage change as seen in awake, severely-bled swine (8, 9) -- even though the normal heart rates of swine are much lower.

Heart rates also increased immediately after hemorrhage in the treated groups and then declined with time. However, the initial increase was less in the TSC-treated animals, and remained less than the controls for the next 20 minutes or so. The differences between the higher dosage TSC group and the control group are statistically significant (p < 0.05) from the time of 3 to 15 minutes after hemorrhage. The differences between the lower TSC dosage group and the control group were not statistically different, although the average was lower for the TSC group. However, the values for all groups were about the same by 30 - 35 minutes post-hemorrhage.

The heart rate of normovolemic animals decreased about 10% after TSC was given, and this effect continued for the next hour. Thus, it appears that TSC results in a decreased heart rate in both hemorrhaged and normovolemic animals.
COMPARISON TO OXYGEN THERAPY

Some insight concerning these results may be gained by comparing our results with another method for increasing oxygen consumption. Breathing pure oxygen was suggested as a treatment for hemorrhagic shock as long as 60 years ago (14). Not only have animal studies shown beneficial effects of oxygen, but human studies have also shown them as well (15,16). In spite of this, however, relatively little research has considered oxygen therapy for hemorrhagic shock, in either animals or humans. A similar study, however, has investigated the use of 100% oxygen for hemorrhagic shock using an awake rat model (17).

In that study, Adir et al. obtained blood pressure and heart rate data for hemorrhaged rats given oxygen therapy (100% oxygen). Their animals had pre-hemorrhage arterial blood pressures similar to those in our study (around 100 mm Hg), which dropped to about 50 mm Hg after hemorrhage. When the hemorrhaged animals were then exposed to 100% oxygen, the blood pressure increased until it reached 80 to 90% of the pre-hemorrhaged value. Once oxygen was discontinued, the blood pressure decreased somewhat before stabilizing at around 70% of the pre-hemorrhage baseline value. They also exposed sham-shock (operated on but not bled) rats to 100% oxygen and found a statistically-significant rise in blood pressure of about 10%; however, the pressure soon returned to the baseline value even though the 100% oxygen was continued. These are very similar to the results seen in our study. TSC increased blood pressure to about 70-80% of the pre-hemorrhage value, with the effect decreasing slightly when the drug cleared, and it also caused a transient rise of about 10% in the blood pressure of normovolemic animals.

It has also been known for years that oxygen therapy lowers the heart rate in humans (18). In fact, a study by Bean in 1945 concluded that the evidence left little doubt that breathing oxygen at atmospheric pressure caused a slowing of the human heart (19). Adir et al. (17) found that the use of 100% oxygen caused a decrease in the heart rate of non-hemorrhaged rats of about 12% (as compared to a decrease of about 10% with TSC). They also found that 100% oxygen resulted in a survival rate (at two days) of 90% for the oxygen-treated animals as compared to 40% for their controls. This is quite similar to our survival rate of 29% for the controls and 100% for the TSC-treated animals (after a period of 4 hours). Thus, an overall comparison of our results with those of Adir et al. (17) shows that similar results come from either using 100% oxygen or injecting TSC. This suggests that the effects of TSC are actually due to the increased oxygen consumption it causes. A remaining question, then, concerns the mechanism by which TSC increases whole-body oxygen consumption.

Unlike hemoglobins and fluorocarbons, TSC does not bind oxygen nor increase its solubility in blood plasma (20). TSC does not alter blood viscosity or red cell deformability (20), nor does it affect 2,3-DPG release or shift the oxyhemoglobin saturation curve. The only oxygen-related variable affected by TSC appears to be the diffusivity of oxygen through liquids such as blood plasma. Recent in vitro testing in our laboratory showed that TSC increases the oxygen diffusion by 30%. Further confirmation of these results comes from computer simulations of oxygen moving through a liquid, which attribute this increase in diffusivity to changes in the (molecular-level) spacing in the liquid which is caused by the TSC (21).

Although changes in diffusion have long been encountered in other situations where they are the controlling factor (22), such a proposed mechanism of action for a drug appears to be novel. This may be because it has commonly been thought that the delivery of oxygen (blood flow rate times blood oxygen concentration) determines the rate of consumption. However, during the past 10 years, it has been suggested that there are situations where diffusion may also control the rate at which oxygen can be consumed (23-25). If hemorrhagic shock were one of these, then increasing the diffusivity of oxygen with TSC could increase oxygen consumption.
CONCLUSION

It would appear that TSC may be very useful for treating hemorrhagic shock. Not only does it increase oxygen consumption in hemorrhaged rats, it also increases blood pressure. Of perhaps more importance, TSC reduces the increase in blood lactate levels which often accompany hemorrhagic shock, and lessens the shift in blood pH. As noted previously, TSC can be given together with an infusion of fluid such as saline. This present study did not utilize fluid replacement in order to learn more about the action of the drug itself. However, a previous study (5) suggests that the volume of fluid infused can be reduced when using TSC, presumably because of the added influence of the drug in those cases.

It is also interesting to note the effect of TSC on organ damage which occurs as a consequence of hemorrhagic shock. It has been found (26) that TSC prevented the levels of the liver transaminase enzymes, GOT and GPT, from increasing, while the levels of those enzymes in controls doubled over 24 hours after hemorrhage. Since these are indicative of liver damage, it would seem that TSC can prevent that from occurring. Support for this has come from histology of the liver and kidney done after hemorrhagic shock in rats (27), which shows far less necrotic cells in those organs if TSC is used.

Diffusion Pharmaceuticals LLC in Charlottesville, Virginia is currently coordinating the synthesis of TSC and toxicological testing. They hope to have it ready for clinical trials in the near future.

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Trans Sodium Crocetinate: Novel Treatment for Hemorrhagic Shock


A Role for Vasopressin during Resuscitation of Traumatic Shock

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SUMMARY

In the past few years, arginine vasopressin (AVP) has emerged as a rational alternative to catecholamines for the hemodynamic support of refractory vasodilatory shock and cardiopulmonary arrest. The therapeutic potential of AVP in traumatic shock is now being evaluated. Our laboratory investigations have revealed an apparent benefit of AVP when compared to standard fluid resuscitation in clinically relevant models of brain injury and chest injury. Further experimental work and subsequent clinical trials appear justified to validate the efficacy of AVP for resuscitation of trauma patients.

1.0 INTRODUCTION

AVP has been used to treat patients for almost 100 years, but some exciting new indications have been suggested in a recent comprehensive review [1]. The basic physiology is well defined. It is a nonapeptide antidiuretic hormone that is formed primarily in the supraoptic nuclei of the hypothalamus and is stored in large secretory granules in nerve terminals in the posterior pituitary gland. Several stimuli release AVP, including increases in plasma osmolarity, volume contraction, trauma, pain, anxiety, and certain drugs (morphine, nicotine, tranquilizers, and some anesthetics). Perhaps the most potent stimuli for AVP release is hemorrhage; a loss of 25% of the blood volume can cause as much as a 50 fold increase in the rate of AVP secretion. The biological actions are mediated by V1 (vascular), V2 (renal) and V3 (anterior pituitary) receptors. The half-life of exogenous AVP is 10-35 minutes. The secondary messenger system at the V1 receptor involves a G-protein coupled phosphoinositide pathway leading to increased cytosolic calcium levels and vascular smooth muscle contraction.

The routine use of vasoconstrictors has historically been discouraged in hypovolemic patients, since organ blood flow and oxygen delivery can be compromised [2]. Unfortunately, early aggressive fluid replacement can also have undesired effects [3-6]. Furthermore, prolonged hemorrhage, cardiogenic shock, and sepsis may evolve into a refractory phase characterized by unresponsiveness to either fluid replacement or catecholamines [7, 8]. Recently, AVP was found to effectively restore systemic circulation in some of these conditions [8-14]. This counterintuitive finding has prompted a resurgence of interest in this hormone. The purpose of this report is to review some recent clinical and laboratory data on the therapeutic use of AVP in these critical conditions and to explore its potential in traumatic shock.

2.0 AVP FOR VASODILATORY SHOCK

AVP has little effect on blood pressure under normal conditions, but the hemodynamic response to exogenous AVP may be augmented in shock states [7]. Malay et al performed a randomized controlled trial with septic shock patients (n=10) in the trauma ICU [9]. Low-dose AVP (0.04 U/min) increased systolic pressure from 98 to 125 mmHg and permitted successful withdrawal of all other catecholamine vasoactive drugs. All patients survived in the AVP group while two died within 24 hours in the control group. Another randomized controlled study in patients (n=10) with vasodilatory shock after cardiopulmonary bypass [10] showed that AVP (0.1 U/min) increased mean arterial pressure (MAP) from 57 to 84 mmHg, and reduced norepinephrine requirement.

3.0 AVP FOR SHOCK STATES: THE RATIONALE

Prolonged shock is characterized by a biphasic AVP response. A large initial peak of plasma AVP (100-1000 pg/mL) is followed by inappropriately low levels (e.g. <50 pg/mL) for the degree of hypotension [1, 8]. Similarly, in critically ill patients during late septic shock, AVP levels (3.1 pg/mL) [11] are within the normal range for well-hydrated humans (< 4 pg/mL) [1]. Postulated mechanisms of the endogenous AVP deficiency in shock states include: depletion of neurohypophyseal stores of AVP; inhibition of AVP release due to impaired autonomic reflex; and the inhibitory effect of high levels of circulating norepinephrine [1]. The endogenous AVP deficiency and the purported hypersensitivity to exogenous AVP provide the justification for low-dose AVP therapy in shock states.

4.0 AVP FOR CARDIOPULMONARY ARREST

Cardiopulmonary arrest may be another indication. Endogenous AVP levels are higher in resuscitated patients than in non-resuscitated patients after cardiopulmonary arrest [15]. Laboratory data and clinical trials support the benefits of AVP on coronary perfusion pressure [12], myocardial blood flow [16], restoration of spontaneous circulation [13, 17], and neurological recovery [17]. In a multicenter randomized controlled trial, AVP was equivalent to epinephrine in the management of ventricular fibrillation, but superior to epinephrine in patients with asystole [14].

5.0 AVP FOR TRAUMA WITH HEMORRHAGE

Investigators have questioned the use of aggressive fluid resuscitation for the treatment of uncontrolled hemorrhage prior to surgical intervention [3, 4]. In this context, a porcine model of uncontrolled hemorrhage was adopted to evaluate the effects of AVP on survival after liver injury [18]. A bolus dose (0.4 U/kg, n=9) was administered followed by 0.08 U/kg/min continuous infusion until the bleeding was controlled, when standard fluid resuscitation was initiated. Eight of nine AVP animals survived more than 7 days, while all fluid resuscitation animals (n=7) and all placebo animals (n=7) died in the hemorrhage period. A similar study was conducted to compare the effect of AVP to epinephrine on short-term survival [19]. AVP (n=7), but not epinephrine (n=7) or placebo (n=7), improved survival in uncontrolled hemorrhage after liver injury. These findings suggest that delayed fluid resuscitation combined with AVP infusion may be beneficial. However, a concern remains about possible visceral ischemia by large dose AVP [7].
6.0 AVP FOR TRAUMATIC BRAIN INJURY (TBI)

Hypotension, hypoxemia and increased intracranial pressure (ICP) after TBI are strongly associated with poor outcome [20, 21]. Although hypertonic/colloid solutions [5] or vasoactive agents [22] have been advocated to maintain cerebral perfusion pressure (CPP), reduce the fluid requirement and minimize ICP changes, the validity of vasoactive agents in this setting is undetermined. In a porcine model, phenylephrine improved CPP but did not increase cerebral blood flow in uncontrolled hemorrhage after TBI [23]. Clinical trials have suggested that norepinephrine is superior to dopamine in optimizing both ICP [24] and cerebral blood flow [22]. There is one case report, in which early use of AVP appeared efficacious for a patient with severe TBI (GCS 4) complicated with hypotension refractory to fluid and sympathomimetics [25].

We have performed two series of studies to evaluate the therapeutic potential of AVP during resuscitation from hemorrhagic shock after TBI. In the first series, anesthetized swine (n=19) received standardized fluid percussion TBI and severe hemorrhagic hypotension with MAP < 20 mmHg and isoelectric EEG for 12 minutes. Three animals died before randomization. The survivors were resuscitated with a clinically relevant protocol [26, 27] including administration of normal saline, blood and mannitol (1 g/kg) to maintain CPP > 60 mmHg. Either continuous AVP (0.1 U/kg/hr) or placebo infusion was administered in blinded fashion. Our data showed that the total fluid required to maintain CPP was reduced by half in the animals receiving AVP, the transfusion requirement was reduced by 40%, cerebrovascular reactivity to carbon dioxide was improved, and ICP were reduced (11±1 vs 23±2 mmHg).

In a second series (n=14), the duration of hypotension and isoelectric EEG after TBI was extended to 20 min, which caused a greater number of primary deaths (n=4). Then the identical resuscitation protocol was initiated with AVP bolus (0.2 U/kg) followed by continuous infusion (0.1 U/kg/hr). All AVP animals (n=5) survived 5 hours after TBI, while 3 animals died within 67min in the placebo group (n=5). Altogether, these data suggest a therapeutic potential for AVP during fluid resuscitation from severe TBI.

7.0 AVP FOR PULMONARY CONTUSION

The deleterious effects of large amount of fluid administration on pulmonary contusion have been suggested in animal models, but the efficacy of limiting fluid, either with hypertonic solutions or with vasoactive agents has not been supported by clinical evidence [6]. In our laboratory, the potential benefits of AVP were examined in a porcine lung contusion model combined with hemorrhagic hypotension [28]. After a blast to the chest with a captive bolt gun and hemorrhage to MAP < 30 mmHg for 20 minutes (n=20), there were 3 deaths. The survivors were resuscitated with crystalloid and randomized to either AVP (0.1 U/kg followed by 0.4 U/kg/hr) or placebo. All AVP animals (n=8) survived 5 hours, while 4 of 9 placebo animals died within 120 min after the injury. With AVP vs placebo, the total fluid required to maintain MAP was reduced by two-thirds, ventilatory mechanics (compliance, airway resistance, and airway pressures) were improved, and ventilation/perfusion was improved.

The major limitation of our animal studies is related to safety and specificity. We observed AVP reduced cardiac index in accordance with decrease in heart rate, and thus O2 extraction was increased and lactate clearance was delayed. These parameters eventually returned to normal within 5 hours, but ischemia of visceral organs and distal extremities cannot be ruled out. Finally, despite previous studies which suggest unique benefits of AVP vs catecholamines [12, 14, 19], it is possible that the benefits we observed are related to reduced fluid requirements and are not AVP-specific. Further experimental work and subsequent clinical trials appear justified to validate the safety and efficacy of AVP for resuscitation in trauma patients.
8.0 ACKNOWLEDGEMENTS

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9.0 REFERENCES


A Role for Vasopressin during Resuscitation of Traumatic Shock


Complement Inhibitor APT070 Dramatically Reduces the Need for Resuscitation and Improves Survival in Controlled Isobaric Rat Hemorrhage Model

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ABSTRACT

The complement system (C) becomes activated during hemorrhagic shock in a rat isobaric hemorrhage model, a process significantly aggravating the outcome of shock. Consequently, inhibition of C activation may have beneficial effects on both survival and metabolic status of animals subjected to shock.

1.0 INTRODUCTION

Earlier data showed that complement activation takes place during hemorrhagic shock in pigs6. The exact cause of this activation is not known, but it is likely that the C5a and C3a anaphylatoxins released during the activation of the complement cascade may have a profound role in the pathophysiological changes that characterize trauma and shock7. It has been hypothesized that by inhibiting the complement system, it may be possible to minimize the harmful effects of hemorrhagic shock. To address this question, we have used an isobaric hemorrhage model in rats for the testing of complement inhibitors.

The complement system is complex with three distinct pathways known to initiate the serine protease cascade and produce anaphylatoxins (Fig. 1). The classical pathway is triggered by antibody–antigen complexes while the alternative pathway includes the generation of C3 convertase by foreign surfaces

* Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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(lacking membrane associated or soluble complement inhibitors) and the stabilization of activated C3 by factor D or B. The third pathway involves lectins that bind to bacterial surfaces and initialize C3 activation. All three pathways lead to the cleavage of C3 and release of C3a.

The activation pathways merge into the terminal pathway with formation of the membrane attack complex (MAC, C5b-9) that lysed foreign particles as well as activates inflammatory cells for particle elimination. Complement activation is strictly regulated by natural inhibitory proteins, and there are numerous synthetic molecules that block the activation chain at different sites (Fig. 2). Thus, various reagents with potential therapeutic applications have been developed to target C activation and function (indicated by red asterisks in Fig. 1) but the work reported here has used a modified version of a CR1 (complement receptor type 1) fragment that has been shown to interfere with the early amplification phase in which C3 convertase generates more C3b. The molecule has been modified to be made more effective by tagging it with a positively charged peptide and hydrophobic anchor that allows it to be concentrated on the cellular surface and protect the cell surface vigorously from complement activation. It is known as APT070 (Adprotec, Inc.) (Linton et al. 2590-97, Smith 1037-41). APT070 effectively blocks and reduces the generation of the activated form of C3, and thwarts the release of both C3a and C5a. APT070 is a chemical conjugation of APT154, a region taken from the Complement Receptor type 1 molecule, APT542, and myristoic acid.

**Classical Pathway**

Antigen+Antibody
(and direct activators of C1)

Activated C1
(C1qr2s2)

C1 pool
(C1qr2s2)

C1 pool
(C1qr2s2)

C4 pool

C4b

C2a

C2 pool

C2b

Anaphylatoxins (C3a, C4a, C5a)

C3 convertase

C3 pool

C3b

C3a

C3bi

Factor B

Bb

Spontaneous
activation or bacterial surfaces
(and direct activators of C3)

C3 convertase

C5 convertase

C5 pool

C5b

C5 pool

C6 pool

C6

C7

C9

C5 pool

C5b

C5 pool

C5b

C6

C7

C8

C9

C5 pool

C5b

C6

C7

C8

C9

Figure 1. The key elements in complement system are the serine protease pro-enzymes, which themselves serve as substrate for the next step in the cascade. It represents one of the most powerful signal amplification system ever known. The products of the cascade are a) the membrane attack complex (MAC), that can bore holes in membranes, and has cytolytic properties, b) the opsonins mainly C3b,C3bi, and c) anaphylatoxins (C3a and C5a) and other signalling molecules such as C3d, C4d and C4dg etc). The man-made inhibitors act in the steps marked by red *.
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2.0 METHODS

2.1 Animals
Sprague-Dawley rats (300-600 g) were from Charles River Laboratories, Wilmington, MD. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals and experiments involving animals adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council.

2.2 Chemicals
Isoflurane (Viking Medical Products, Medford Lakes, NJ); pentobarbital (Sigma, St. Louis, MO); 6 IU / mL heparin (Elkins-Sinn, Cherry Hill, NJ); 1Vlg F(ab)2 (Centeon Pharm. GmbH, Wien, Austria); APT070 (ADPROTECH Co., Little Chesterfort, UK), Physiological saline solution (Gibco, Grand Island, NY).

2.3 Equipment for Data Collection
Digital balance (PM300, Metler-Toledo, Columbus, OH); Peristaltic pump (P730, Instech Inc., Plymouth Meeting, PA); Radiometer, Automated Blood Analyzer (ABL 7000, Radiometer America Inc., Westlake, OH); Amplifiers (BPA-400A, Digi-Med, Louisville, KY); MIO6325 DAQ board (National Instruments, Austin, TX); Pentium class PC; DataLyser and REDIREC proprietary data acquisition and analysis software, proprietary isobaric hemorrhage computerized data acquisition and process control software developed at WRAIR using LabView (National Instruments, Austin, TX).

2.4 Induction of isobaric hemorrhagic shock in anesthetized, unconscious rats
Sprague-Dawley rats were temporarily anesthetized with 5%v/v isoflurane gas. The rats were weighed (300-600 g), and single dose of 50mg/kg pentobarbital bolus was injected intra-peritoneally to anesthetize the rat.

Figure 2a. Isobaric hemorrhage: the timeline in the anesthetized, unconscious rat model.

Once the rat was unconscious, tracheotomy and bilateral femoral artery cannulations were performed. The femoral artery cannula was linked to a computer-controlled peristaltic pump (P730) system and a reservoir to maintain desired mean arterial blood pressure (MABP) through the withdrawal of blood under the control of the hemorrhage software. All measurement signals were recorded every five seconds for subsequent analysis. The pentobarbital-anesthetized animals were allowed to stabilize during a control period of 20 minutes, (Fig 2a). Bleeding followed this period, and was conducted with computer-controlled peristaltic pump. The
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2.5 Induction of isobaric hemorrhagic shock in resuscitated, conscious rats

Male Sprague-Dawley rats were anesthetized with isoflurane (5% induction, 1.5-2% maintenance) and cannulas were inserted surgically into femoral artery and vein. The cannulas were then lead subcutaneously to the neck area, exteriorized, passed through a metal spring cannula guard and connected to a two-channel fluid swivel. The swivel connected the venous cannula to the peristaltic pump and the arterial cannula to the blood pressure sensor. The animals recovered from the anaesthesia for two hours and then underwent the hemorrhage and resuscitation while conscious and freely moving. After a 20 min control period, blood was withdrawn under computer control lowering MABP over 15 min to 40 mmHg where it was held for 30 min. The animals received lactated Ringers to during a prolonged hypotensive resuscitation (MABP supported at 60 mmHg for 4 hrs) followed by full resuscitation to 80 mmHg and 24 hr survival (Fig. 2b). Treated animals received the APT070 complement inhibitor (10 mg/kg bolus in 0.7 mL) one minute before the start of the hypotensive resuscitation (at 64 min). Control animals received an equivalent volume of saline at the same time point. Blood samples were taken prior to hemorrhage (baseline), 65 min (end of hemorrhage period), 335 min (end of resuscitation) and the last sample was collected when the animal was nearly moribund or at 24 h, when the observation period ended. The blood samples were analyzed using a blood gas analyzer (ABL 735, Radiometer America Inc., Westlake, OH) to determine blood chemistry.

Figure 2b
Unanaesthetized, survival rat model of isobaric hemorrhagic shock, with resuscitation. Blood pressure data, shed blood volume, the volume of resuscitation fluid and survival time is recorded. Rats recovered 2 h after anaesthesia before start of experiment.

2.6 Statistical analysis

Students’ unpaired t-test was used to compare the mean values in treated and untreated groups, as implemented in GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).
3.0 RESULTS

Complement activation is found early in hemorrhaged rats, as measured by a decrease in residual hemolytic activity of the serum using the Ch50 analysis. The shed blood volume reached its maximum at about 90 min and since blood was only removed and no resuscitation fluid was given up to that time, there was no significant plasma dilution to account for the decrease in the hemolytic activity. Therefore, it is concluded that strong complement activation precedes the development of decompensated hemorrhagic shock (Fig. 3).

**Figure 3.** Complement activation in hemorrhaging rats, representative data of n>10

**Figure 4.** Shed blood profile in hemorrhage experiment. Shed blood reached a maximum after 90 min and infusion of resuscitation fluid was necessary to maintain the targeted blood pressure of 40 mmHg.
Shed blood is the amount of blood removed from a rat to lower its blood pressure to 40 mmHg. More blood is removed, if the animal can keep its blood pressure at a higher level. The apparent increase in the amount of shed blood in rats pre-treated with APT070 while all of the pre-treated rats survived confirms our hypothesis, that blood loss in itself is not the most important factor for the early deaths (difference in death rate by 225 minutes is statistically significant, P<0.001). As Figure 4b indicates, APT070 treatment reduced the need for infusion of lactated ringer solution as well, blood loss induced severe hypoxia and physiological changes characteristic to the late phase.

The blood chemistry of rats undergoing hemorrhage show several large and potentially important changes. Blood lactate concentration increases are due to the development of tissue hypoxia and the consequent switchover from aerobic to anaerobic respiration. Reduced hepatic flow may also contribute to lactate elevation since the liver under aerobic conditions normally takes up lactate produced by other tissues, Fig. 5a). It is worth noting that the APT treated rats lost slightly more blood, and if fact are expected to have more severe hemorrhage, however the survival times and blood chemistry illustrated that the opposite is the case, the are better of than the controls, and complement inhibiton is highly beneficial.

Figure 5. Changes in blood chemistry in rats undergoing hemorrhage.
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Under normal conditions, potassium is actively stored in cell cytoplasm, requiring energy to maintain this state. Rats in the untreated and control groups have increased potassium levels, as hypoxia sets in and energy supply is insufficient to maintain the active storage of the potassium ions (Fig. 5b). Due to a higher shed blood volume (and therefore, more severe hypoxia and impaired energy production), the blood potassium level is expected to be more severe. However as we show in the presentation, it is not the case. Blood pH is normally slightly alkaline (7.4). During hemorrhage and other hypoxic conditions, lactate is produced through anaerobic respiration and contributes to shifting the plasma to acidic pH (Fig. 5c) the acidosis is also marked by negative base excess values, indicating that the bicarbonate/CO$_2$ buffer system cannot compensate for the observed shift in pH (Fig. 5d). At the baseline measurement, all groups are within a normal range. This also indicates changes in gas exchange and breathing patterns. During hemorrhage the APT070 pre-treated animals were partially protected from these severe changes as we show in the presentation. Plasma in hemorrhagic rats shifts to acidic pH (Fig. 5c) and metabolic acidosis is also marked by negative base excess values, indicating that the bicarbonate/CO$_2$ buffer system can not compensate for the observed shift in pH (Fig. 5d).

4.0 DISCUSSION & CONCLUSIONS

In the isobaric hemorrhage model, hemorrhage occurs in three distinct phases. The first, or compensated stage, occurs when bleeding has begun, but the rat’s cardiovascular system is still capable of maintaining blood pressure above the 40 mmHg preset threshold. Approximately 45 minutes into the compensated stage, blood loss reaches a plateau, at which point the cardiovascular system collapses; in a sense, it is no longer able to maintain blood pressure above 40 mmHg and requires blood or resuscitative fluid to be continually infused for the animal to survive.

Earlier measurements indicated that the complement system is activated during hemorrhage. Since C5a and C3a are anaphylatoxins that are implicated in inducing hemorrhagic shock, a theory was developed that inhibiting the anaphylatoxins would prevent the adverse affects of hemorrhagic shock. APT070 blocks complement activation on cell surface, and strongly reduce the release of the anaphylatoxins C3a and C5a. Its demonstrated benefits in our model hold promise for using this C inhibitor as adjunct therapy for hemorrhagic shock. However, further studies are necessary to bear out this proposition.

5.0 REFERENCE LIST


Permissive Hypotension Strategies for the Far-Forward Fluid Resuscitation of Significant Hemorrhage

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ABSTRACT

Acute hemorrhage accounts for about 50% of the deaths on the battlefield in conventional warfare. In addition, hemorrhage is the primary cause of death in 30% of injured soldiers who die from wounds. With future combat strategies focused around the Objective Force Warrior, greater dispersal of troops and fighting in urban settings and on non-linear battlefields, the likelihood of longer evacuation times for combat casualties is suggested. As a consequence of these conditions and the logistic limitations of weight and cube, fluid resuscitation research within the Army’s Combat Casualty Care Research Program is focused to investigate limited- or small-volume fluid resuscitation strategies, including permissive hypotension, in far-forward areas for the treatment of severe hemorrhage. The goals are to improve battlefield survival and to reduce or prevent early and late deleterious sequelae. Utilizing both anesthetized and conscious large (swine) and small (rodent) animal models, current efforts are focused on evaluating available crystalloid and colloid fluids such as lactated Ringer’s, Hespan and Hextend. In addition, other studies are evaluating hemoglobin therapeutics as well as hypertonic/hyperoncotic fluids. Preliminary data suggest that colloid containing fluids offer volume sparing effects over standard isotonic crystalloids, but under these animal model conditions, no obvious advantage of an oxygen-carrying fluid has been observed. Studies to evaluate limitations of hypertonic fluids under these conditions are currently in progress. As there are little data available on the consequences of permissive hypotension coupled with longer evacuation times for the military, these studies have important implications towards the development of optimal fluid resuscitation strategies for stabilization of the combat casualty.

1.0 INTRODUCTION

Acute hemorrhage accounts for about 50% of battlefield deaths in conventional warfare, and for 30% of casualties who die from wounds [1]. Overall these data estimate that 65% to 80% of casualties may require some amount of fluid. In addition, lessons learned by the British in the Falkland Islands War, the Israelis in their past conflicts and the Indians in their Northern India military skirmishes, confirmed that prompt resuscitation improves survival [2,3]. In a recent consensus conference, Butler et al. [4] recommended fluid resuscitation for anyone who was unconscious (suggesting a systolic blood pressure less than 50 mmHg) or for any casualty with a change in mental status.

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1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Permissive Hypotension Strategies for the Far-Forward Fluid Resuscitation of Significant Hemorrhage

It is well recognized that limitations exist in providing fluid resuscitation to the injured in far-forward combat environments. Weight and cube limitations restrict the availability of large volumes of crystalloid resuscitation fluids for far-forward use, and there can be significant time delays and failure rates in obtaining intravenous access of peripheral veins. All the above are coupled to the noise and confusion of battle. To compound the problem, evidence from experimental animals suggests that interventions to re-establish homeostasis may need to be initiated within 30 minutes after injury to assure survival, [5] offering additional challenges to attempts to improve survival on the battlefield.

Future combat scenarios under the objective (future) force where the troops are more dispersed, imply that evacuation times of casualties will be prolonged. Evacuation times that exceed 24 hr may be common, particularly if from urban environments, as was learned in Somalia [6]. It has even been suggested that the time until air evacuation from battlefields of the future could approach 96 hours. Special operations forces currently operate under the assumption that evacuation of casualties may not be possible for up to 72 hours. Taken together, the implication is that at a minimum, several hours may pass before any surgical intervention is possible to treat the injured soldier. As indicated by Bellamy, [1] mortality increased from 20% to 32% when evacuation times of casualties was increased from immediately to 24 hours. Although his data did not extend beyond 24 hours, the mortality rate would be expected to continue to rise beyond this point, but whether this rise would be linear or exponential, is unknown.

2.0 PERMISSIVE HYPOTENSION

Based on this information, one of the goals of the US Army’s Combat Casualty Care program is to develop a strategy to improve field fluid resuscitation for the treatment of significant hemorrhage in combat casualties expecting longer evacuation times and limited availability of resources. The concept of permissive hypotension, or fluid resuscitation to a blood pressure lower than normal, as a far-forward treatment strategy for special operations forces grew out of a workshop held at the 1998 Special Operations Medical Association meeting but, permissive hypotension was recognized as a reasonable approach in the care of combat casualties in both World Wars I and II [7,8]. Thus, permissive hypotension remains a logical choice for far-forward resuscitation of casualties.

Until very recently, the standard practice for trauma resuscitation in civilian urban settings involved the infusions of large volumes of fluids to try to normalize blood pressure. Today, this practice is being challenged, especially for treating hemorrhagic-shock victims with penetrating injuries [9,10]. Even for blunt trauma patients, the wisdom of rapid volume infusion is being questioned [11]. It has been argued that resuscitation to baseline or normal blood pressure can increase bleeding and worsen outcome because of severe hemodilution and disruption of newly forming blood clots. Thus, it is hoped that permissive hypotensive resuscitation can improve outcome, yet avoid these adverse hemostatic effects [9,10,12,13]. For example, studies in experimental animals have shown that in the treatment of uncontrolled hemorrhage from a vascular injury, restoring blood pressure to 40 or 60 mmHg resulted in longer survival compared to animals resuscitated to the baseline mean arterial pressure of 80 mmHg or animals that received no fluid [14,15]. Providing some fluid even before surgical repair of the injury, also appeared to produce better outcomes than delaying all fluid until after surgery [14]. Recently, a study in our laboratory showed that fluid resuscitation with lactated Ringer’s (LR) to a mean arterial pressure (MAP) of 70 mmHg in rats improved hemorrhage-induced vascular hyporeactivity to norepinephrine better than LR resuscitation to baseline MAP during the 4-hr study period [16]. Rats were anesthetized and hemorrhage to a MAP of 50 mmHg for 60 min. This degree of hemorrhaged corresponded to about 19 + 2 ml/kg body weight. The rats were then resuscitated with different fluids (Table 1) to achieve and maintain a MAP of 70 mmHg and monitored for up to 4 hr or until
death. An additional group received LR infusion to return MAP to pre-hemorrhage levels. Resuscitation to baseline MAP with LR resulted in severe hemodilution and deterioration of vascular responsiveness to norepinephrine.

Table 1: Infusion volume of each fluid to maintain MAP of 70 mm Hg after hemorrhage in rats

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Volume infused (ml/kg)</th>
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<tbody>
<tr>
<td>LR</td>
<td>156±23</td>
</tr>
<tr>
<td>HS-LR</td>
<td>100±14*</td>
</tr>
<tr>
<td>Hespan</td>
<td>17±2*</td>
</tr>
<tr>
<td>Hextend</td>
<td>24±5*</td>
</tr>
<tr>
<td>LR-BL</td>
<td>399±30*</td>
</tr>
</tbody>
</table>

1Data expressed as mean ± SEM of n=7/group
2 Lactated Ringer’s group
3 5% Hypertonic saline during first hr and LR thereafter
4 LR resuscitation to baseline MAP

*P<0.05 as compared to the LR group

Nevertheless, the adequacy of hypotensive fluid resuscitation has recently been questioned. For example, studies have suggested that hypotensive crystalloid resuscitation to a MAP of 60 to 70 may be inadequate to prevent metabolic derangements associated with hemorrhagic shock [17,18]. It should be noted that over the last decade of research into hypotensive resuscitation, the majority of these studies have only followed animals for a few hours and LR or normal saline has been the primary fluid evaluated [19,20]. However, a study in rats, [14] pigs, [15] and dogs [21] have now included an observation time of 72 hours or longer after a hypotensive period. Since not all animals in the hypotensive resuscitation groups survived in some of these studies, further investigation warrants use of different fluids, resuscitation to a higher blood pressure, or resuscitation to better physiologic endpoints in an attempt to improve outcome. The limits of permissive hypotension as a fluid resuscitation strategy remain unknown.

3.0 SMALL VOLUME RESUSCITATION

To compensate for the logistic problems of providing enough crystalloid fluids on the battlefield to resuscitate the injured soldier adequately, the U.S. Army initiated studies to investigate the potential efficacy of resuscitation fluids that could be effective in small volumes. This led to an extensive effort to evaluate 7.5% NaCl/6% Dextran-70 (HSD). Results from pre-clinical and clinical studies have shown that HSD could be at least as effective as LR for the treatment of significant hemorrhage [22-27]. In experimental animals resuscitated from a controlled hemorrhage with a bolus dose of HSD, the volumes required were only 1/10-1/12 the volume of LR to achieve similar hemodynamic effects [22-24]. The differences in volume requirements of various fluids are illustrated in Figure 1. The premise here is that for treating a 1L blood loss, 3L of LR would be the standard fluid resuscitation strategy. In contrast, only 1L of a colloid solution such as Hextend (illustrated) or Hespan would be required. Combining a colloid with a hypertonic crystalloid further reduces the fluid requirement such that only a 250 ml bag of HSD (illustrated) would provide similar resuscitation as the 3L bag of LR. The implication of this research strategy on reducing the logistic burden on the battlefield is obvious and potentially offers a wider range from hypotensive resuscitation to full restoration of baseline blood pressure, but with much smaller volumes.
The treatment of significant hemorrhage requires fluid resuscitation, but it is recognized that the presence of hypotension, environmental and tactical conditions, limited expertise of the medic and/or the presence of mass casualties can lead to significant time delays and failures in gaining access to peripheral veins in the far-forward combat arena. Based on evidence to suggest that intraosseous (IO) infusion is a viable route for the emergency injection of drugs and fluids [28] and that the technique was easy to learn by military first responders, the US Army, through in-house research activities and outside contracts, has examined the intraosseous route as an alternative means of infusing resuscitation fluids for the treatment of hemorrhagic hypotension in experimental animals [2,30-32]. These studies have focused on HSD and have observed that a single dose of HSD induced essentially identical hemodynamic effects through the IO route as the IV route [30-32]. In addition, where studies with IO infusion of isotonic crystalloids indicated that such administration could not resuscitate from hemorrhagic hypotension in a timely manner, [33] IO administration of HSD could be effective [31,32].

A pilot study was initiated to determine whether HSD, infused through the IO route, could be used in the context of permissive hypotension to resuscitate animals subjected to an uncontrolled hemorrhage [34]. Anesthetized, splenectomized animals were bled 25 ml/kg (about 37% of estimated blood volume) from the femoral artery over a 30 minute period. An uncontrolled hemorrhage was induced by pulling the aortotomy
wire, and the animal was left undisturbed for 15 minutes. Fluid resuscitation with HSD or LR was initiated through an IO sternal access device until a systolic blood pressure of 70 mmHg was achieved. Pressure was maintained at this level with the appropriate fluid over a 2-hour experimental period. The preliminary results of these studies indicated that the volume of HSD required to maintain systolic blood pressure at 70 mmHg was less than 10% of the volume of LR needed, similar to the data obtained with bolus infusions of HSD [23,24]. Although the IO technique appears relatively safe, [2,28,32] the safety of multiple infusions of hypertonic fluids has not been established [35].

4.0 CURRENT STUDIES

Currently in our laboratory, hypotensive resuscitation research has expanded to evaluate US FDA-approved fluids or other investigational products. In one study, anesthetized, splenectomized swine were hemorrhaged 20 ml/kg over a 4 min 40 sec period followed by an additional 8 ml/kg after a 30 min compensation period. The second hemorrhage was over the same time period and each hemorrhage period duplicated the blood loss profile of an uncontrolled aortotomy hemorrhage. Thus, the model mimics an uncontrolled hemorrhage, yet retains the reproducibility of a controlled hemorrhage. Also, this model is lethal if left untreated. Fluid resuscitation was begun 30 minutes after the first hemorrhage. The second hemorrhage was then begun to mimic rebleeding that may occur with resuscitation. Fluid resuscitation is continued as needed to achieve and maintain a systolic blood pressure of 80 mmHg. Animals were monitored for 3 hr after the start of fluid infusion or until death. Figure 2 illustrates preliminary results comparing LR resuscitation versus no treatment on blood pressure in these animals [36]. Of note, resuscitation with lactated Ringer’s (LR) to a systolic blood pressure of 80 mmHg resulted in 75% survival from an otherwise lethal hemorrhage, and the volumes required were 3.4 times the shed blood volume, exceeding the typical 3:1 ratio of resuscitation fluid to blood volume loss often used in standard resuscitation. These results suggest that in hypotensive resuscitation, crystalloid fluid alone may be insufficient to properly resuscitate from severe hemorrhage. Other fluids under evaluation in this study include Hespan and Hextend as colloids, and a hemoglobin therapeutic as an oxygen carrier. It should be noted that Hextend is now carried by special forces medics. One of the goals of this study was to evaluate whether any of these fluids, when used in hypotensive resuscitation, was superior in improving hemodynamic and metabolic responses to severe hemorrhage. Preliminary results in table 2 show the volumes of the different fluids necessary to achieve and maintain a systolic blood pressure of 80 mmHg, and illustrates the expected volume sparing effects of colloids compared to a crystalloid. However, these colloids resulted in similar survival rates as LR in these experiments. As described above, similar volume sparing effects of colloids were observed in table 1 to maintain MAP at 70 mmHg after hemorrhage in rats. In addition, there are parallel studies using these fluids in conscious pigs and rats to evaluate the effects of permissive hypotension for up to 24 hr. In the conscious pig model chronically instrumented, splenectomized pigs are hemorrhaged 37 ml/kg following the same uncontrolled hemorrhage profile described above. In this model, fluid resuscitation begins 10 min after hemorrhage and continues to achieve and maintain a systolic blood pressure of 80-82 mmHg. After 24 hr, the animals receive their shed blood back and are allowed to recover. Animals are monitored for up to 72 hr after start of resuscitation or until death to begin to evaluate potential complications of prolonged hypotensive resuscitation. In the conscious rat model, animals are hemorrhaged to 40 mmHg (MAP) and held there for 30 minutes. At this point they are given fluid as required to raise their MAP to 60 mmHg and they are given fluid as required to stay above this level for an additional 4 hours. Thereafter they are given sufficient fluid to raise their MAP to 80 mmHg and they are monitored for an additional 20 hours.
Figure 2: Systolic, diastolic and mean arterial pressure in hemorrhaged swine receiving either no treatment or fluid resuscitation with lactated Ringer’s (LR). Data represent mean ± standard error for 3-5 animals per group.
Table 2: Infusion volume of each fluid to maintain systolic blood pressure of 80 mmHg after hemorrhage in pigs

<table>
<thead>
<tr>
<th></th>
<th>No Resuscitation</th>
<th>LR²</th>
<th>Hespan</th>
<th>Hextend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume infused (ml/kg)</td>
<td>0</td>
<td>88.8±17.7</td>
<td>33.2±16.3*</td>
<td>33.6±9.7*</td>
</tr>
<tr>
<td>5% NaCl</td>
<td>16.0±5.9*</td>
<td>10.4±2.8*</td>
<td>29.7±0.8*</td>
<td></td>
</tr>
</tbody>
</table>

¹Data expressed as mean ± SEM of n=2-5/group
²Lactated Ringer’s group
³Hypertonic saline dextran
⁴Hemoglobin oxygen carrier fluid

* p< 0.05 from lactated Ringer’s group

5.0 CONCLUDING REMARKS

Current studies now investigate fluid resuscitation practices under the concept of permissive hypotension. However, much remains to be investigated to determine the optimal fluid that can be used in small volumes, yet improve outcomes even in situations where definitive care is delayed for many hours after injury. It is interesting to note that after years and resuscitation of thousands of patients for the treatment of hemorrhage, little reliable evidence exists to suggest how much fluid to give or the clinical endpoints to guide resuscitation [37]. Although the results of some animal studies seem promising, the long-term effects of permissive hypotension and the lasting superiority of one type of fluid over another are unknown. To this end, other studies under the Resuscitation task area of the Combat Casualty Care research program investigate the genetic responses to hemorrhage and resuscitation. Employing state-of-the-art microarray technology, these studies use genomics to evaluate the metabolic consequences of hemorrhage to provide a better understanding of the potential complications of prolonged hypotensive resuscitation, and to provide potential ways to recognize casualties that are not tolerating the prolonged hypotension. Other studies are also aimed at the early recognition of casualties that may require alternative resuscitation strategies or priority evacuation from the battlefield. In addition, working under the premise that hemorrhagic shock results in complement activation, preliminary studies in our laboratory suggest that early use of a complement inhibitor may reduce fluid needs and improve outcome in hemorrhaged rats [38]. Although evidence suggests that resuscitation to a systolic blood pressure of 80 mmHg may be inadequate to improve cerebral perfusion after head injury, [39,40] the addition of adjuncts or a “designer” fluid might improve outcome when used with permissive hypotension. As noted, to date most fluid resuscitation studies evaluating permissive hypotension have generally utilized crystalloids such as LR or normal (physiologic) saline. Recently, Burris et al. [41] suggested that at least short term outcome can be improved by resuscitating to a lower blood pressure with a hypertonic saline-hetaastarch fluid than with LR.
6.0 SUMMARY

In summary, research in our laboratory over the years with HSD and the IO administration route, as part of the Combat Casualty Care fluid resuscitation task area, suggest that innovative means can be explored in resuscitating injured soldiers from severe hemorrhage in the far-forward combat environment. It is anticipated that our research in the area of permissive hypotensive resuscitation will help identify therapeutic windows and complication rates with currently available fluids. The successful implementation of hypotensive resuscitation strategies for far-forward treatment of severe hemorrhage will conserve the limited resources available, decrease rebleeding complications and improve the likelihood that injured soldiers will reach medical treatment facilities, thereby reducing the number killed in action.

7.0 REFERENCES


Permissive Hypotension Strategies for the Far-Forward Fluid Resuscitation of Significant Hemorrhage


18. Wu X, Stezoski J, Safar P, Tisherman SA. During prolonged (6 h) uncontrolled hemorrhagic shock (UHS) with hypotensive fluid resuscitation, mean arterial pressure (MAP) must be maintained above 60-70 mmHg in rats. *Crit Care Med* 2003; 12(Suppl):A40.


ABSTRACT

OBJECTIVES: To compare the physiologic effects of BPH vs. hetastarch (HEX), the current resuscitative fluid used by U.S. Special Forces, in delayed resuscitation HS models simulating battlefield injuries. METHODS: After induction of HS in controlled (catheter withdrawal) and uncontrolled (liver injury) hemorrhage swine models, the effects of BPH, HEX, and no resuscitation (NON), followed by hospital-like care after a 4 hour “evacuation delay”, were compared. Standard physiologic parameters were followed for 72 hours. Hemostasis was evaluated by routine coagulation assays, thromboelastography, collagen/ADP-coated membrane aperture closing time, and platelet aggregation ADP-release. Leukocyte adhesion and immunophenotype were compared using FACS. Plasma cytokines were assayed by ELISA and Western Blot. RESULTS: In controlled HS, 100% (8/8) of BPH, 88% (7/8) of HEX, and 63% (5/8) of NON pigs, survived to
72 hours (p=0.04). MAP (p=0.01), SVRI (p=0.08), and MPAP (p=0.06) were higher in BPH pigs as compared to HEX. By 90 minutes, cardiac index reached baseline in the BPH group (4.2 ± 0.3 L/min/m²) but was 1.4-fold greater than baseline in the HEX group (6.7 ± 0.5 L/min/m², p<0.001). BPH pigs had a lower fluid requirement (716 vs. 1226 ml, p=0.004) in the prehospital phase. 12% of BPH vs. 100% of HEX pigs required blood transfusions (p=0.002) in the hospital phase. Although transcutaneous tissue oxygenation (TCOM) levels were higher in BPH pigs (i.e., 34.6 ± 5.0 vs. 15.2 ± 3.0 mmHg at 90 minutes, p<0.001), lactate, urine output, and creatinine levels were similar (p=NS). In uncontrolled HS, 7/8 (87.5%) BPH, 1/8 (12.5%) HEX, and 1/8 (12.5%) NON pigs survived 72 hours (BPH vs. HEX p=0.01). BPH pigs had higher systemic and pulmonary artery pressures and had comparable cardiac outputs, but were less tachycardic in comparison to HEX pigs. Baseline TCOM levels were restored more rapidly in BPH pigs (45 vs. 220 minutes) and lactic acidosis was less severe (3.2 ± 0.7 vs. 6.7 ± 4.4 at 60 minutes). Although BPH pigs received similar amounts of IV fluid, urine output was higher (2.3 ± 1.5 and 1.0 ± 1.1 ml/kg). HEX pigs had higher blood loss than BPH pigs (51.8 ± 3.2 and 43.2 ± 4.1 ml/kg respectively). In comparison with HTS, BPH had no significant effects on coagulation and platelet function parameters. In controlled HS, expression of PMN beta 2-integrins (CD11b, CD18), and the lymphocyte alpha 4-integrin (CD49d), were lower in BPH- than HEX-pigs (~2.7, 4.8, and 1.5-fold). Increased elaboration of the anti-inflammatory Th2 cytokine, IL-10, was seen in BPH pigs (~1.7-fold). The CD4/CD8 ratio and the plasma level of the pro-inflammatory cytokine, IL-2, were not significantly different. CONCLUSIONS: In comparison with HEX, BPH restored tissue oxygenation, decreased lactic acidosis, diminished proinflammatory neutrophil activation, and improved survival, without increased hemorrhage or deleterious effects on hemostasis, in controlled and uncontrolled HS models. Clinical trials should be completed to definitively compare the efficacy and safety of BPH and traditional asanguinous resuscitative fluids, for the resuscitation of HS casualties.

1.0 INTRODUCTION

Hemorrhage accounts for the preponderance of salvageable combat casualty mortality, especially with evacuation delay. After securing hemostasis, blood transfusion may be life-saving pending surgical stabilization, however, transfusion capability is costly, rarely supports echelons I/II units and USN ships, and the walking blood bank is impractical. Thus, a “bridging” volume replacement fluid with O₂ transporting properties is urgently needed to improve survival of hemorrhagic shock casualties aboard US Navy vessels and on the battlefield of the 21st century. A low volume hemoglobin substitute that is room temperature stable, has no blood typing and banking requirements (i.e., universal compatibility), is easy to administer, and is efficacious and safe as a “bridging” replacement fluid for early resuscitation of hemorrhagic shock (HS) combat casualties, should fill that current therapeutic hole [1]. Hemoglobin based oxygen carriers (HBOC) transport O₂ and provide colloid volume replacement (something in between crystalloid fluid and RBC transfusion) and thus, they are potentially ideal resuscitative fluids for hemorrhagic shock casualties in the field, aboard US Navy vessels, and for Special and other operations in which delayed blood transfusion and prolonged evacuation times are expected.

2.0 STUDY OBJECTIVE

To compare the efficacy and safety of HBOC-201 (bovine polymerised hemoglobin [BPH], Hemopure®, Biopure Corp. MA) with 6% hetastarch in balanced salt solution (HEX, Hextend®, Abbot Laboratories, IL) and no resuscitation in swine models of HS with controlled and uncontrolled hemorrhage incorporating a four hour delay to definitive medical treatment (Figure 1).
3.0 MATERIALS AND METHODS

3.1 Animal husbandry and preparation

The experiments reported herein were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals”, Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the NMRC Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC). Swine were allowed to acclimate to the animal facility for 1 week with free access to feed and water. Feed and water were withheld 12-14 hours prior to initiation of the experiment to induce dehydration.

3.2 Injury, hemorrhage and resuscitation procedures

3.2.1 Anesthesia: Animals were sedated and anesthesia induced with intramuscular ketamine hydrochloride (33 mg/kg), atropine sulfate (0.05 mg/kg), and mask ventilation with isoflurane (3.0%) and 100% O₂ to facilitate endotracheal intubation. Anesthesia was maintained via isoflurane (1%-2.5%) in 21% O₂. Pigs were ventilated for anesthesia-induced apnea (Ohmeda 7800 series ventilator, Datex, Madison, WI) (12-15 breaths/minute; tidal volume 5-10 ml/kg; and FiO₂ 0.21).

3.2.2 Instrumentation: The right external jugular vein and carotid artery were dissected and isolated. A 9 F introducer sheath was placed in the external jugular vein using Seldinger technique and a 7.5 F pulmonary artery catheter (PAC; Edwards Life Sciences, Irvine, CA) was inserted for continuous hemodynamic and cardiac output (CO) monitoring. A 20G angiocath was placed in the carotid artery and mean arterial pressure (MAP) was continuously transduced. Urine was collected via bladder catheterization. All surgical procedures were performed under aseptic techniques.

3.2.3 Tissue Injury and Controlled Hemorrhage: A 3-5 cm lower abdominal incision was made and the left rectus abdominis muscle located. The rectus sheath was bluntly mobilized and a Kocher clamp placed on a standardized portion of the muscle in the center of the incision. The Kocher clamp was closed for 5 minutes to create a soft tissue injury and pigs were hemorrhaged 40% or 55% of estimated total blood volume (EBV) via the external jugular vein and/or the carotid artery to induce HS. All “shed” blood was collected in blood bags containing citrate phosphate dextrose (CLX, Medsep Corp., Covina, CA) for later re-infusion.
3.2.4 Liver Injury and Uncontrolled Hemorrhage: A standardized liver injury was created by placing a ring clamp over the left lower lobe, ~50% in width and ~0.75-2.0" from the apex, adjusting for relative size of the liver and weight of the pig. The clamp was closed and an 11 blade was used to lacerate the lobe from the top of the clamp through the remaining width. The liver injury denoted the start of the pre-hospital phase (Time 0). After 1 minute, the clamp was removed and the remaining tissue excised, resulting in ~25% lobectomy, consistent with a grade III liver injury. Bleeding was spontaneous, unhampered, removed via intraperitoneal suction, and quantified by weight.

3.2.5 Resuscitation: Pigs were randomly allocated to one of three treatment groups: Hemoglobin based oxygen carrier (HBOC, HBOC-201®, Biopure Corp., Cambridge, MA); 6% hetastarch in LR (HEX, Hextend®, Abbott Laboratories, Abbot Park, IL); or no fluids (NON). Five minutes following controlled hemorrhage or at 15 minutes into uncontrolled hemorrhage, resuscitated pigs were administered 10 ml/kg of HBOC or HEX over 10 minutes. Additional infusions of 5 ml/kg were provided at 30, 60, 120, and 180 minutes post-injury if hypotension (MAP < 60 mmHg) or tachycardia (HR > baseline value [Time 0]) were observed. Fluids were infused at room temperature.

3.3 Post-operative clinical observations and treatment
Hospital arrival was simulated at 4 hours. Animals were administered 10 ml/kg autologous shed blood or allogeneic packed red blood cells (PRBC) for anemia (Hb <7 g/dL) and/or 10-20 ml/kg normal saline (NS) for hypotension. 13 mg/kg cephazolin (antibiotic), and 0.01 mg/kg buprenorphine (analgesic), were administered. The PAC was removed and the jugular vein introducer was secured for postoperative blood sampling and fluid administration. The arterial and bladder catheters removed and surgical sites repaired as necessary. Surgical incisions were closed and surgical dressings applied. Animals were extubated and recovered from anesthesia. Vital signs and general status were assessed 24, 48, and 72 hours post-injury. Pigs received 10 ml/kg NS, autologous shed blood or PRBCs as needed for anemia or hypotension as well as antibiotics and analgesia. Pigs were euthanized 72 hours post-injury for necropsy and histological analysis.

3.4 Data Collection and Analysis
Standard invasive and noninvasive hemodynamic parameters were monitored for 240 minutes during the simulated pre-hospital phase. In the liver injury, blood loss was measured by weighing collection canisters at 5 and 15 minutes (pre-resuscitation), and 20, 30, 60, and 240 minutes (post-resuscitation). Sponge weight was included in total post-resuscitation blood loss. Transcutaneous tissue oxygenation (TCOM or tcpO₂) was noninvasively measured with a TCM4 Tina monitor (Radiometer, Copenhagen, Denmark) using four Clark type polarographic electrodes (data represent mean values) positioned bilaterally on the upper torso and on the inner thighs. Blood gases (ABG and MVBG) were measured with an automatic analyzer (ABL 705, Radiometer, Copenhagen, Denmark). Blood samples were collected for complete blood counts (CBC, Pentra 60 C+, ABX, France), chemistries (Vitros 250 Analyzer, Ortho), and coagulation assessments (PT, PTT, StatCompact Diagnostica Stago, Asniere, France). Thromboelastography (TEG) was used to evaluate clot formation dynamics (TEG, Haemostasis Analyzer, Haemoscope Corp, Skokie, IL). 20 µl of 0.25 mM CaCl₂ and 340 µl of whole blood were pipetted into an oscillating cup. A pin connected to a torsion wire transmits the motion signal generated by clot retraction; this is integrated into a digitally based score. The reaction time (TEG-R) corresponds with initiation of fibrin formation and depends mainly on plasma factors. TEG-K and TEG-α are measurements of the kinetics of clot formation and reflect platelet adhesion on newly formed fibrin and rate of fibrin polymerization, respectively. TEG-MA measures maximal clot strength and shear modulus, and is dependent on platelet number and function, as well as plasma proteins to a lesser extent [2]. TEG-Ly (done at T 30 minutes) measures fibrinolysis due to tissue plasminogen activator (t-PA) activity, and
is indicative of the presence of fibrin degradation products (FDP). Computed indices such as clot firmness (TEG-G) and the coagulation index (TEG-CI) are also reported. TEG-CI was defined as TEG CI = 0.0184 * TEG-K + 0.1655 * TEG-MA – 0.0241 * TEG-α - 0.2454 * TEG-R - 5.022. [15]. TEG-G was derived as TEG-G= 5000*TEG-MA/(100-TEG-MA). Leukocyte adhesion and immunophenotype were compared using FACS. Plasma cytokines were assayed by ELISA and Western Blot.

3.5 Statistics

Physiology data are expressed as mean ± standard error of the mean (SEM) for animals alive at time of measurement. Hematology data are presented as mean ± standard deviation or as otherwise stated. Data were analysed with a two-tailed paired Student’s t-test, or equal variance Student’s t-test. Statistical significance was considered for p< 0.05.

4.0 RESULTS

4.1 Physiology

4.1.1 Tissue Injury with 40% EBV Controlled Hemorrhage: 100% (8/8) of HBOC-201-, 88% (7/8) of HEX-, and 63% (5/8) of NON-resuscitated pigs, survived to 72 hrs (Figure 2, left column). Mean arterial pressure (MAP), systemic vascular resistance (SVRI), and mean pulmonary arterial pressure (MPAP) were higher in the HBOC-201 group. By 90 minutes, cardiac index (CI) was restored to baseline in the HBOC-201 group and was 1.4-fold greater than baseline in the HEX-group following resuscitation. HBOC-201- treated pigs had a lower fluid requirement than HEX-treated pigs (716 vs. 1226 ml) in the pre-hospital phase and required fewer blood transfusions (12% vs. 100%) in the hospital phase. Urine output and creatinine levels were comparable in HBOC-201- and HEX- treated pigs. Tissue oxygenation levels (TCOM) were highest in the HBOC-201 group. In summary, except for decreased CI, the hemodynamics, lactic acid, base deficit, cutaneous tissue oxygenation, urine output, and survival were equivalent or better in HBOC-201-treated swine, despite lower IV fluid and blood transfusion requirements.

4.1.2 Liver Injury with Uncontrolled Hemorrhage: In order to simulate resuscitation in combat with severely delayed evacuation, NMRC evaluated HBOC-201 in a liver crush/laceration injury model (similar to Manning and Katz’s model, [3], [4]) with extension of evacuation delay to 4 hours, spontaneous ventilation (FiO2 0.21), and with accurate quantification of hemorrhage [5]. HBOC-201 was compared with HEX and no treatment (NON). Additional arterial catheter blood withdrawal was not done and hemorrhage into the abdominal cavity was measured. Resuscitation fluids were infused for MAP < 60 mm Hg and/or tachycardia. HBOC-201 stabilized hemodynamics, increased cutaneous tissue oxygenation, decreased base deficit and lactic acidosis, increased survival, decreased blood loss, and decreased fluid and blood transfusion requirements (Figure 2, right column).
Figure 2: Physiology data for controlled (left) and uncontrolled (right) hemorrhage models

- **Survival**
  - HBOC-201, HEX, NON

- **Mean Arterial Pressure**
  - HBOC-201, HEX, NON

- **Cardiac Index**
  - HBOC-201, HEX, NON

- **Tissue Oxygenation**
  - HBOC-201, HEX, NON

- **Mean Fluid Index**
  - HBOC-201, HEX, NON

- **Blood Loss**
  - Post-resuscitation to 60 min, 4 hours, Total Blood Loss

- **Figure 2**
4.2 Hematology: Thromboelastography (TEG):

In both HS models (controlled and uncontrolled hemorrhage), the natural course of HS without resuscitation (NON-pigs) was characterized by an absence of significant changes in TEG-R, TEG-al, and TEG-MA (TEG-mean amplitude) (the former reflecting coagulopathic and latter reflecting thrombopathic alterations). In contrast, TEG-G and TEG-CI (clotting index) remained stable during the simulated prehospital phase but increased subsequently during the simulated hospital phase (Figure 3).

In the HS model with controlled hemorrhage, TEG-R began to increase in HBOC-201-pigs during the late prehospital phase and was greater than in NON- or HEX resuscitated pigs at 24 hours. A similar but mirror-image trend was seen for TEG-al. TEG-MA values were similar across treatment groups but a trend was appreciated with NON>HBOC-201>HEX. TEG-G increased during the hospital phase at 48-72 hours in HBOC-201-pigs. The TEG-CI appeared to be more affected in HBOC-201-resuscitated pigs, especially at 24 hours.

In the HS model with uncontrolled hemorrhage, as aforementioned, comparisons across treatment groups is hampered by the differential survival rates in the HBOC-201 and control groups. Nevertheless, possible trends were noted towards higher TEG-MA, TEG-G, and TEG-CI in HBOC-201- in comparison with NON or HEX resuscitated pigs. In the HBOC-201 group, in which survival was high, despite lower fluid requirements (and thus less hemodilution), the overall pattern of TEG responses was similar to that noted in controlled hemorrhage. TEG-R increased and TEG-al decreased by 24 hours; TEG-MA decreased slightly initially and then stabilized; TEG-G and TEG-CI decreased during the pre-hospital phase and stabilized during the hospital phase (TEG-G exceed baseline by 24 hours).

4.3 Immunology: Leukocyte adhesion markers

In the swine HS model with controlled hemorrhage, the natural course (NON-treatment) of HS was shown be characterized by ~2-2.5-fold increased expression of PMN beta-2 integrins, CD11 and CD18 (Figure 4). Resuscitation with HEX increased beta-2 integrin expression even higher to ~3-5-fold the baseline value; in contrast, HBOC-201-treatment averted a significant rise in PMN beta-2 expression (p=0.001 and 0.01, respectively, at 4 hours). Lymphocyte alpha-4 integrin expression increased >1.5-fold in HEX-, but did not
increase in HBOC-201-treated pigs (p>0.05) (PMN alpha-4 integrin expression was not quantified in the controlled hemorrhage model).

Figure 4. CD11 and CD18 data from controlled hemorrhage model

In contrast to observations in the controlled hemorrhage model, in the swine HS model with uncontrolled hemorrhage, expression of PMN CD11b and CD18 and of lymphocytic CD49 increased ~2-3-fold in HBOC-201-pigs; PMN CD49 remained stable. As there were few survivors in the control groups in this severe HS model, treatment-effect comparisons across groups could not be completed. However, significant effects on adhesion marker expression were not obvious in NON- and HEX- resuscitated pigs (except possibly for PMN CD49).

5.0 DISCUSSION AND CONCLUSIONS

As 90% of war fatalities occur on the battlefield and 60% of potentially salvageable deaths are related to rapid exsanguination, immediate restoration of blood volume and O₂ content may be lifesaving, but expeditious blood transfusion is rarely available for combat casualties (especially in the asymmetric battlefield) [6]. Pre-evacuation standard of care relies on attempts at extrinsic hemostasis and resuscitation with asanguinous fluids, but complications of free radical generation, immune activation, reperfusion injury, irreversible shock, MOF, and coagulopathy and thrombopathy are common. Thus, a whole blood-like bridging volume replacement fluid with O₂ transporting properties as well as hemostatic, immunomodulating, antiapoptotic, and antioxidant properties that does not require a cold chain, is likely to improve outcome of HS casualties. Moreover, in keeping with requirements of operational agility, efficiency, and limited medical footprint in SeaPower 21 (i.e., Sea Basing), optimal stabilization of combat casualties is vital. Recent work in this area has led to the development of a comprehensive database of the physiological, hematological, immunological, and histopathological consequences of HS with controlled and uncontrolled hemorrhage in swine.
In these experiments, we have demonstrated that HBOC-201 improved hemodynamics and tissue oxygenation, decreased lactic acidosis, and improved survival in HS swine models. Despite moderate vasoactivity, hemorrhage was not increased in HS with uncontrolled hemorrhage due to solid organ injury.

TEG data from the controlled hemorrhage model suggest that in comparison with controls, HBOC-201-resuscitation may induce mild coagulopathy but decrease thrombopathy. The pattern of TEG data from the uncontrolled hemorrhage model was similar except that in comparison with HEX-resuscitation, TEG-CI was higher with HBOC-201, suggesting diminished coagulopathy. Taking together, these data suggest that the hemostatic effects of HBOC-201-resuscitation in HS are mixed and are unlikely to be clinically significant.

Furthermore, in moderately severe HS with controlled hemorrhage, resuscitation with HBOC-201 was “immuno-protective”, preventing significant increases in leukocyte adhesion marker expression. In the severe HS model with uncontrolled hemorrhage, leukocyte adhesion markers increased despite HBOC-201 resuscitation, however, the relative increase, in comparison with controls, cannot be assessed in this model. As Ortegon showed in vitro that HBOC-201 stimulation of beta-2 integrin expression is dose-dependent, higher blood concentrations of HBOC-201 in the uncontrolled hemorrhage model may explain elevated adhesion marker expression. However, it is hypothesized that HBOC-201 may in fact have been immuno-protective but that early mortality in the comparator groups preclude such comparisons.

In comparison with HEX, HBOC-201 restored tissue oxygenation, decreased lactic acidosis, diminished pro-inflammatory neutrophil activation, and improved survival, without increased hemorrhage or deleterious effects on hemostasis, in controlled and uncontrolled HS models. Mild enhancement of HS-induced coagulopathic changes were observed. HBOC-201 increased PMN and lymphocyte adhesion marker expression and apoptosis, and elaboration of both pro- and anti-inflammatory cytokines. Clinical trials should be completed to definitively compare the efficacy and safety of HBOC-201 and traditional asanguinous resuscitative fluids for the resuscitation of HS casualties.

6.0 ACKNOWLEDGEMENTS

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The opinions contained herein are the ones of the authors and are not to be construed as official or reflecting the views of the Navy department, or Department of Defense, or the U.S. Government.

The experiments reported herein were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals”, Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the WRAIR/NMRC Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the American Association for Accreditation for Laboratory Animal Care (AALAC).
7.0 REFERENCES


Orthopaedic Field Experience at a Level II Navy Surgical Facility during Operation Iraqi Freedom

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ABSTRACT

A review of the orthopaedic surgical experience at a level II Navy field hospital during Operation Iraqi Freedom was undertaken. A retrospective data review was performed to evaluate include demographics of casualties treated; mechanism of injury; and procedures performed.

Results confirm that a majority of the injuries sustained on the battlefield will be to the musculoskeletal system. Both battle and non-battle injuries were administered to. The surgical environment remains austere at the level of the echelon II field hospital, requiring tolerance and improvisation. Management of the wounds remains unchanged, i.e. debridement and stabilization, and may be referred to in present day terms as damage control orthopaedics. Our results differ from the etiology of the injuries seen from other reports of recent campaigns. The majority of the battle injuries attended to at this facility were due to missile wounds as opposed to blast injuries as seen in other recent campaigns. Despite this the majority of wounds remained orthopaedic. The Orthopaedic surgeon is a vital asset to our fighting force in the field.

1.0 OBJECTIVES

Review of the field orthopaedic surgical experience at a Navy level II facility assigned to the US Marine Corps during Operation Iraqi Freedom.

1.1 METHODS

A retrospective review was performed of the orthopaedic procedures performed at Bravo Surgical Company covering the period of 3 April 2003 to 4 May 2003 (28 operational days). Data was retrieved from operating room and personal logs. The data reviewed included all surgical cases performed in the Bravo Surgical Co.
OR and the orthopaedic procedures performed at Forward Resuscitative Surgical System 5 (FRSS 5) while it was co-located with Bravo Surgical Co (while the FRSS and Surgical Company were co-located all patients were staged through the Surgical Company’s resuscitation area, but varied as to which operating room they were taken to).

The data was reviewed to evaluate: (1) casualties attended to by an orthopaedic surgeon; (2) make-up of casualties i.e. Iraqi vs. U.S. Forces; (3) procedures performed; (4) cause of injury i.e. battlefield or non-battle injuries. Only those patients with which direct contact was made and who subsequently received intervention by the orthopaedic surgeon are presented. Patients managed via verbal consultation with non-orthopaedic colleagues are not presented.

The environment was a Navy level II surgical facility (i.e. field hospital) in support of the US Marine Corps during Operation Iraqi Freedom. Tents were used for resuscitations areas, operating rooms (Fig. 1a), and holding wards. The floors were the desert sand and included unexpected visitors (Figs. 1b and 1c). The mission was for the Surgical Company to be in position so that life and limb saving procedures could be performed prior to the patients being transported to more definitive care. Equipment available included digital x-rays, military individually packed external fixation devices (Fig. 2), dressing material, and rudimentary surgical equipment (transportability being the limiting factor). The Surgical Company has a 72-hour holding capacity by doctrine and as such all attempts were made to transfer patients to higher levels of care within that constraint.

1.1.1 RESULTS

Bravo Surgical Co. had 66 casualties treated in the operating room. 41/66 (62%) of these casualties were attended to by an orthopaedic surgeon (Fig. 3), a general surgeon attended to the remaining casualties. Staffing of Bravo Surgical Co included 2 general surgeons, 3 ob/gyns, and 1 orthopaedic surgeon. Staffing of FRSS 5 included 1 general surgeon and 1 orthopaedic surgeon.

An orthopaedic surgeon treated a total of 53 casualties from 3 April 2003 to 4 May 2003 (Table 1); on 3 April and from 8 April to 13 April Bravo Surgical Company and FRSS 5 were co-located and both authors attended to procedures performed on those days. 27/53 (51%) of the patients and 41% of the procedures reviewed were attended to by both authors (AT and CE), the remaining casualties reviewed were attended to by AT.

41 casualties were treated in the Bravo Surgical Co. OR, 7 casualties in the FRSS 5 OR, and 5 casualties were treated at Bravo Surgical Co outside the formal operating room (Fig. 4). 26/53 (49%) of the casualties treated were Iraqi; 27/53 (51%) were U.S. Forces (Fig. 5). 39/53 (74%) of the casualties sustained battlefield injuries: 27 from missile wounds, and 12 from blast injuries (Fig. 7). 14/53 (26%) of the casualties sustained non-battle injuries: 6 MVA/MCA, and 8 occupational or recreational injuries (Fig. 8). No patient was operated on more than once, as all casualties (Iraqi and US), were transferred to higher echelon of care within 48 hours.

124 total procedures were performed on the 53 casualties. The procedures included: fasciotomy /compartment release (61); I & D and stabilization of open wounds / fractures (24); amputations (15); ex-fix (9); arthrotomy (6); closed reduction and stabilization (6); radial artery repair (1); sagittal band repair (1); removal of hardware (1) (Fig. 9). All wounds were left open and dressed with a wet to dry dressing. 79/124 (63%) procedures on 35/53 (66%) of the casualties were performed in the first 11 days (9 operational days). Further delineation, by body region, of fasciotomies / compartment releases, amputations, and external fixators can be seen in Figs. 10, 11, and 12 respectively.

External fixators were limited in supply and were used most often to manage unstable injuries that were not otherwise amenable to splinting (Figs. 12a and 12b). Severity of wounds and their management are represented in Figs. 13a through 15c.
With some severe wounds improvisation was necessary for optimal management (Figs. 15a, 15b and 15c). In this case dental acrylic was used, as it was incidentally discovered that the material used by our dental colleagues for capping teeth was similar in character to PMMA used in orthopaedic practice.

1.1.1.1 CONCLUSIONS

Shortfalls in this review include lack of follow-up as all pts were transferred from our facility in short order and received further care at the next echelon of care. Another deficiency is that data on all orthopaedic injuries that were treated at the Surgical Company are not presented, as many of the non-operative orthopaedic injuries were treated by non-orthopaedic colleagues, i.e. FP’s, Dentists, and PA’s via verbal consultation since the tempo of operations was too great to allow for direct intervention by the surgeon.

A consequence of any military campaign will be taking of casualties. Recent campaigns have shown that 60-70% of battlefield injuries will be to the musculoskeletal system\(^1\)\(^-\)\(^5\). The majority of penetrating injuries sustained in recent wars are reported to have been the result of blast injuries.\(^1\)\(^,\)\(^3\)\(^,\)\(^6\) Our experience does confirm that the majority of injuries sustained on the battlefield are to the musculoskeletal system. The cause, however, of the majority of wounds was a result of missile rather than blast injuries as seen previously. This was probably due to the way this campaign was waged, with an early and heavy ground assault. The orthopaedic surgeon is an essential and vital asset in the field setting. The majority of the surgical expertise that will be needed in the field for future campaigns will be from an orthopaedist.

The type of surgery performed could be called damage control orthopaedics, in keeping with the principles used to manage femur fractures with temporary external fixators prior to definitive osteosynthesis in the multiply injured civilian trauma patient.\(^7\)\(^-\)\(^11\) The same principles are applied in the field (i.e. temporary stabilization with delayed definitive fracture fixation). The limiting factor in the field not only being the severity of the injuries, but also of the environment in which the treatment is rendered. The flow of casualties, as expected, followed the pace of the battles being waged and our experience showed the high intensity of the battles being waged in a short duration of time. Despite advances in our specialty, modern day field orthopaedics remains a practice in an extremely austere environment with only rudimentary and limited equipment requires adherence to the principles of battlefield wound management\(^2\)\(^,\)\(^12\) and necessitates tolerance, improvisation and innovation on the part of the surgeon.

Fig. 1a Operating Room
Orthopaedic Field Experience at a Level II
Navy Surgical Facility during Operation Iraqi Freedom

Fig. 1b Operating Room

Fig. 1c Operating Room

Fig. 2 Individually packed military external fixator
Orthopaedic Field Experience at a Level II Navy Surgical Facility during Operation Iraqi Freedom

Fig. 3

Casualties Treated in Bravo OR

Fig. 4

Patients Operated Upon by Orthopaedic Surgeon

Fig. 5

US vs Iraqi

Total number of patients

US Forces

Iraqi
Orthopaedic Field Experience at a Level II Navy Surgical Facility during Operation Iraqi Freedom

Fig. 6

Battle vs Non-Battle Injuries

- Pts with Battle Injuries: 39
- Pts with Non-Battle Injuries: 14
- Total number of Patients: 53

Fig. 7

Battle Injuries

- Total # of Patients: 53
- Pts with Blast Injuries: 16
- Pts with Missile Injuries: 37

Fig. 8

Non-Battle Injuries

- Total # of Patients: 53
- Recreational: 2
- Occupational: 10
- MCA: 1
- MVA: 14
Orthopaedic Procedures Performed

- Fasciotomy / Compartment Releases: 61
- Amputations: 24
- Application of External Fixators: 15
- Closed Reduction and Stabilization: 9
- Arthrotomy: 6
- Radial Artery Repair: 6
- Sagittal Band Repair: 1
- Removal of Hardware: 1
- Total # of Procedures: 124

Fig. 9
Orthopaedic Field Experience at a Level II Navy Surgical Facility during Operation Iraqi Freedom

**Fig. 10**

**Fasciotomy / Compartment Release**

<table>
<thead>
<tr>
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<tr>
<td>Thigh</td>
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<tr>
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<tr>
<td>Foot</td>
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</tr>
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</tr>
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<td>Hand</td>
<td>12</td>
</tr>
<tr>
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</tr>
<tr>
<td>Guyon Canal</td>
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<tr>
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**Fig. 11**

**Amputations**

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<tr>
<td>Talus</td>
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<td>Total Amputations</td>
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</table>
Fig. 12a

Fig. 12b fasciotomy and external fixator bridging elbow for GSW to distal humerus
Fig. 13a GSW to Hand

Fig. 13b wound debrided & wrist disarticulation performed

Fig. 14a High Energy GSW to knee with 2º injuries to proximal tibia from bone fragments. Wounds grossly contaminated with dirt, rocks, etc.
Fig. 14b debridement and stabilization prior to transfer completed

Fig. 15a 4x6cm segmental defect radius and ulna s/p high energy GSW
Fig. 15b placement of intercalary spacer using dental acrylic

Fig. 15c dental acrylic
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References

Le Percy Fx un fixateur externe mono-latéral à usage unique utilisable en traumatologie civile et militaire

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RESUME

L’intérêt de cette communication est de faire partager l’expérience de trois Service de chirurgie orthopédique militaires Français dans l’utilisation d’un nouveau type de fixateur externe spécialement développé pour la traumatologie : «Le Percy Fx».

Le fixateur externe reste une arme de choix pour le traitement des traumatismes balistiques des membres. Depuis les années 80, le service de Santé des Armées Français a développé conjointement avec certains chirurgiens orthopédistes une gamme spécifique de fixateurs externes : «Le FESSA» : Fixateur Externe du Service de Santé des Armées. Si ce matériel constitue encore la référence militaire en matière de fixateur externe pour le traitement des fractures ouvertes prises en charge dans les « trauma-centre » militaires Français et dans les antennes chirurgicales il n’en reste pas moins qu’il a certains inconvénients. En effet, réalisé totalement en métal donc lourd il offre peu de latitude dans l’implantation des fiches et il n’est pas compatible avec d’autres fixateurs utilisés par les services de santé des pays de l’OTAN. Utilisant comme base de travail le cahier des charges du «fixateur externe universel» défini par les membres de l’OTAN, les chirurgiens orthopédistes de l’hôpital d’Instruction des Armées Percy ont mis au point à l’aide du département de recherche et de développement du laboratoire DEPUY un nouveau type de fixateur externe pour la prise en charge des fracas balistiques des membres d’origine civile ou militaire. Le résultat est un nouveau type de fixateur externe mono-latéral d’usage unique, radio-transparent et amagnétique : le « Percy Fx ».

Le Percy Fx un fixateur externe mono-latéral
à usage unique utilisable en traumatologie civile et militaire

Ce fixateur, dédié uniquement à la traumatologie et ses complications est un fixateur mono-latéral, utilisant peu de composant compatibles à la fois pour les traumatisms survenant au membre supérieur et au membre inférieur. Presque complètement radio-transparent il est réalisé en fibre de carbone et en matière composite. Une étude biomécanique a démontré une rigidité intermédiaire entre le FESSA® (+ rigide) et l’ORTHOFIX® (- rigide). Après qu’une étude prospective clinique de 26 cas réalisés en accord avec les lois bioéthiques Françaises ait montré l’intérêt de ce fixateur, trois équipes chirurgicales l’ont utilisé pour traiter des fractures ouvertes, des fractures comminutives, et des infections. Une série rétrospective de 100 cas est présentée par les auteurs avec des résultats comparables aux séries de la littératures.

Aussi efficace que les autres fixateurs externes, le « Percy Fx » apporte des avantages peu fréquents : Radio-transparent, amagnétique, compatible avec de nombreux fixateurs, son faible coût rend possible un usage unique. Par ce fait, il nous semble particulièrement adapté à la prise en charge des traumatismes ouverts des membres en pratique civile et militaire.

1.0 INTRODUCTION

Le fixateur externe est depuis son invention un outil incontournable dans la prise en charge des blessés de guerre des membres. Dès les années 80 le Service de Santé français s’est doté d’un matériel spécifique1,2 qui a démontré depuis longtemps sa fiabilité en traumatologie. Toutefois, le poids important de ce matériel, son caractère très directif dans l’implantation des fiches et la volonté de maîtriser au mieux les risques d’infections nosocomiales ont motivé l’équipe de chirurgiens orthopédiste de l’hôpital Percy à mettre au point un nouveau concept de fixateur externe destiné à la traumatologie : « le Percy Fx »2. Après avoir exposé les différentes étapes franchies jusqu’à la mise au point définitive du « Percy Fx » les auteurs rapportent une étude clinique rétrospective de 100 cas.

2.0 MATÉRIEL ET MÉTHODES

2.1 Présentation du matériel :

En 1997 l’équipe de chirurgiens orthopédistes de l’hôpital Percy a mis au point un nouveau type de fixateur externe avec l’aide technique du département de recherche et développement du laboratoire DEPUY : « le Percy Fx »3,4. Il s’agit d’un fixateur externe mono-latéral inspiré du FESSA (Fixateur Externe du Service de Santé des Armées). Ce fixateur est constitué de barres de carbone à haute résistance de 12 et 18 mm de diamètre (fig 1). Celles ci sont reliées à des fiches auto taraudeuses auto perforantes cylindriques en acier 316 L de 3, 4,5 et 6 mm de diamètre implantées dans l’os (fig 2) par un système simple de plaquettes porte fiche crantées Pf1,Pf2 (fig 3) solidarisées à un collier de diamètre 18 ou 12 mm cranté lui aussi (fig 4) « clippé » sur la barre (fig 5). Plaquettes et colliers réalisés en matière composite rechargée en fibre de verre sont solidarisés par des vis de 6 mm de diamètre et de 45 ou 60 mm de long (fig 6). Ce sont avec les fiches les seuls éléments métalliques donc radio-opaques du dispositif. La possibilité pour les colliers de glisser le long des barres mais aussi de tourner autour et la mobilité de l’interface collier plaquettes permet une orientations des fiches dans les trois plans de l’espace. De petites barres traversées de part en part par une longue vis coaxiale de 120 mm de long constituent les « barres de renvoie épiphysaire » (fig 7) permettant les montages épiphyso-métaphysio-diaphysaire. L’ensemble des composants permet un nombre innombrable de montages tout en laissant au chirurgien une grande liberté dans l’implantation des fiches. Le matériel est servi par un ancillaire comprenant un vilebrequin à nez interchangeable adapté au diamètre des fiches, un manche de tournevis à cliquet permettant le serrage et desserrage simple et rapide des vis, enfin des guides gigognes protecteurs de partie molles (fig 8). Ce fixateur constitue un matériel polyvalent, modulaire, de faible coût,
léger amagnétique en partie radio-transparent et appareillable à de nombreux fixateurs utilisé en Europe et aux États Unis.

2.2 Étude biomécanique :

Pour valider le concept une étude biomécanique a été réalisée dans le service du Professeur Lavaste à l’Ecole Nationale Supérieure des Arts et métiers de Paris. Elle comprenait une étude statique des différents montages et dynamique des différents éléments de liaison. L’étude statique s’est prolongée de 1997 à 1999 comparant la résistance tridimensionnelle des éléments réalisés en carbone en aluminium et en acier et ceci par rapport aux résultats obtenus lors d’études précédentes sur d’autres fixateurs déjà commercialisés. L’étude dynamique effectuée en 1999 avait pour but de déterminer la résistance en fatigue des pièces de liaison (colliers de serrage, plaquettes porte fiche). Un banc d’essai composé d’une machine de traction compression INSTRON 1185-IOT (fig 9) et de son armoire de commande associée à 6 capteurs couplés à une carte d’acquisition STYREL ST816 ont permis de faire des mesures statiques. Un autre banc d’essai utilisant une machine de traction compression INSTRON 8501 a permis de tester la rigidité dynamique des différents montages en comparaison par rapport aux autres fixateurs. L’étude initiale réalisée en 1997 a permis de valider le concept d’utilisation de barre de carbone en lieu et place du métal utilisé jusqu’alors. Les deux études effectuées en 1998 et 1999 ont montré une résistance des liaisons à plus de un million de cycles à 5 Hz d’une force appliquée de 70% de la charge maximale sans dégradation irréversible. L’ensemble de ces résultats permet de situer le Percy Fx entre le FESSA (+ rigide) et l’ORTHOFIX (- rigide) en matière de résistance.

2.3 Étude clinique préliminaire :

Une étude prospective de 24 cas réalisée conformément aux lois de bioéthique Françaises a permis de valider cliniquement le concept. Dix huit hommes et 6 femmes ont bénéficiés de 24 montages utilisant le Percy Fx 2 fois au bassin, 4 fois au fémur, 8 fois au tibia, 6 fois à la cheville, 4 fois au poignet et à l’avant bras. Cette étude a permis de répondre aux questions du cahier des charges en montrant une implantation rapide, une polyvalence adaptée aux différents types de situations rencontrées en clinique, une pose simple, une possibilité conservée de traitements adjuvant (lambeaux, greffes osseuses) et une durée de traitement comparable aux autres fixateurs. Patients et chirurgiens semblent unanimes pour saluer les avantages de ce fixateur.

2.4 Série :

Après ces premiers résultats encourageants le « Percy Fx » a été utilisé de façon systématique dans plusieurs hôpitaux militaires Français. La série rétrospective comprend 100 cas opérés dans trois hôpitaux d’instruction différents : HIA Percy, HIA Lavéran, HIA St Anne. Il s’agissait de patients présentant une fracture ouverte (fig 10) grave une fracture fermée complexe comminutive (fig 11) ou une infection sur matériel d’ostéosynthèse interne pour lesquels l’indication d’une fixation externe avait été retenue selon des critères habituels. Cette série comprend 68 hommes pour 32 femmes d’un âge moyen de 49 ans (extrêmes entre 19 et 97). On dénombre 55 fractures ouvertes pour 34 fractures fermées comminutives , et 16 poses de fixateur pour sépsis. 36 Montages ont été réalisés au membre supérieur (1 épaule, 2 bras, 3 coudes (fig 12), 4 avant bras, 23 poignets) et 73 au membre inférieur (6 bassins, 1 hanche, 7 fémurs, 3 genoux, 34 jambes(fig 13), 22 chevilles. 85 patients ont été traités en première intention et 15 en traitement secondaire prenant le relais d’un protocole thérapeutique en cours. La durée moyenne de maintient du fixateur a été de 4 mois (comprise entre 21 jours et 485 jours), respectivement 7 mois pour les fractures ouvertes , 3 pour les fractures fermées comminutives et de 4,5 mois pour les infections. Nous avons notés 14 complications en rapport avec l’utilisation de la fixation externe dont 8 infections sur fiche, une perte de réduction après montage.
défectueux, et une torsion de fiche après traumatisme. Il ne semble ressortir aucune complication spécifique à l’encontre du « Percy Fx ».

3.0 RÉSULTATS

La durée de traitement quelque soit l’indication n’est pas supérieure à celle nécessaire avec d’autres fixateurs externes déjà commercialisés. De même nous n’avons pas noté plus de balayage de fiches ou de sépsis sur fiche qu’avec le FESSA. La temps d’intervention pour la mise en place initiale du montage pour une indication équivalente est moins longue avec le « Percy Fx » qu’avec le FESSA. Comme pour le FESSA il est nécessaire de resserrer les vis de solidarisation du montage entre le 15ème et le 21ème jour mais à la différence du FESSA il n’est ensuite plus nécessaire de le refaire. Nous n’avons noté que trois fractures de matériel au niveau des plaquettes porte fiche Pf1 et toujours au moment du serrage initial en cours d’intervention. Le suivi radiologique des patients a été facilité par la radio transparence des éléments les plus volumineux du dispositif. Certains patients qui avaient déjà été traités par fixateur externe ont apprécié la légèreté du matériel. Dans trois cas nous avons gardé certaines fiches de fixateurs externes implantées dans un autre centre (autre hôpitaux, autres pays) en les utilisant pour un montage optimisé avec le Percy Fx et ceci grâce à la grande polyvalence des plaquettes porte fiche(fig14). En outre, dans cinq cas on a pu réaliser un montage hybride utilisant à la fois des éléments de FESSA et des éléments de Percy Fx pour améliorer une prise épiphysaire, permettre la réalisation d’un lambeau ou assurer une meilleure réduction (fig 14). Chaque fois qu’un lambeau ou des greffes de peau ont du être réalisés (fig 15) le Percy Fx n’a pas été considéré comme gênant par l’opérateur, de même chaque fois qu’un apport osseux a été nécessaire. Les soins de fiches n’ont pas posé de problèmes particuliers, de même le nettoyage du fixateur externe est au dire du personnel soignant plus simple avec le Percy Fx qu’avec les autres fixateurs externes en particulier le FESSA. Le fait qu’il s’agisse d’un fixateur mono-latéral facilite également les pansements itératifs.

4.0 DISCUSSION :

De l’avis de tous les chirurgiens orthopédistes et ceci quelque soit leur nationalité, le fixateur externe reste l’arme de choix dans le traitement des fracas de guerre des membres, même si, certaines publications signalent qu’un traitement différent est parfois possible. La publication récente d’articles décrivant de nouveaux dispositifs de fixation externe prouve si il le fallait que le fixateur externe universel n’est pas encore sur le marché. Le fixateur idéal devrait associer à la fois des qualités de rigidité suffisante de modularité importante d’une rusticité compatible avec un exercice en milieu hostile (antenne chirurgicale) tout en ayant un coût le plus réduit possible. En outre, il faut que ce matériel puisse être compatible au mieux avec les fixateurs des armées Européennes et Américaines. Toutes ces caractéristiques sont énoncées in extenso dans le cahier des charge du « fixateur idéal » prôné par les différents services de santé des armées de l’OTAN. Ce texte a constitué d’ailleurs la base de travail dans l’imagination et la réalisation du « Percy Fx ». Les chirurgiens qui ont « inventé » le « Percy Fx » ce sont largement inspiré du Fixateur Externe du Service de Santé des Armées mis au point par leurs pères 20 ans auparavant. Par sa grand rusticité et sa fiabilité ce matériel a d’ailleurs acquis depuis longtemps ses lettres de noblesse. En apportant en plus la légèreté, la rapidité de pose, la radio transparence, le faible coût et surtout une très grande souplesse dans l’implantation des fiches le « Percy Fx » se rapproche à notre sens du fixateur idéal de l’OTAN. Les tests biomécaniques ont montré une grande rigidité dont on connaît le rôle dans la consolidation des lésions les plus instables. Nous ne déplorons dans cette série qu’une seule perte de réduction secondaire (survenue d’ailleurs lors d’un montage défectueux) (fig16). L’étude clinique préliminaire a également démontré un pourcentage de guérison identique à celle obtenue avec d’autres fixateurs et ceci dans
Le Percy Fx un fixateur externe mono-latéral à usage unique utilisable en traumatologie civile et militaire

des délais tout à fait classiques. La série que nous présentons ici confirme ces données. En outre, le caractère radio transparent du « Percy Fx » constitue un confort indéniable dans le suivi des fractures. Il permet en effet de noter précocement l’apparition d’un cal débutant et d’éviter au besoin les artéfacts parasites en cas d’examen tomodensitométrique. La polyvalence des plaquettes porte fiche a démontré à chaque fois qu’il en a été besoin sa compatibilité « universelle » avec d’autre fiches de fixateurs existant : Orthofix®, Fixateur Italien, Fixateur Allemand. En effet, trois patients évacués secondairement après prise en charge dans des structures de santé étrangères ont pu bénéficier d’une reprise ou d’un complément d’immobilisation par des éléments du « Percy Fx » sans difficultés particulière et ceci tout en conservant certaines fiches initiales. Le faible nombre de pièces, utilisable à la fois au membre supérieur et au membre inférieur est également un avantage indéniable réduisant le poids d’emport du matériel (contraintes logistiques) et facilitant la mise en œuvre. Même si elle n’a pas été mesurée à chaque fois les différents opérateurs qui ont eu à utiliser le « Percy Fx » ont après une courte d’apprentissage de un ou deux cas noté une durée de mise en place plus rapide que pour le FESSA même pour un opérateur entraîné à ce matériel. L’utilisation de fiches auto-tarodeuses et auto-perforantes fixées sur des portes fiches à clip explique la vitesse de mise en place. En outre, la possibilité d’orienter les fiches dans les trois plans de l’espace permet de rattraper l’implantation d’une fiche intermédiaire rendue difficile par des fiches mal positionnées en amont ou en aval (fémur). Les montages réalisés doivent toutefois rester en accord avec les lois fondamentales de la biomécanique un échec ne pouvant alors être imputé qu’au manque de clairvoyance de l’opérateur. L’ancillaire proposé par le laboratoire qui commercialise ce fixateur est complet mais on peut regretter l’absence de tournevis dynamométrique pour optimiser le serrage des vis et éviter le bris de plaquettes Pf1 lors du montage initial comme nous avons pu le constater au tout début de notre propre expérience. Enfin, le faible coût de ce matériel constitue pour les pays où les fixateurs sont fréquemment réutilisés une façon de diminuer le risque de transmission d’infections. En effet, peu coûteux le fixateur peut être jeté sans scrupule. Cette propriété va à la fois dans le sens du confort et de la sécurité du malade mais aussi dans celui de la maîtrise des dépenses de santé.

5.0 CONCLUSION :

Le « Percy Fx » a démontré depuis le début de son utilisation des qualités comparables à celles des grands fixateurs mono-latéraux actuellement sur le marché. Une étude biomécanique a pu confirmer scientifiquement sa fiabilité et le situer parmis les leaders de sa catégorie. L’étude clinique que nous présentons confirme l’intérêt de ce dispositif. Cliniquement comparable aux autres sur les résultats il apporte un plus dans le suivi par sa radio-transparence, un plus dans le confort du malade par sa légèreté, un plus dans sa mise en place par son faible nombre de pièces et son montage rapide, enfin un plus dans sa compatibilité avec les autres fixateurs externes utilisés par les différentes armées de l’OTAN. En outre il répond aux exigence logistique de transport par son faible poids et aux exigence de prévention des infections par la possibilité d’un usage unique.

6.0 BIBLIOGRAPHIE :

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FIGURES :

Fig 1 : Barres de carbone de 18 mm et 12 mm de diamètre

Fig 2 : Fiche de 6mm auto-tarodeuse auto-perforante.
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Fig 3 : Plaquettes porte fiche type Pf1 (bleu) et Pf2 (verte).

Fig 4 : Colliers crantés de 12mm (gris) et de 18mm (noir).
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Fig 5 : assemblage de l'ensemble fiches plaquettes colliers barr

Fig 6 : vis d'assemblage de 40mm, 60mm, et 120mm.

Fig 7 : barres de renvoi épiphysaires.
Fig 8 : guides gigognes protecteurs de parties molles.

Fig 9 : banc d'essai pour l'évaluation biomécanique du fixateur.
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Fig10 : Percy Fx sur une fracture stade IIIb de Gustilo

Fig 11 : Percy Fx sur une fracture fermée comminutive du pilon tibial
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Fig 12 : Percy Fx au coude pour arthrodèse sur fracas balistique.

Fig 13 : Montage monoplan deux barres sur la jambe.
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Fig 14 : Montage mixte FESSA initial avec adjonction secondaire d’un montage epiphysaire par Percy fx.

Fig 15 : Lambeau neuro-cutané sural latéral réalisé sur une jambe en présence d’un fixateur de type Percy Fx.

Fig 16 : L’absence d’entretoise verte (flèche blanche) sur ce montage de bassin pour « open book » traumatique explique la perte de réduction précoce.
Le Percy Fx un fixateur externe mono-latéral à usage unique utilisable en traumatologie civile et militaire
Military Speedfix – A New Versatile External Fixator for Combat Injuries

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ABSTRACT

External fixators are the first choice for severe comminuted fractures in long bones on the battlefield. The applied fixators of the different medical services in the NATO are mostly bulky in shape, difficult to mount, unstable or too complex to understand.

It was the intend of the NATO working group STANAG 2469 to improve the standards for the external fixation of long bones. The Italian proposal for a general fixation system was opposed by the US and German delegies in terms of lack of reducability after mounting, questionable stability and applicability.

The German group favours a special military fixator on the basis of the Speedfix (Smith&Nephew). A special design allows the fixation of the clamp for one fragment in one step by one screw or leverarm. The weight, versatility and stability of the new fixator were tested and found to be stable enough also for the femur. It is reduducable after mounting and easy to understand (one bar system). The price is also comparable to the common systems on the market.

The clinical tests are now under observations. The acceptance by the surgeons is high, the duration of implantation short, and the stability excellent. So we conclude, that the new military speedfix can probably replace the older systems.

INTRODUCTION

The only option of stabilisation complex fractures of long bones in the field is the external fixator. It allows in a simple and undangerous way the primary stabilisation, also in multiple trauma patients. The general advantages of the external fixation in long bones is the facilitation of transport, reduction of fractur pain and the protection of soft tissues and vessels. Also the symptoms of shoc an be reduced. No further extension device is necessary in comminuted or defect fractures. It is also assumed that the stabilisation away from the fracture zone could prevent infection and facilitates the bone and soft tissue debridement. Complex systems cannot fulfill the requirements for military purposes in many ways.

So it was proposed to design not a simple, but simple to understand system, that is versatile to handle, variable for all locations on long bones, ist should have only one screw for tightening one element, has to be reducable and inexpensive.

The International Standardization Agreement Ratification –STANAG 2469 / EXTERNAL FIXATION DEVICES FOR BONE INJURIES describes a single bar external bone fixator that is able to hold long bone fragments in place for transportation of the casualty. The fixator has to be stable enough to avoid secondary dislocation during transportation, and must allow the alignment also when it is in place. External fixation in the field is usually necessary for the lower extremities and pelvis. Upper extremity injuries can usually be initially treated with splinting or casting until they reach a definitive care facility. For transport, single bar fixation may be adequate depending on fracture and patient characteristics. It is

not, however adequate for definitive fixation. Additional bars may be added without anesthesia to enhance stability. Additional pins or significant fracture manipulation would require anesthesia. However, since these are usually open fractures the patient will most likely be returning to the operating room for further debridement.

**SOLUTION**

We designed a new versatile fixator for long bones that can be implanted easy and in a short time by a relatively simple procedure, can be locked by only 1 clamp screw per element, allows reduction in all directions when it is in place. It is tested for the stability that can withstand bending forces and rotational moments also at a femur site. This unit is consisting of a single bar fixator, 4 (schantz) pins 5mm or 6mm in diameter, and 2 multipin clamps along with insertion instruments. This is to be used at the (Forward Surgical Team) FST level. This system generally conforms with the principles described in the STANAG but is fundamentally different in that it allows fracture realignment independent of pin placement (fig 1 and 2).

![Figure 1: Speedfix (Smith&Nephew) 4 screws per fixation element.](image1)

![Figure 2: “Military Speedfix” Prototype 1 screws per fixation element (Dr.Ing.W.Veith, Heidelberg-Germany).](image2)
Overview of the Hemostasis Research Program: Advances and Future Directions

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ABSTRACT

The mission of the Combat Casualty Care Research Program of the US Army Medical Research and Material Command is to reduce the morbidity and mortality resulting from injuries on the battlefield through the development of new life-saving strategies, surgical techniques, biological and mechanical products, and the timely use of telemedicine technologies. One of the major areas of focus of the Combat Casualty Care Program is hemorrhage control. This article provides an overview of the Hemostasis Research Program and its accomplishments to date, and makes suggestions for areas of basic research in the future. The Hemostasis Research Program is based at the US Army Institute of Surgical Research (ISR) and includes collaborations with extramural research laboratories.

Figure 1: Level of care available pre-and post-evacuation. Adapted from [1].

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1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
1.0 THE NEED: DECREASED COMBAT MORTALITY

Due to improvements in survival after evacuation from the battlefield, overall mortality from combat wounds has decreased during the past hundred years. Although mortality has decreased for those dying of wounds after evacuation (DOW), there has been little improvement in the number of those killed in action (KIA) (Figure 1). Furthermore, the percentage of the wounded that die on the battlefield increases with prolonged evacuation [1], an increasingly likely scenario under the Objective Force as troops are more dispersed throughout the combat arena.

Hemorrhage is the leading cause of death from wounds on the battlefield (accounting for over 50% of deaths) [1] and the second leading cause of death in civilian trauma [2]. Although some soldiers killed on the battlefield are clearly unsalvageable and become KIA within minutes of impact, it appears that approximately one-third of KIA would be salvageable (Figure 2) with the development and fielding of new methods for early intervention [3]. Data supporting the position that a salvageable population of KIA exists were obtained in Oman in 1973 [4] and Panama in 1989 [5]. In both instances, the stationing of emergency medicine physicians at casualty collection points provided advanced medical care closer to the point of wounding and resulted in lower KIA rates than in previous conflicts. Given the possibility of a significant population of salvageable KIA, the hemorrhage control program has focused primarily on providing new methods, drugs, or devices to those present or near the point of wounding, such as the wounded soldier himself, his buddy, the combat lifesaver, or the combat medic.

![Figure 2. Percentage of deaths on the battlefield occurring at different times from wounding in Viet Nam. Making the assumption that deaths occurring within the first 15 minutes are not salvageable, approximately 33% of KIA deaths are potentially salvageable. Adapted from [3].](image)
2.0 HEMOSTASIS RESEARCH PROGRAM OBJECTIVES

Figure 3 depicts the current state of field hemorrhage control and the objectives we strive to meet by 2010. Approximately 20% of hemorrhagic deaths are due to compressible wounds (i.e., those that are accessible to direct pressure), treatable with pressure dressings, tourniquets, and mechanical surgical methods. However, the vast majority (approximately 80%) of hemorrhagic deaths on the battlefield are due to intracavitary hemorrhage, which is not accessible for direct compression (e.g., within the pelvic, abdominal or thoracic cavities). Currently, no method other than surgical intervention can treat intracavitary hemorrhage. The mission of the Hemostasis Research Program is to develop procedures, devices or agents that may be used by the soldier himself, a buddy, a combat medic or higher echelon medical personnel to control compressible and non-compressible hemorrhage under far-forward situations. We will therefore discuss in subsequent sections the devices, drugs, and methods that are currently being developed or evaluated to accomplish this objective.

![Figure 3. Current (2004) and future (2010) field hemorrhage control.](image)

2.1 One-Handed Tourniquet

One need identified by the soldier in the field has been a tourniquet that can be self-applied by a wounded soldier with one hand. Project development was originally funded by the US Special Operations Command (USSOCOM), but was subsequently “handed off” to the Hemostasis Research Program for further development. To this end, Calkins et al. [6] developed a needs survey that asked Special Operations corpsmen to rank desired characteristics in a tourniquet. A comprehensive search of commercially available tourniquets or patented tourniquet designs was subsequently performed and several novel tourniquet designs were also developed. These tourniquet designs were then evaluated as to how well they met the desires of the user community as expressed in the survey, as well as how well they performed under austere far-forward conditions. Special Operations corpsmen then tested seven tourniquet designs for successful tourniquet placement and time required for placement. Preliminary field-testing results suggested a ratchet design as the best tourniquet available for field use. It was later recognized that this was not the optimal design [6].
Subsequently, two new prototype designs (cinch and wrap) were developed and modifications were made to the ratchet design by investigators at the Walter Reed Army Institute of Research (WRAIR). These three designs were then evaluated based on their effectiveness, application time, size and weight. Furthermore, laboratory testing on artificial limbs assessed the pressure distribution resulting from the tourniquet application. An *ad hoc* panel containing both scientists and combat medics then convened at the ISR, and this panel concluded that the cinch design best met the requirements of the user community. With slight modifications, this tourniquet was produced and sent to field users (USSOCOM, AMEDD Center and School, US Navy and Marine Corps) for ongoing evaluation. Subsequently, a manufacturer was found and large-scale manufacturing was begun.

Special Forces medics in Afghanistan and Iraq have used the one-handed tourniquet. Additionally, an expert panel was convened to promulgate guidelines for field use of tourniquets as well as to address the potential for field removal of the tourniquet if used in combination with advanced hemostatic dressings. Researchers within the Soft Tissue Research Area at ISR are currently investigating the ability to enhance tissue salvage utilizing tourniquets with further improvements.

### 2.2 Advanced Topical Hemostatic Dressings

Under the auspices of the Hemostasis Research Program, a variety of topical hemostatic dressings have been tested for their potential applications to trauma using both severe liver injury and arterial injury models in swine [7-9]. As a result of these evaluations, two dressings were approved for external use in the field, the American Red Cross Dry Fibrin Sealant Hemostatic Dressing (under an IND protocol) and the FDA-approved HemCon Chitosan bandage. The ability of these bandages to act as the primary hemostatic mechanism for internal use (in lieu of surgical intervention) over prolonged periods of time (e.g., in the event of extended evacuation) is currently being tested at the ISR. For a detailed description of the development and evaluation of these dressings, please see the paper by Kheirabadi *et al.*

### 2.3 Intracavitary Hemostatic Agent

A major issue to be addressed by the Hemostasis Research Program is a method to control bleeding from non-compressible truncal wounds, for which surgical intervention is currently the only effective treatment. The concept of an intracavitary hemostatic agent was advanced to treat such an injury in the field. The hypothesis was that a hemostatic material could be infused into a closed body cavity by a trocar, spread throughout the closed cavity, and interact with the bleeding sites to stop hemorrhage. In the gunshot wound, for example, foam could be delivered through the hole left by the penetrating object and administered close to the injury site to reduce internal bleeding. In order for this to be a viable option, however, a putative intracavitary hemostatic agent must: 1) produce hemostasis without applied pressure when administered directly to an actively bleeding site; 2) distribute uniformly throughout the body cavity when it is introduced into a closed cavity and stop hemorrhage upon contact with actively bleeding tissues. In addition to providing hemostatic efficacy, it has been proposed that an intracavitary hemostatic agent might also provide enough intracavitary pressure to provide some level of tamponade, thereby aiding in hemorrhage control. It is important to emphasize, however, that intracavitary pressure cannot be increased to the point at which function of vital organs is compromised.

Initially, several liquid hemostatic agents were tested in internal hemorrhage models in small animals to evaluate their ability to provide hemostasis without applied pressure. Surgical liquid fibrin sealant (FS), for example, was transformed into a foam and sprayed directly onto the bleeding surface of a lacerated liver in rodents. Although an encouraging decrease in blood loss was observed, application of the FS foam did not
increase survival rate 1 hour post-application [10]. Furthermore, experimentation with the same formulation of FS foam in a rabbit model of partial liver resection could not replicate the reduction in blood loss seen in the rat liver injury model (Kheirabadi, unpublished data).

In subsequent work, the formulation of the active components of FS was substantially modified to provide a highly adhesive fibrin foam that would attach firmly to rabbit liver slices even if the tissues were covered with fresh blood. In rabbits, direct application of this modified FS foam on the bleeding surface of the lacerated liver was partially effective in reducing the hemorrhage and improving survival. Infusion of the FS foam into a rabbit model of closed-abdomen bleeding, however, had only a marginal effect in reducing hemorrhage or improving survival rate. In other studies using a variety of means of making the foam (e.g., chemical, propellant gas), FS foam did not prove to be effective in reducing bleeding under practical application circumstances (i.e., without producing large increases in intracavitary pressure that disrupted vital organ function).

Currently, we are in the process of testing other potential hemostatic agents that might be used as intracavitary agents. Although not a foam, FloSeal (Baxter Biomedical) consists primarily of collagen and thrombin. When applied directly to the bleeding liver injury in the open abdomen rat model, FloSeal was able to reduce blood loss by almost 25%, but did not significantly increase survival time. In a closed-abdomen model of internal hemorrhage, however, FloSeal neither reduced blood loss nor improved survival time in rats.

There are two major obstacles to the effectiveness of any such agent when infused into a closed body cavity. The first is the inability of these agents to distribute thoroughly in the cavity and reach the entire injured surfaces before they become activated. The second major obstacle is the inability of these agents to penetrate through the pooled blood around the organs and move against free-flowing blood to interact with the injured tissues themselves. Hence, although intracavitary foams/liquid agents offer an alluring solution to hemorrhage from non-compressible wounds, there are as yet many obstacles to overcome and it is unclear at this point whether this potential solution to non-compressible hemorrhage is possible using current technology.

### 2.4 Hemostatic Drugs

As indicated above, there is currently no treatment available to the field medic for severe non-compressible hemorrhage. One approach to this problem is to identify a drug(s) that could be administered intravenously that might act systemically to decrease bleeding. The concept of using intravenous drugs to enhance or augment the body’s innate clotting mechanisms during situations in which blood loss is expected is not new. Indeed, drugs have been used in the treatment of bleeding complications for over 30 years. For example, the FDA-approved drugs epsilon-amino caproic acid, tranexamic acid, aprotinin, and desmopressin (DDAVP) have been used to reduce bleeding complications and blood loss in a variety of clinical situations including cardiac surgery, hepatic surgery, oral surgery, knee and hip arthroplasty, and in patients with bleeding disorders [11]. More recently, recombinant factor VIIa (rFVIIa) has been used very effectively in hemophilic patients for controlling acute bleeding episodes and during surgical procedures [12].

Another objective of the Hemostasis Research Program is to screen these FDA-approved drugs for their potential use in non-compressible traumatic hemorrhage. Preliminary results suggest that epsilon-amino caproic acid, tranexamic acid, aprotinin, or desmopressin (DDAVP), when used alone or in combination, neither decreases bleeding time nor increases survival time following severe liver injury in rodents (Ryan et al., in preparation).
A more promising candidate to provide hemostasis following trauma is rFVIIa. A growing number of case reports document the successful use of rFVIIa to decrease blood loss in trauma patients [13]. In laboratory models using pigs with normal coagulation function, however, rFVIIa does not appear to decrease blood loss following severe liver injury, aortic injury, or liver avulsion [14-17]. Administration of rFVIIa in these pigs does, however, activate clotting mechanisms and increases the pressure at which rebleeding occurs during resuscitation, suggesting that rFVIIa may strengthen the nascent clot [15, 16]. In pigs with abnormal coagulation function induced by reduction of body temperature (hypothermia) and dilution of blood (hemodilution), rFVIIa decreased the severe blood loss following severe liver injury in two studies [18, 19] but not in another [20]. It therefore appears that rFVIIa use may be beneficial in the coagulopathic soldier in whom bleeding cannot be stopped by other means. Ongoing laboratory investigations will further delineate the conditions under which this drug might be useful.

In addition to rFVIIa, we are studying the ability of a new drug, Factor Xa-PCPS (phosphatidyl choline-phosphatidyl serine vesicles), to reduce bleeding in swine models of trauma. We have established collaborative agreements with investigators at Haematologic Technologies, Inc. and the University of Vermont, who developed the drug. In preliminary laboratory tests, this drug stopped cuticular bleeding in both normal and hemophiliac dogs [21]. As other new hemostatic agents are developed, they will likewise be tested in trauma-relevant animal models for their potential to decrease bleeding and to save the lives of wounded soldiers on the battlefield.

### 2.5 Mechanisms of Early Trauma-related Coagulopathy

As alluded to above, early responses (within 24 hours) to trauma and subsequent resuscitation, especially under battlefield conditions, may include hypothermia, hemodilution and acidosis. Such conditions induce coagulopathies in which normal coagulation function is altered and disrupted. As part of the Hemostasis Research Program, we are investigating trauma-related coagulopathy in a complex setting designed to more closely model combat injuries sustained on battlefields. Our goal is to identify the changes of coagulation activity, platelet function, and fibrinolysis using our existing animal model and techniques, as well as to develop new techniques and understandings of the basic physiological mechanisms underlying the development of coagulopathy.

We are currently studying physiological mechanisms underlying the development of such coagulopathies. In our initial project, we have developed an in vivo swine model to study coagulopathy with hypothermia and acidosis, as well as other in vitro methods using physiologically relevant agonists. One of these in vitro methods is a measurement in minimally altered pig blood that illustrates the clot process over time. We have found that acidosis and hypothermia (alone and in combination) cause significant increases in bleeding time and decreases in fibrinogen concentration and platelet level. These were associated with significant increases in prothrombin time (PT) and activated partial thromboplastin time (aPPT), which are measures of clotting via the extrinsic and intrinsic coagulation pathways, respectively. However, platelet function was unchanged when temperature was decreased from 39 to 32°C. Development of this in vivo animal model and new in vitro methods has expanded our ability to characterize the coagulopathic state in greater detail than that provided by previous investigations. These initial results have provided valuable information about the mechanisms underlying coagulopathy induced by hypothermia and acidosis, and will be expanded to include investigations into coagulopathies associated with hemodilution or combinations of this trauma-induced triad (hypothermia, acidosis and hemodilution). Our comprehensive approach is aimed towards gaining insightful information and directions for pharmaceutical intervention to correct trauma-related coagulopathies and thereby save lives of wounded soldiers.
2.6 High Intensity Focused Ultrasound (HIFU) Device

In addition to screening potential hemostatic agents, the ISR is currently evaluating other means to reduce non-compressible hemorrhage. Ideally, a combat medic or other care provider would have a device to non-invasively visualize a source of internal bleeding and to safely cauterize it. Although this sounds like science fiction, investigators at the National Center for Physical Acoustics at the University of Mississippi and the Applied Physics Laboratory at the University of Washington are working toward such a device. These investigators are developing a hand-held device that will 1) incorporate a computerized Doppler system to locate bleeding structures and 2) focus sound waves to cauterize the bleeders without damaging the overlying tissues [22]. So far, acoustic hemostasis has been shown to be effective in sustaining hemostasis (up to 60 days) in exposed splenic lacerations [23]. Currently, these investigators have developed animal models in which blood vessel and liver injuries are made non-invasively. These injuries are subsequently visualized and cauterized using non-invasive methods. The Hemostasis Research Program has a collaborative agreement with the Applied Physics Laboratory, thereby leveraging industry and academic investments to meet Army needs. Investigators from the two programs interact on a regular basis and HIFU will eventually be tested in animal models of trauma at the ISR.

3.0 INTEGRATED APPROACHES

It should be emphasized that the hemostatic tools discussed above are not conceived as disparate solutions to the problem of bleeding; rather, the concept is that these tools can be used to complement each other in the field. Figure 4 depicts two scenarios in which the use of hemostatic tools could be integrated. In the first, a soldier with a bullet wound to the thigh that includes femoral artery injury could be treated at the level of self/buddy aid by applying a tourniquet, an advanced hemostatic dressing or both. If the tourniquet accomplishes vascular control, an advanced hemostatic dressing might then be applied and the tourniquet released, thereby improving the possibility of limb salvage. The battalion surgeon might then use HIFU to cauterize the arterial injury, if necessary. In the second scenario, a soldier with a shrapnel wound that produces severe liver laceration could be treated by a combat medic using an intracavitary hemostatic agent and/or an intravenously administered hemostatic drug. The battalion surgeon might then opt to use HIFU to cauterize the wound and, if the soldier had developed coagulopathy, additional intravenous agents might be administered to reverse the coagulopathy. The goal of the Hemostasis Research Program is to provide an array of advanced hemostatic tools to care providers, thereby allowing greater flexibility in patient treatment based on immediate clinical/combat demands.
Figure 4. Possible scenarios for integrated use of hemostatic tools. Current pre-evacuation treatment for scenario one is gauze dressing and use of field expedient tourniquet or belt. For scenario two, there is currently no efficacious pre-evacuation treatment available.
4.0 FUTURE DIRECTIONS

As the Army moves toward the Objective Force and the troops are more dispersed on the battlefield, units must be more self-sustaining in terms of all support requirements, including medical needs. Evacuation of casualties may be prolonged, requiring the need for medical care through a progression of post-traumatic states over a period as long as 96 hours. A scenario can be envisioned, for example, in which the casualty first needs enhanced hemostasis and then needs treatment for hypothermic and acidotic coagulopathy. Next, the patient may progress to a hyperfibrinolytic state, a state of inappropriate intravascular activation of the coagulation system, or a septic state. Superimposed upon these conditions may be ischemia-reperfusion injury due to resuscitation or a massive inflammatory response. Other confounding factors on the future battlefield that may impact coagulation function include the use of artificial oxygen carriers (blood substitutes), immune modulators, performance enhancers or other such drugs. The ability to manipulate the hemostatic mechanisms and the way they interact with other systems will therefore be critical to optimize the potential for recovery and survival of the casualty.

Before we can manipulate the hemostatic mechanism, however, we must first understand how the coagulation system responds to trauma and how it impinges upon other systems such as the immune system. In essence, we must better understand the integrated physiological responses to traumatic injury to guide us in the subsequent development of life-saving strategies. For example, it is currently known that thrombin generation (which is necessary for coagulation) activates the inflammatory system, which can lead to detrimental tissue effects subsequent to hyperinflammation. Modulation of this activation at the level of the endothelial cell may thus allow us to increase coagulation function while decreasing inflammation. As another example, there is very little information currently available about how physiological states that soldiers routinely experience (e.g., dehydration, sleep deprivation, cold stress, heat stress, acute exercise or combinations of these) affect the coagulative response to subsequent trauma. Although no data currently exist that specifically address the effects of these physiological factors on trauma responses, related studies of elite athletes suggest that such stressors may have a profound effect on trauma-related hemorrhage and we see an urgent need to expand on this knowledge base. Finally, when tissue responses to the ischemia produced by blood loss are more fully understood, it may also be possible in the future to supplement hemostatic mechanisms by simultaneously manipulating such responses either through conventional pharmacological means or by new techniques such as gene therapy. Thus, the need for more basic research to understand the physiological mechanisms underlying the response to trauma exists and will continue to grow. The Hemorrhage Research Program will move to fill this need by performing both clinical and laboratory research to understand the interactions between physiological systems and their response to trauma. Further understanding of the basic mechanisms underlying coagulation and subsequent physiological responses will provide future solutions that not only reduce bleeding in the short term but will also decrease long-term detrimental effects. Our vision is to continuously improve upon hemostatic tools so that the KIA rate in future battles will progressively decrease.

5.0 REFERENCES


Surgical Tourniquet Technology Adapted for Military and Prehospital Use

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ABSTRACT

In groundbased tactical situations and prehospital settings, the tourniquet is used as a life-saving hemorrhage control device. It must completely stop blood flow, must be fast and simple to self-apply, and must require minimal training. However, an improperly designed or used tourniquet can cause loss of the limb, compromised limb salvage, and systemic effects harmful to the patient; all may result from excess tourniquet pressure, excess tourniquet time, or a tourniquet that is too narrow. Most existing prehospital tourniquets use technology from the 1800’s: mechanically tightened narrow bands that apply uncontrolled pressures (e.g. Spanish Windlass). In contrast, the modern pneumatic tourniquet is considered indispensable in common surgical procedures, and is now used thousands of times each day to completely occlude arterial blood flow in limbs continuously throughout surgeries lasting several minutes to several hours, and longer using appropriate techniques. Injury or compromise of limb recovery due to pneumatic tourniquet use in surgery is rare. The purpose of this study was to test a newly developed prehospital pneumatic tourniquet, which is based on proven surgical tourniquet designs. The new device has an inflatable bladder, a manual inflator, and a locking clamp. It weighs 220 g, packs to 570 cm³, and fits arms and thighs up to 85 cm circumference. To demonstrate effectiveness at safe pressures, the new tourniquet was applied to adult volunteers. Arterial occlusion (indicated by Doppler stethoscope) was reached at an average pressure of 229 mmHg (SD 32, range 165-302, n = 32) on the thigh and 140 mmHg (SD 17, range 106-175, n = 32) on the upper arm. The tourniquet was then used on thighs in 21 surgical procedures at the normally used pressures of 300-350 mmHg. Good hemostasis was maintained in all cases and there were no complications. Users found the tourniquet very easy to apply after a single demonstration. To demonstrate single-handed self-application after minimal training, volunteers were given one demonstration and then timed during self-application to the upper arm and thigh using their non-dominant hand only. First-time application and inflation averaged 29 seconds for the arm (range 22-40, SD 6.8, n = 7 volunteers) and 36 seconds for the thigh (range 24-62, SD 12.5, n = 7 volunteers). After 5 minutes of additional demonstration and practice, average times improved to 23 s (arm, range 12-47, SD 8.3, p = 0.035, n = 16 volunteers) and 26 s (thigh, range 18-36, SD 5.2, p = 0.042, n = 16). To demonstrate effectiveness when access over the distal end of the limb is impossible, volunteers unthreaded the tourniquet, passed it around the limb, and inflated to occlusion. Average times were 43 seconds (arm, range 26-63, SD 10.7, n = 16) and 43 seconds (thigh, range 23-82, SD 13.4, n = 16). The new prehospital pneumatic tourniquet studied proved safe, effective, and reliable in volunteer testing and in clinical evaluations. In contrast to most existing prehospital tourniquets, the new device occludes blood flow at safe pressures over adequate width, as has been proven safe in routine limb surgery. Manual and electronic controllers for the new prehospital pneumatic tourniquet are also described and discussed.

1.0 INTRODUCTION

It is recorded that surgeons used constricting devices for amputation in Roman times, and in 1718 Jean Louis Petit developed a screw-type device (a ‘tourniquet’) which tightened a cloth band around the limb to achieve hemostasis. In 1864 tourniquets were first used for surgical procedures other than amputations. In 1904 Cushing introduced a pneumatic tourniquet for the surgical setting to provide more even and controllable pressure to the limb compared to previous mechanical tourniquets, thus allowing more reliable occlusion and reducing the chance of tourniquet-induced injuries. Pneumatic tourniquet systems (which include a tourniquet controller that supplies and/or regulates pressure and a pneumatic tourniquet cuff applied to the limb) have since been developed and refined. In 1982 the modern automated, microprocessor based tourniquet controller was introduced which featured greatly improved regulation of pressure, time display, warnings of various hazardous conditions, automatic self-test of calibration, and other patient safety related functions [18,21]. Wider, better fitting pneumatic tourniquet cuffs which include many other improvements in design have also been developed for better safety and performance, and to minimize the pressure required to maintain occlusion and thereby minimize the chance of injury [7, 19, 20, 24, 27, 28]. Due to these improvements in safety, efficacy, and reliability these modern tourniquet systems are now used in virtually all modern operating rooms in an estimated 20,000 procedures each day worldwide. Pneumatic tourniquet related complications are rare [23], and the pneumatic tourniquet has been placed in the lowest risk category for medical devices by the Food and Drug Administration (FDA), the US medical product regulatory body.

However in the pre-hospital, emergency, and military settings, established tourniquet techniques still employ a non-pneumatic constricting band and a mechanical device (such as a windlass, buckle, or ratchet mechanism) to allow the user to generate the high tension required to occlude blood flow. For example, most emergency medicine guidelines describe a windlass technique wherein the user ties a strap or cloth band around the limb, inserts a stick or rod under the band, and twists the stick to tighten the band until blood flow stops. In the surgical literature, it has been shown that narrow tourniquets must apply very high pressures to the limb to occlude blood flow [10]. High pressures have been shown to cause nerve, muscle and soft tissue damage, and in particular the resulting steep pressure gradients developed in the limb are thought to be the primary mechanism of nerve injury [11, 25, 26]. Applications of high pressure, even for a brief period, can cause limb paralysis [1, 30].

Due to these hazards, most emergency medicine guidelines have specified the non-pneumatic tourniquet as a ‘last resort’ method to control life-threatening bleeding [2, 4, 6, 15, 17, 29, 31], although the life-saving value of an effective tourniquet is recognized [12, 17, 32]. Current commercially available tourniquets for emergency settings (MacMillan Tourniquet, CMC Rescue Inc., Santa Barbara, CA: Prämeta Emergency Tourniquet 910A, Prämeta, Cologne, Germany) and some tourniquets under development [3, 5] utilize narrow, non-pneumatic constricting bands. Various non-pneumatic strap-type tourniquets and commercially available venous tourniquets (designed to assist in intravenous catheter placement) have been shown to be ineffective for occluding arterial blood flow [5]. In emergency situations where life-threatening bleeding from an extremity must be controlled quickly and pressure bandages cannot be used effectively, an effective tourniquet may save the victim’s life. However, such a strap or cloth-and-stick type emergency tourniquet may not be effective, and if it can be applied tightly enough to be effective it may reduce the chance of successful limb salvage.

A pneumatic emergency tourniquet, a new manual pneumatic tourniquet system, and a prototype miniature electronic tourniquet monitor, all based on modern pneumatic surgical tourniquet technology, have been developed by tourniquet specialists Delfi Medical Innovations Inc. (Vancouver BC, Canada; www.delfimedical.com) for emergency and pre-hospital use. These new tourniquets have designs that are
closely based on the proven designs of pneumatic surgical tourniquets that are widely used in hospital settings (see US patents 5312431, 5454831, 5578055; US Patent Application 20030139766), and thus have similar safety and reliability to modern surgical tourniquet systems, but are adapted for portability and use in settings without external power sources. In this article we report on the development of the new pneumatic emergency tourniquet, manual tourniquet system, and electronic tourniquet monitor, results of volunteer testing, and results of use in surgery. Essential tourniquet design details from proven commercial surgical tourniquets that have been incorporated in the new devices are described below.

2.0 MATERIALS AND METHODS

2.1 Emergency Tourniquet

The new emergency tourniquet consists of a pneumatic bladder having similar width (3.5”) to many commonly used surgical tourniquets, a securing clamp, and a manual hand-bulb type inflator and deflation valve (Fig. 1). Many crucial features are closely based on the proven, patented designs of surgical tourniquets, as described below. The tourniquet weighs 210 grams (7.5 ounces) and is about 4” x 3” x 3” when packed. The securing clamp allows the user to self-apply the tourniquet using one hand only and allows adjustment to fit limbs from 8 to 85 cm (3 to 34”) in circumference, encompassing over 97.5% of arms, thighs, and lower legs encountered in the population. The bladder is formed from sheets of urethane-backed nylon radio-frequency sealed together; this material and assembly technique are used in surgical tourniquet cuffs which normally last for hundreds of surgical procedures over several years. The selected width of the pneumatic bladder provides a width/limb circumference ratio greater than 0.10, allowing occlusion at pressures similar to those used in surgery [10]. Unlike a blood pressure cuff, the tourniquet bladder completely encircles the limb and applies inward radial pressure evenly around the entire limb circumference, which is essential for complete occlusion. To prevent the tourniquet from rolling down the limb as it is inflated, selected areas along the edges of the bladder are non-inflating. The bladder design allows sufficient inward expansion upon inflation to provide occlusion in most cases when the tourniquet is applied over clothing, is not applied snugly as directed, or is applied to an obese limb. The tourniquet is packed with both ends of the bladder threaded through the clamp to form a loop which can be passed over the distal end of the injured limb, moved proximally into position, pulled snug and clamped, then inflated. If access over the distal end of the limb is restricted, one end of the bladder can be unthreaded from the clamp, wrapped around the limb, rethreaded through the clamp, passed around the limb at the desired location, and rethreaded through the clamp.

2.1.1 Volunteer Test Method

A convenience sample of 16 volunteers was recruited among research center staff members (15 male, median age 27, age range 19-50). All volunteers were civilians with no prior training in emergency medicine. Two volunteers had previous experience with the tourniquet, and the remaining 14 had not seen the tourniquet in use before the test. Each volunteer was timed from the moment of picking up the tourniquet until locking the clamp with the tourniquet snugly applied around the limb under the following conditions:

- First attempt passing the looped tourniquet over the distal end of the limb, after a 30 second demonstration by the investigator.
- Second attempt as above, after up to 5 minutes of additional demonstration of optimal techniques and practice (up to two applications).
- Third attempt in which the volunteer unthreaded the tourniquet bladder, wrapped it around their limb, rethreaded the bladder through the clamp, tightened the bladder around the limb, and locked the clamp. Volunteers were shown the optimal technique and allowed up to two practice applications before their timed attempt.
The above sequence of tests was performed on each volunteer’s dominant arm and on one thigh. Note that volunteers were considered ‘experienced’ after their first attempt on either the arm or thigh, therefore there are only 7 true ‘first attempts’ for each limb. Limb occlusion pressure (LOP, the tourniquet pressure at which the distal pulse was no longer audible) was measured after the first and second attempts on each limb by gradually inflating the tourniquet and monitoring the distal pulse in the limb using a Doppler stethoscope. After their third attempt, the time required for each volunteer to manually inflate the tourniquet approximately to their LOP was measured. For each attempt, the sum of the application time and the inflation time is taken as the total time to occlusion. In all tests, volunteers used their non-dominant hand only and attempted to limit movement of the affected limb. All tests were done with the volunteer sitting on a flat surface. The tourniquet was applied over the volunteer’s clothing; full length single layer fabric trousers on all thighs and up to two layers of clothing on arms. At the beginning of each application the tourniquet was rolled up as packaged, but was not sealed in its plastic pouch; time required to rip open the pouch is not included in the results. All LOP measurements were taken at the posterior tibial and radial arteries by a single experienced technician using a Doppler stethoscope (Versatone D-9, Medsonics, Mountain View CA USA). A hand operated pressure regulator (Inflatomatic 3000, Zimmer, Dover OH USA) was used to inflate and increase tourniquet pressure during the LOP measurements, and pressures were monitored using a digital gauge with 1 mmHg resolution (Cecomp Electronics, USA).

2.1.2 Surgical Evaluation Method

To confirm that blood flow could be reliably occluded at pressures similar to those used in surgery, the emergency tourniquet was used in surgical procedures to the knee and lower leg. This technique of evaluation on surgical patients is not possible for unproven strap-type tourniquets, because of concerns about possible tourniquet-related injuries, but was possible for the emergency tourniquet because the design was closely based on proven surgical tourniquets. The tourniquet was applied to the thigh and inflated to 300 mmHg (for small and average sized thighs) or 350 mmHg (for large thighs) for each case (the typical range of thigh tourniquet pressures used in standard surgical tourniquets [13]). The surgeon in charge noted the quality of the bloodless field throughout the procedure. For these clinical cases the hand-bulb inflator was removed and the emergency tourniquet was connected to the standard automatic tourniquet instrument normally used in the operating room.

Figure 1: Emergency tourniquet in use (left) and packed (right)
2.2 Manual Tourniquet System

A new manual tourniquet system has been developed for applications where the self-application ability and extreme compactness and light weight of the emergency tourniquet are not essential (e.g. medic kits, far-forward surgical teams, ground and air ambulances, and other applications such as industrial and marine medical aid kits). The manual tourniquet system requires no batteries or external power sources. It consists of a 4” wide by 34” long pneumatic bladder, hook-and-loop fasteners to secure the bladder around the limb, a manual hand-bulb type inflator with deflation valve, a pressure gauge, and a quick-release locking connector to allow connection to a hospital-type or portable automatic tourniquet system at any time during use. The bladder includes a plastic stiffener having a selected width and stiffness to prevent the bladder from rolling down the limb and to direct expansion of bladder radially inwards upon inflation. The design of the cuff is closely based on the proven design of surgical tourniquet cuffs that are used safely and effectively many times each day in hospital operating rooms around the world.

2.2.1 Evaluation Method

Extensive testing was not required for the manual tourniquet system because the bladder design has been well proven in surgery and its performance on typical thighs and lower legs has been reported in the literature [19, 20]. The system was tested on the upper arms of two volunteers for LOP. The system was also applied to one volunteer’s arm, thigh, lower leg, and ankle to check for movement down the limb.

2.3 Electronic Tourniquet Monitor

The prototype electronic tourniquet monitor is a portable, self-contained, water-resistant unit weighing 90 grams (3.2 ounces) that may be used with the emergency tourniquet (described above) or with other pneumatic tourniquet cuffs to monitor tourniquet pressure and time (Fig. 2).

The monitor has a field-replaceable battery (shelf life 5 years and service life of 100 two-hour uses) and a hand-bulb type inflator. Like modern surgical tourniquet controllers, the monitor accurately displays tourniquet pressure, allows the user to set a desired pressure set point, and gives audio and visual warnings if the tourniquet pressure drifts outside of a tolerance zone around the set point. Elapsed tourniquet time is also...
displayed, and audio and visual warnings are triggered if tourniquet time exceeds a selected limit. The monitor powers up automatically upon tourniquet inflation and shuts down automatically after being deflated for a pre-set period. An audio-visual low battery alarm is activated when there is still sufficient battery life for several uses.

3.0 RESULTS

3.1 Emergency Tourniquet

3.1.1 Volunteer Test
Volunteers with no previous experience were able to pass the tourniquet over the distal end of the limb, slide it proximally into position, then lock and inflate it to occlusion pressure in an average of 29 seconds for the arm (range 22-40, SD 6.8, n = 7 volunteers) and 36 seconds for the thigh (range 24-62, SD 12.5, n = 7 volunteers). After up to 5 minutes additional demonstration and two practice applications (and including results from two additional volunteers with previous experience with the tourniquet), average times improved to 23 s for the arm (range 12-47, SD 8.3, p = 0.035, n = 16) and 26 s for the thigh (range 18-36, SD 5.2, p = 0.042, n = 16). Volunteers were also able to unthread the emergency tourniquet, pass it around the limb, rethread it, then lock and inflate it to occlusion pressure in an average time of 43 seconds for the arm (range 26-63, SD 10.7, n = 16) and 43 seconds for the thigh (range 23-82, SD 13.4, n = 16) after up to two practice applications. In all 96 timed applications, 3 took longer than 60 seconds; one volunteer required 62 seconds to complete a thigh application on their first attempt and two other volunteers required 63 and 82 seconds to unthread, wrap, and apply the tourniquet to the arm and thigh respectively (see Figs. 3 and 4). Occlusion was achieved at an average pressure of 229 mmHg (SD 32, range 165-302, n = 32) on the thigh and 140 mmHg (SD 17, range 106-175, n = 32) on the arm.

3.1.2 Surgical Evaluation
The new tourniquet was used in 22 surgical cases. One surgeon performed 6 arthroscopic anterior cruciate ligament reconstructions and 12 other knee arthroscopic procedures, and a second surgeon performed 4 below knee open surgeries using the emergency tourniquet. A satisfactory bloodless field was achieved in all cases. Eight thighs were noted to be large or obese, and on 4 of these patients 350 mmHg was used. In the remaining 18 cases 300 mmHg was used. Pressure was not increased during the procedure in any of the cases. Both surgeons noted that there was no difference in occlusion compared to the surgical tourniquet cuffs normally used at the clinic, and that no problems of fit or function of the tourniquet were encountered. In several cases staff noted that the new tourniquet was easier to apply than standard surgical tourniquet cuffs, particularly on large limbs.
Surgical Tourniquet Technology Adapted for Military and Prehospital Use

Figure 3: Average time to self-apply with non-dominant hand only, arm

Figure 4: Average time to self-apply with non-dominant hand only, thigh
3.2 Manual Tourniquet System

On two volunteers with 25-28 cm (10-11”) circumference upper arms, the system occluded blood flow at 140 mmHg, which is well below the 200 to 250 mmHg that is typically used on upper arms in surgery. In additional thigh, lower leg, and ankle applications on one volunteer the tourniquet remained stable and did not slide down the limb. The bladder design has previously been shown to be stable and effective in surgery on a wide variety of patients.

4.0 DISCUSSION

Current emergency tourniquet techniques and devices are based on centuries-old narrow constricting strap approaches, and narrow tourniquets have been shown to require high pressures to occlude blood flow [10]. These high pressures are developed by increasing the tension in the strap during application, and accordingly most non-pneumatic devices include a windlass, ratchet, or other mechanical arrangement to assist the user in tightening the strap. Wider tourniquets occlude blood flow at lower pressures and have been found to transmit pressure more effectively to deep tissues [7, 22], but increasing the width of the strap in a non-pneumatic tourniquet greatly increases the tensioning effort required as a greater area of tissue must be compressed. In a recent study of a variety of commercially available and custom self-applied tourniquets, Calkins found that all of the non-pneumatic, strap-type devices tested (typically 1-1.5 inches wide and including several ratchet-assisted devices) failed to occlude blood flow in a substantial number of trials [5]. Furthermore, pressures are difficult to measure and regulate with strap-type tourniquets and although a strap tension indicator has been suggested (U.S. Patent No. 4,243,039), strap tension has not been shown to be a reliable indicator of pressure applied to a limb around its circumference. High, uncontrolled tourniquet pressures increase the chance of further injury to the limb [1, 25, 26, 30] and therefore will likely decrease the chance of successful limb salvage. Due to these hazards, narrow, non-pneumatic strap-type tourniquets cannot be ethically tested on healthy volunteers in the civilian and medical setting. In contrast, surgical pneumatic tourniquets are routinely used on healthy volunteers in studies.

Some references specify that a wide material should be used to encircle the limb and warn against using rope, cord, or twine [2, 4, 6, 29]. However wide cloth bands or similar materials tightened using a windlass tend to concentrate the pressure applied to the limb along the midline of the band; a similar effect has been observed under elastic bandages used as tourniquets [22].

Another hazard of tourniquet use is excessive continuous tourniquet occlusion time, which can result from attendants forgetting or not being aware that a tourniquet is in place, not knowing when it was applied, or leaving tourniquets in place continuously during a long evacuation to hospital care [8, 33]. Although most emergency medicine guidelines warn of the hazards of excessive continuous tourniquet time, the user is typically advised to leave the tourniquet in place until hospital care is available [2, 6, 15, 29, 31].

Due to the hazards associated with currently known emergency tourniquets and techniques, their use is typically recommended only as a ‘last resort’ [2, 4, 6, 15, 17, 29, 31]. Using a blood pressure cuff as an emergency tourniquet is suggested in some emergency medicine references [2, 4, 15], but these cuffs are typically not designed to completely occlude all arterial flow, have inflatable bladders that do not completely encircle the limb, are generally too wide, are not intended to be pressurized for extended periods of time, and can fail under typical prehospital conditions [9]. Despite these limitations and risks inherent in current emergency tourniquets and techniques, the life-saving potential of an effective tourniquet has been recognized in military [5, 12, 16] and some emergency medicine literature [4, 17, 32]. One civilian emergency medicine guideline states that a tourniquet can be extremely useful when applied properly, but recommends only wide, pneumatic types providing even pressure distribution around the limb [17].
These limitations and risks have largely been addressed in the surgical setting, and the pneumatic tourniquet has become standard equipment in the modern operating room [14]. Pressure on the limb is generated by inflation pressure rather than circumferential tension, and is therefore more easily controlled and more evenly applied around the circumference of the limb. The required pressure can be generated in a tourniquet of any size, and modern tourniquet bladders have been developed to fit various limb sizes. Pneumatic pressure can be accurately regulated and both elapsed time and pressure can be monitored and displayed. Alarms and other safety features are often incorporated to prevent hazardous conditions such as excessive tourniquet pressure and time. Bladder width is typically 3 – 5 inches for adult limbs, providing a sufficiently high width/circumference ratio to occlude most limbs at less than 350 mmHg. Modern bladder designs completely encircle the limb, allow sufficient inward radial expansion, produce acceptable pressure gradients at the tourniquet edges, and include stiffeners or other means to direct expansion of the bladder radially inward and to prevent rolling and sliding of the tourniquet down the limb. Bladder materials are durable enough to withstand daily use and hundreds of inflation cycles. However until now this modern surgical tourniquet technology has not been adapted to the emergency and pre-hospital setting. Surgical tourniquet systems generally require an external power source and are not portable or rugged (with the exception of the P.T.S. Portable Tourniquet System, Delfi Medical Innovations Inc., Vancouver BC, Canada www.delfimedical.com). A variety of surgical tourniquet bladder sizes are normally required to fit all limbs of all patients, and they are not well suited for self-application.

The new pneumatic emergency tourniquet described in this study offers significant improvements in safety and efficacy over existing emergency tourniquets. The design of this new pneumatic emergency tourniquet was closely based on the proven design of surgical tourniquets that are used safely and effectively many times each day in hospital operating rooms around the world. This resulted in reliable occlusion at pressure levels that are routinely used in surgery without complications. To allow the emergency tourniquet to be rolled up to a small packed size, the bladder design has been adapted to eliminate the stiffener used in most surgical tourniquets. The new tourniquet utilizes a clamp rather than hook-and-loop type fasteners to secure the bladder snugly around the limb; this allows the tourniquet to fit the majority of limbs likely to be encountered, enables one-handed application, and improves reliability in wet or dirty conditions. In the current study, most untrained volunteers were able to successfully apply the new emergency tourniquet within a minute, and application times improved after about 5 minutes of additional demonstration and practice. Blood flow was occluded on all volunteers at pressures similar to those used routinely in surgery [13]. These application times are comparable to those recorded by Calkins, although the Calkins study did not investigate untrained volunteers and scenarios where access over the distal end of the injured limb was impossible [5]. In surgery, the new emergency tourniquet maintained occlusion at 300-350 mmHg throughout typical surgical procedures, which included repositioning and manipulation of the limb. Such evaluation on surgical patients, and demonstration of the low pressures required to stop blood flow, was only possible for the emergency tourniquet because the design was closely based on proven surgical tourniquets. Such evaluations would not generally be possible for non-pneumatic strap-type tourniquets, because of concerns about reliability and about the risk of tourniquet-related injuries to the underlying limb due to high pressures and high pressure gradients.

The new manual tourniquet system described in this study provides a surgically-equivalent pneumatic bladder designed in a size that will fit the great majority of limbs and a completely self-contained manual inflator, valve, and pressure gauge. It is portable, does not require any batteries, external power, or compressed air, and can also be used with automatic tourniquet systems.

The new electronic tourniquet monitor is the first portable, self-contained device that provides tourniquet pressure and time monitoring and safety alarm functions similar to those of modern surgical tourniquet technology.
systems. It is a more sophisticated alternative to the pressure gauge used with the manual tourniquet system described above and can also be used with the new emergency tourniquet or with other pneumatic tourniquet cuffs. Safety is improved by assisting the user in maintaining safe tourniquet pressures and times; as tourniquet pressure changes due to changing conditions such as ambient temperature or movement of the limb, audio-visual alarms indicate if the pressure deviates excessively from the selected pressure. The user may then manually inflate or deflate the tourniquet back to the selected level. The elapsed time monitor, display, and alarms reduce the risk of excessive continuous tourniquet time. The automatic power-on, power-off, and low battery indicator features minimize the user inputs required to operate the device and help ensure that the monitors and alarms function at all times when the tourniquet is inflated. The device does not require external power or compressed gas sources.

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6.0 REFERENCES


Hemostatic Agents for Control of Intracavitary Non-Compressible Hemorrhage: An Overview of Current Results

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ABSTRACT

The majority (~80%) of hemorrhagic deaths on the battlefield are due to intracavitary hemorrhage that is not accessible for direct compression and cannot be treated with externally applied hemostatic agents. In an attempt to address this issue, a project was initiated to evaluate the efficacy of different hemostatic products when introduced into a closed hemorrhaging body cavity. Two thrombin-based hemostatic agents have been tested thus far in rat and rabbit models. In the initial phase, these agents were tested by direct and immediate application over severe parenchymal injury without compression in open-abdomen models. In the second phase of the project, the hemostatic agents were infused 5 minutes after a liver injury in closed-abdomen injury models. In the phase 1 open abdomen studies, both hemostatic agents decreased blood loss when compared with placebo-treated control animals. This decreased blood loss corresponded to an increase in survival rates that was not, however, statistically significant. In the phase 2 closed abdomen study neither hemostatic agent was able to produce a significant change in blood loss or survival rates when compared to control animals. The hemostatic properties of both hemostatic agents involve binding with injured tissues. Such characteristics require contact of these agents with damaged, bleeding tissues. In the closed abdomen model, contact is made difficult by ongoing hemorrhage and pooled blood. The failure of both products to demonstrate efficacy may also have been due to model design. In the severe liver injury model, bleeding is most severe in the first few minutes after the injury that unless treated promptly the consequences cannot be reversed by later interventions. Additional studies in more appropriate models with alternative hemostatic agents will further evaluate the potential for intracavitary approach to treat the noncompressible hemorrhage.

1.0 INTRODUCTION

Hemorrhage is the greatest threat to survival in the first 24 hours following traumatic injury [1]. It accounts for nearly 50% of all deaths on the battlefield and 39% of civilian trauma deaths [2-5], most of which occur before patients reach the hospital [6-7]. Traditional methods available to pre-hospital personnel for controlling hemorrhage, such as applying tourniquets or pressure dressings, or clamping, are practical for extremity or superficial truncal wounds. However, these types of wounds account for only 10% of hemorrhage-related combat deaths and even fewer hemorrhage-related civilian deaths [8-9]. The majority of hemorrhagic deaths...
on the battlefield (up to 90% in Vietnam war) are due to intracavitary hemorrhage that is not accessible to
direct pressure and cannot be controlled by these traditional methods [8]. This has left first responders with no
means to treat truncal hemorrhage other than fluid resuscitation. Paradoxically, the administration of
intravenous fluids, by diluting coagulation factors and platelets, can further exaggerate bleeding. Even after
arrival in a hospital, patients with acute bleeding from truncal injuries can present significant challenges to
surgeons who possess multiple surgical techniques, sophisticated equipment, and variety hemostatic materials
[10]. Currently, surgical interventions are the only available methods for controlling noncompressible
hemorrhage and preventing death. Developing methods to control or reduce intracavitary hemorrhage within
the first critical hours following injury, before patients reach the hospital could have a significant impact on
morbidity and mortality rates for trauma patients.

1.1 Intracavitary Intervention

A novel concept for treating intracavitary hemorrhage was first introduced by Holcomb and colleagues [11].
They proposed the creation of a hemostatic agent for direct infusion into a bleeding body cavity. The
hemostatic agent would then self-expand throughout the closed cavity and interact with bleeding sites to stop
hemorrhage. In a penetrating injury the hemostatic agent would be introduced through the hole left by the
wounding mechanism. To implement this concept, the hemostatic agent would have to: 1) reduce blood loss
and increase survival when applied directly on an active bleeding site without manual compression; 2) spread
efficiently and reach the internal injuries in the closed cavity (e.g. abdomen); 3) remain active during and
after indirect application and interact with bleeding sources to reduce overall blood loss and related fatalities.
To put this concept to the test two hemostatic products, fibrin sealant (FS) foam and FloSeal were considered.
FS foam contained human fibrinogen, human thrombin, and calcium chloride (CaCl₂), and was
reconstituted in saline and transformed into foam with the aid of a chemical reaction. FloSeal was
obtained from commercial sources and contained bovine-derived thrombin and collagen particles. Both
hemostatic agents were evaluated in modified rat or rabbit liver injury models.

2.0 OPEN-ABDOMEN TREATMENT OF PARENCHYMAL BLEEDING (DIRECT
APPLICATION)

FS foam and FloSeal were first tested in open abdomen models that allowed easy access to the wounds and
direct application of hemostatic agents on the injuries. A positive outcome in this initial phase of the study
was a prerequisite for further evaluation of the agents.

2.1 FS Foams

2.1.1 First Formulation

In the initial proof-of-principle study [11], a formulation of fibrin sealant foam with high thrombin (36 IU/ml)
and low fibrinogen (1.9 mg/ml) concentration was developed and tested. The foam was rapidly prepared and
sprayed directly on the bleeding surfaces of rat liver tissues immediately following sharp resection of 60% of
the median hepatic lobe. Subsequently, animals were resuscitated (3.3 ml/kg) to maintain their blood pressure
at pre-injury levels and observed for 30 minutes or until death. The FS foam treatment decreased blood loss
(~50%) compared with untreated or placebo foam (IgG replaced fibrinogen) treated injuries (P<0.01).
However, this reduced blood loss did not influence the survival of the treated group. A tight binding between
fibrin clots and injured hepatic tissue was observed which might have been responsible for the overall
reduction in blood loss. But when the FS foam was introduced into a closed-body cavity at a site distal to the
liver injury, no change in blood loss or survival was observed among the treated animals (unpublished data). The high thrombin activity and rapid clotting may have prohibited this formulation of FS foam from dispersing sufficiently throughout the cavity to reach the bleeding targets. Hence, the first formulation was considered inappropriate for intracavitary treatment purposes.

2.1.2 Second Formulation
A new formulation for FS foam was designed and tested extensively in the laboratory. The concentration of individual components of this foam were optimized based on an *in-vitro* tissue adhesion test developed in our laboratory. The new FS foam formula contained high concentrations of human fibrinogen (20 mg/ml) and albumin (10 mg/ml) with low levels of thrombin activity (3.3 IU/ml) and CaCl₂ (0.9 mg/ml) and polymerized more slowly than the previous formula. This foam attached firmly to rabbit liver slices even if the tissues were covered with fresh blood (not anticoagulated).

2.1.3 Rabbit Model
To evaluate the hemostatic efficacy of this foam *in-vivo*, a new liver hemorrhage model was developed in rabbits. This species offered several advantages over rats: 1) the larger circulating blood volume (200-250 ml) in mature animals (3 kg) permitted blood sampling during operation without affecting the blood pressure; 2) higher bleeding volumes in rabbits minimized adverse effects of small variations in final blood/blood clot collections and blood loss measurements; 3) less efficient blood clotting ability and lower survivability of rabbits with hemorrhage provided better conditions for testing hemostatic agents [12].

2.1.4 Anticoagulants
During the course of model development different anticoagulant regimens were tested. The parenchymal injury consisted of sharp resection of 1/3 of the middle and left lobes and 1/4 of the right lobe of the liver without damaging the gall bladder and bile ducts. Subsequently, the abdominal incision was closed and animals were resuscitated with lactated Ringer’s (LR) solution at a constant rate (1 ml/kg/min) to maintain mean arterial pressure at 80% of baseline value (pre-injury level). This relatively consistent liver injury and resuscitation protocol nonetheless produced wide variations in blood loss and resulted in only 30% mortality among rabbits. Ressecting larger portions of the liver frequently resulted in rapid exsanguination and no opportunity for hemostatic treatment.

Therefore, in order to produce persistent liver bleeding with relatively large and reproducible blood loss in rabbits, anticoagulant treatment was incorporated into the protocol. Initially, heparin (200 IU/kg) was used as an anticoagulant. The above-noted liver injury in heparinized animals caused persistent bleeding with 100% mortality within a 60-minute observation period. When FS foam was tested in the heparinized model, no hemostatic benefit and no interaction between the fibrin clot and bleeding tissues was observed. It became evident that the presence of heparinized blood in the wound may have inhibited the activity of the thrombin in the foam and prevented cross-linking of fibrin to tissues. Therefore, a new anticoagulant, heparinoid, which has little or no effect on thrombin activity, was selected. The anticoagulant activity of heparinoid is predominately due to the inhibition of Factor Xa activity in blood.

2.1.5 FS Treatment in Heparinoid-Treated Rabbits
The hemostatic potential of the new formulation of FS foam was reexamined in heparinoid-treated rabbits. Animals were injected intravenously with a dose of anticoagulant (sodium danaparoid, 50 anti FXa
activity/kg) and liver injuries were induced. The bleeding tissues were sprayed with FS foam or placebo foam in which hemostatic components were replaced with an equal quantity of human IgG. The rapid and direct treatment of the injuries with FS foam produced a 37% decrease in blood loss ($P=0.07$) and a 23% increase in percent survival (Fig. 1a, b). The average survival time in the FS group was 53.3 ± 4.2 min versus 41.3 ± 7.7 min in the placebo-treated group ($P=0.34$, Fig. 1c). Although these outcomes were not statistically significant, (mainly because of limited number of animals in each group), the numerical trends provided sufficient positive evidence to justify further testing of the hemostatic capacity of the FS foam in a closed-abdomen model.

![Figure 1: Open-Abdomen Model. Effects of direct and immediate application of placebo or fibrin sealant foam after severe liver injury in rabbits on: a) blood loss into the abdominal cavity; b) survival time after injury; and c) percent survival.](image)

### 2.2 FloSeal

This FDA-approved hemostatic agent is commercially available (Baxter International Inc). It consists of specially engineered collagen particles and bovine thrombin. Upon exposure to blood, the gelatin (collagen) granules expand ~ 20% to provide some tamponade [13], and thrombin converts endogenous fibrinogen to fibrin that adheres the gelatin granules to the wound. To aid hemostasis, gentle compression of FloSeal mixture over the wounds with moist gauze for 2 to 3 minutes is strongly recommended. This agent has been used in humans to reduce bleeding in a variety of surgical procedures including vascular surgery, cardiac valve replacement and partial nephrectomy [13-15]. It was shown to be more effective in controlling blood loss than a combination of Gelfoam and thrombin in a clinical study involving cardiac surgery [16].

FloSeal was also tested as a hemostatic agent for intracavitary noncompressible hemorrhage using the previously described rat liver injury model (unpublished data). FloSeal paste was prepared according to the manufacturer’s instructions by dissolving thrombin in saline and then thoroughly mixing the dissolved
thrombin with Gelatin Matrix. Five ml of the mixture or vehicle solution (saline) were applied directly over the cut liver surfaces immediately after injury without additional compression and the abdominal cavity was closed. Animals were resuscitated (LR, 3.3 ml/min/kg) to maintain their blood pressure at pre-injury levels and they were monitored for 90 minutes or until death. FloSeal treatment significantly reduced the blood loss (p<0.01) and numerically increased percent survival (26.7 vs 6.3%; P=0.17) and average survival time (37.7±9.0 vs 20.7±5.2 min; P=0.12) among the rats (Fig 2 a, b, c). There was no association between the volume of resuscitation fluid used and blood loss (P=0.45). This was the first evidence of the hemostatic benefit of FloSeal for major bleeding when used without the aid of manual compression, an essential requirement for any hemostatic agent under consideration for treatment of noncompressible hemorrhage.

![Figure 2: Open-Abdomen Model. Effects of direct and immediate application of saline (control) or FloSeal after severe liver injury in rats on: a) blood loss into the abdominal cavity; b) survival time after injury; and c) percent survival.](image)

**3.0 CLOSED-ABDOMEN TREATMENT OF PARENCHYMAL BLEEDING (INDIRECT APPLICATION)**

**3.1 FS Foam**

The efficacy of slow polymerizing FS foam (2nd formulation) was further tested in a closed-abdomen injury model in rabbits. Following heparinoid injection and laparotomy, three frontal lobes of liver were sharply resected as described before and the abdominal incision was closed with suturing. Five minutes following liver injury, the rabbits were either treated with FS foam or left untreated to bleed freely from the injuries (controls). For foam treatment, a plastic tube was inserted into the abdomen (on suture line) and aimed toward the liver. About 100 ml of FS foam was prepared in a large syringe (140 ml) and infused into the cavity through the implanted tube. Animals were resuscitated with LR as in previous experiments (1 ml/min/kg) to maintain their blood pressure at 80% of pre-injury levels and monitored for 60 minutes or until death at which time blood, blood clots and foam clots were collected and weighed to estimate total blood loss (corrected for the weight of the foam).

Infusion of FS foam into the abdomen distal to the bleeding tissues did not reduce blood loss or improve percent survival in the rabbits (Fig. 3a). The only difference was a larger variation in blood loss of individual rabbits in the foam group compared with control rabbits. Inspection of the wounds after completion of the
experiments revealed that in most cases (4/6) foam did not spread well enough to penetrate and cover the resected areas in the left and right lobes of the livers. It reached and attached firmly to the middle lobe where blood had drained away from the tissue.

### 3.2 FloSeal

The potential intracavitary application of FloSeal was tested in the rat liver injury model. Following sharp resection of 60% of the median hepatic lobe the peritoneal cavity was closed. FloSeal was prepared as for the open abdomen experiments except it was diluted four-fold with saline to reduce FloSeal viscosity and to improve its distribution throughout the abdomen. Five minutes after the liver injury, FloSeal was infused into the closed abdomen ~ 3 cm distal to the bleeding site. Animals were resuscitated and monitored up to 60 min. This procedure did not reduce blood loss ($P=0.43$) or improve survival ($P=0.66$) when compared with the saline-treated control rats (Fig. 3b). When the surgery was performed again in a presumably positive control group in which the injured tissue was clamped and the bleeding was abruptly and completely stopped after 5 minutes, there was no improvement in blood loss or survival of animals as compared with saline-treated or untreated groups.

![Figure 3: Closed-Abdomen Model. a) Effects of indirect and delayed (5 min) application of fibrin sealant foam after liver injury in rabbits on internal blood loss and survival. b) Effects of indirect and delayed (5 min) application of saline or FloSeal after severe liver injury in rats on blood loss into the abdominal cavity and percent survival.](image-url)
4.0 DISCUSSION

Despite positive results of FS foam and FloSeal when applied directly without manual compression in an open-abdomen model, these agents were ineffective in preliminary evaluations of indirect application in the closed-abdomen models. The reasons for hemostatic failure may be similar for both products. For FS, the interaction and binding of fibrin clot with the injured tissues is an essential step for achieving hemostasis. This interaction may also be a component of FloSeal’s hemostatic ability. The poor distribution, weak and incomplete binding of FS foam with injury sites appeared to be the main cause of its ineffectiveness.

There were several factors that may have impeded the distribution of the foam and FloSeal in the closed abdomen. First, the five-minute delay in application of the hemostatic agents was implemented in both intracavitary studies to more closely reflect a realistic battlefield situation in which no medical care would be available to soldiers immediately after wounding. Such a delay, however, might have resulted in significant blood pooling around the liver, rendering the injury sites less accessible for treatment. In addition, significant blood loss during this crucial period may have made the later intervention essentially futile. This was clearly demonstrated in the control group of FloSeal study in which complete cessation of bleeding five-minutes after injury had no effect on the final measurements. Second, the flowing blood from the tissues could have also impeded the penetration and interaction of the agents with the bleeding sites. Third, in rabbits, the ineffectiveness of the foam may have been related to the anatomical location of the liver wound. The close proximity of the peritoneal wall to the right and left lobes and the natural drainage pathway of the blood on the lateral sides of the abdomen might have prevented the dispersion and contact of the foam with those tissues. Although foam covered the middle lobe, and perhaps stopped the bleeding in this region blood flow from the middle lobe might have shifted to the lateral lobes and bleeding continued with the same intensity from these lobes that were not covered by the hemostatic agent. The four-fold dilution of FloSeal necessary for closed-abdomen usage might have also weakened the interaction of this agent with damaged tissue and reduced its hemostatic efficacy.

Theoretically, intracavitary injection of hemostatic agents could be a successful method for treating noncompressible hemorrhage, but the preliminary testing with FS foam and FloSeal in the animal models described above did not show any potential benefit. Although the models seemed appropriate for the initial hemostatic testing of the agents with direct application, they did not meet some bleeding characteristics necessary for indirect and delayed application of the products. The following options and changes will be considered in future studies to further investigate intracavitary treatment of noncompressible hemorrhage.

- Development of a new noncompressible hemorrhage model with a slow but persistent bleeding profile in rabbits that would be responsive to delayed treatment with hemostatic agents.
- Addition of a high-pressure liquified gas propellant to hemostatic products to facilitate introduction and distribution of the agents into the cavities. The propellant gas will have to be vented rapidly from the abdominal cavity to prevent sustained high insufflation pressure and its potentially harmful effects (e.g., abdominal compartment syndrome).
- Intracavitary infusion of gel/foam preparations of true hemostatic agents (e.g. thrombin, reptilase). Unlike fibrin sealant products, these agents promote hemostasis by stimulating the patient’s own blood to clot and seal injuries to stop the bleeding. With the use of these agents, the blood pooling around the injuries could actually be beneficial and promote hemostasis instead of acting as a physical barrier.
- Development of an integrated approach that combines intravascular and intracavitary treatments. For example, the essential coagulation precursors such as fibrinogen and phospholipid membranes may be
injected intravascularly as additives to fluid resuscitation procedures, and hemostatic enzymes infused into cavities to induce coagulation and control bleeding.

5.0 SUMMARY

The intracavitary administration of hemostatic agents offers a novel concept for treatment of noncompressible hemorrhage. However, the preliminary testing of this concept with two hemostatic agents failed to show measurable benefit in reducing parenchymal bleeding. The reasons for this failure appeared to be more related to the hemorrhage models, treatment conditions, and the agent formulas (designed for direct application) used for evaluating this hypothesis rather than the concept itself, but clearly, there are several technical problems associated with testing of such a novel approach that have yet to be overcome. Future studies with alternative hemostatic agents will focus to resolve these issues and further evaluate the potential benefit of intracavitary treatment of noncompressible hemorrhage in clinically relevant and potentially treatable models.

6.0 REFERENCES


Alterations in Coagulation Induced by Hypothermia and Acidosis in Swine

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Abstract

Although clinical coagulopathy is associated with acidosis and hypothermia, the underlying mechanisms by which these factors alter the coagulation process remains unclear. The primary purpose of this study was to investigate the contributory effects of acidosis and hypothermia to the development of coagulopathy. Twenty-four pigs were randomly divided into control (pH 7.4, 39°C), acidotic (pH 7.1, 39°C), hypothermic (pH 7.4, 32°C), and combined acidotic-hypothermic (pH 7.1, 32°C) groups (n=6/group). Acidosis and hypothermia were introduced by infusion of 0.2M HCl and using a blanket with circulating cold water (4°C), respectively.

Measurements were compared before (pre) and 10 min after the induction of acidosis and hypothermia (post). Development of coagulopathy was defined as a significant increase in splenic bleeding time in vivo.

Coagulopathy developed shortly after the induction of acidosis and/or hypothermia. Splenic bleeding time was prolonged by 41%, 57%, and 72% in acidotic, hypothermic, and the combined groups (p<0.05, pre vs post in each group), respectively. Hypothermia caused a delay in the onset of thrombin generation, whereas acidosis caused both a delay in the onset of thrombin generation and an impairment in thrombin generation rate. The reaction time (latency time for initial clot formation) of the thromboelastogram (TEG) was prolonged in the hypothermia and the combined groups, but not in the acidotic group. The α-angle (the rapidity of fibrin build up and cross linking) of the TEG was reduced in the acidosis and combined groups, but not in the hypothermia group. We conclude that acidosis and hypothermia cause coagulopathy via different mechanisms.

1.0 INTRODUCTION

The association of hypothermic coagulopathy with high mortality has been well described [1-10]. As many as 66% of trauma patients arrive in emergency departments manifesting hypothermia (temperature < 36°C) [2]. Approximately 80 % of non-surviving patients have had a body temperature of less than 34°C [9]. Furthermore, investigators have reported a 2.4-fold increase of blood loss in post-laparotomy patients whose body temperature was 33.8±0.5°C as compared to that of patients whose temperature was 36.1±0.7°C, despite similar injury severity [4]. The effect of hypothermia on coagulation has been hypothesized to result from inhibition of enzyme activities and platelet function, as well as increased fibrinolysis. The inhibition of the enzyme activity has been estimated by the effect of cold on the prothrombin time (PT) and activated partial

1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

thromboplastin time (PTT). Prolonged PT has been found in hypothermic patients and experimental animals, as well as in plasma samples cooled in vitro [10-15]. Changes in platelet function with cold are less consistent, but shear induced platelet activation is markedly depressed at temperatures below 34°C [16]. However, no changes in fibrinolysis have been reported under hypothermic conditions [10, 11, 15]. These data suggest that the effects of cold on coagulant activity are complex, but general mechanisms related to the development of coagulopathy are known.

The role of acidosis in the development of clinical coagulopathy is poorly described, though it has been implicated [17-19]. With the limited studies performed, inconsistent results have been reported [20-23]. Decreased clotting time was observed when lactic acid was added to heparinized human and dog blood in vitro [20]. Similar decreases in PT and PTT were reported in dogs following the infusion of 1M lactic acid [21, 22]. However, increased PT and PTT, as well as decreased fibrinogen levels and platelet counts were reported by Dunn et al [23] in dogs after the infusion of HCl solution. Decreased activities of factor VIIa - tissue factor (FVIIa/TF) complex and factor Xa -factor Va (FXa/FVa) complex on phospholipid surfaces were reported by Meng et al, when pH was decreased from 7.4 to 7.0 [24]. Thus, the effect of acidosis on the coagulation process remains to be clarified.

The essence of blood coagulation is the production of fibrin from fibrinogen, and thrombin has a central role [25]. Thrombin activates platelets, as well as cofactors, enzymes in the clotting process, and inhibitors of the fibrinolytic process. In the initial phase, small amounts of thrombin are produced by the activation of FVIIa/TF complex and factor Xa. Afterward, there is a propagation phase with generation of large amounts of thrombin, which result from the production of prothrombinase complex on the surface of activated platelets. At the same time, thrombin generation is subject to inhibition from antithrombin III, thrombomodulin activated protein C, and tissue factor pathway inhibition. This complex mechanism enables rapid clot formation upon tissue injury, but inhibition of clot formation away from the site of the injury. Thus, changes of thrombin generation kinetics under different circumstances provide information about the mechanisms of underlying alterations in coagulation. To the best of our knowledge, changes of thrombin generation kinetics under acid and hypothermic conditions have not been investigated.

The primary purpose of this study was to investigate the individual and combined contributions of hypothermia and acidosis to the development of a clinical coagulopathy in vivo. To understand the mechanisms involved, thrombin generation kinetics were quantified under acidic, hypothermic, and acidic and hypothermic combined conditions in swine.

2.0 METHODS

2.1 Animals

This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas (A-00-006). A total of 24 crossbred Yorkshire swine (body wt. 40.6 ± 3.9 kg) were randomly allocated into normal control, acidic, hypothermic, and acidic and hypothermic combined groups (n=6 in each group). After an overnight fast, animals were pre-anesthetized with Glycopyrrolate (0.1 mg/kg) and Telazol (6mg/kg), followed by 5% Isoflurane in 100% Oxygen by mask for the surgical procedures. The right femoral artery and the right external jugular vein were cannulated for blood sampling and fluid infusion, respectively. Arterial blood temperature and pH were monitored in vivo using intraarterial sensors precalibrated according to manufacturer’s instructions (Paratrend 7- Trendcare system, Diametrics Medical Inc, Roseville MN) placed via a 20 gauge carotid artery cannula. Artery blood pressure and heart rate were monitored using an ex vivo pressure transducer connected to the same cannula.
2.2 Experimental Design

After baseline blood samples and coagulation measurements were taken (the “pre” sample), hypothermia (32.0°C) was induced using recirculating water at 4°C via a water-pumped blanket until the animal’s body temperature reached 32.0-32.5°C. Acidosis (pH 7.1) was induced by infusion of 0.2 M HCl in Lactated Ringers solution at a rate of 0.4-0.8 ml/kg/min. The rate of infusion was slowed below pH 7.3 to facilitate achieving the target pH of 7.1. In combined group, hypothermia and acidosis were induced simultaneously to reach 32.0°C temperature and pH 7.1.

Blood samples were taken before the induction of acidosis and hypothermia (pre) and 10 min after target pH and temperature were achieved (post). Blood samples were collected by inserting a 25 cm single-use catheter made from Tygon® tubing (Saint-Gobain Performance Plastics, Akron, Ohio) into the self-sealing port of the femoral catheter introducer. Blood was gently withdrawn to minimize shear induced platelet activation. The first 3 ml of blood withdrawn were discarded at each sampling time.

Splenic bleeding time was measured before the induction of acidosis and hypothermia and after target pH and temperature were achieved using #11 scalpel blades positioned into a right-angle clamp to obtain a 3 mm cutting depth. Splenic bleeding cessation time was recorded and blood from the incision was collected using pre-weighted gauze.

2.3 Analyses

Hemoglobin (Hgb), Hematocrit (Hct), and Platelet (PLT) counts from citrated blood were measured using a Pentra 120 hematology analyzer (ABX Diagnostics, Inc., Irvine, CA). Prothrombin time (PT), Partial Prothrombin Time (PTT), and Fibrinogen concentration from citrated plasma were determined at 37°C using the ACL Futura Coagulation System (Instrumentation Laboratory, Lexington, MA).

Thrombelastography (TEG): Whole blood (300 µl) was drawn from the femoral artery into an Eppendorf repeater pipet (Brinkmann Instruments Inc. Westbury, NY) and transferred directly into saline preloaded cups of Model 5000 TEG’s (Haemoscope, Skokie, IL). TEGs were run under the same pH and temperature conditions as the pig from which the blood samples were taken following the manufacture’s instructions.

Thrombin generation kinetics was determined from thrombin-antithrombin III complex (TAT) concentration using minimally altered whole blood, following the procedure described by Rand et al.[26]. This approach has been validated in previous reports [25, 27]. Briefly, blood samples were withdrawn from the femoral artery using an Eppendorf repeater pipet with a 25ml syringe barrel. The syringe barrel was wrapped with 500 ml saline bags for insulation. The blood was added (1ml each) to twenty-four 12 x 75 mm polystyrene tubes with 3/16-inch holes in the side at the midpoint of the tubes to start the reaction. All tubes were preloaded with 29 µl of 20 mmol/L HEPES, 150 mmol/L NaCl, and 5 mmol/L CaCl2 at pH 7.4 (for control and the hypothermic groups) or pH 7.1 (for the acidic and combined groups) and 50 µl of normal saline or a 1:100 dilution of pig thromboplastin. The assay tubes were fixed and rocked continuously in Thermal Rockers (Lab-Line Instruments, Inc. Melrose Park, Illinois) set to either 39°C or 32°C. Whole blood clotting was stopped at different time points by adding 1ml of quench solution (50mmol/L EDTA and 10mmol/L L-benzamidine in HEPES-buffered saline). After quench, tape was placed over the holes in the tubes. The tubes were vortexed and centrifuged at 4000 g for 15 min. Supernatant was collected and concentrations of TAT was measured using Enzygnost TAT micro enzyme immunoassay kits (Dade Behring, Marburg, Germany) following the manufacture’s instructions.
2.4 Statistical Analysis

Data were analyzed using SAS statistical software. In each study group, comparisons were made in all measurements on a pre/post basis using one-way ANOVA. The slope parameter was tested against zero to determine significant changes within a group. Between group comparisons were made with appropriate adjustments for multiplicity using Tukey adjustment. The statistical significant level was set at \( p < 0.05 \). Data are expressed as Means ± SEM.

3.0 RESULTS

All 24 animals survived the procedures to the end of the experiment. Hypothermia (32.0 ± 0.3°C) or acidosis (pH 7.11 ± 0.02), or both, were successfully induced in all animals as intended, within 2 hours. Mean arterial pressure (MAP) in each group remained unchanged during the study. Heart rate (bpm) was increased in the acidotic group from 105±5 to 147±17 (p<0.05), but not altered in other groups. The hematocrit, hemoglobin, plasma total protein, and plasma Na+ and K+ levels remained unchanged in all groups. Plasma fibrinogen concentration was decreased in the acidotic and combined groups (p<0.05), but was not decreased in the hypothermic group (Fig.1). The platelet count was decreased in the acidotic, hypothermic, and combined groups (p<0.05, Fig. 1).

Splenic bleeding time was significantly prolonged by 41%, 57%, and 72% in the acidotic, hypothermic, and combined groups, respectively (P<0.05, Fig 2). However, changes were not demonstrated in the PT measured at 37°C (Table 1). The PTT was prolonged in the acidotic and combined groups (p<0.05), but not in the hypothermic group (Table 1).

Table 1: In Vitro Clotting Time Measurements

<table>
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<th>Control</th>
<th>Acidosis</th>
<th>Hypothermia</th>
<th>Combined</th>
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<tr>
<td>Pre</td>
<td>10.2±0.1</td>
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<td><strong>PTT (sec)</strong></td>
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<tr>
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</tr>
<tr>
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<td>19.0±1.5‡</td>
<td>13.5±0.4</td>
<td>19.0±0.8‡</td>
</tr>
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PT- prothrombin time measured at 37°C.
PTT – partial activated thromboplastin time measured at 37°C.
* pre vs post within each group (p<0.05). ‡ Control vs experimental groups (p<0.05).
Figure 1: Changes of plasma fibrinogen concentration (mg/dl) and platelet count ($10^3/\mu l$) in experimental groups. * $P<0.05$, pre vs post within each group.
The independent and combined effects of acidosis and hypothermia on thrombin generation are shown in Fig. 3. Each curve represents the average value (n=6/group) of thrombin content assayed as TAT at 10 different quench times. Acidosis caused a moderate delay in the onset of thrombin generation. This delay was further prolonged in the hypothermic and combined groups. After initial thrombin was generated, the thrombin generation rate was primarily inhibited by acidosis. At 4 min, thrombin generation in the acidotic, hypothermic, and the combined groups was 47.0±4.9%, 12.5±4.7%, and 5.7±1.3% of the average value in the control group, respectively (p<0.05, control vs the hypothermia or combined group). At 7 min, thrombin generation in the acidotic and combined groups was 60.4±4.2% and 43.5±4.0% of the average value in the control group, respectively (p<0.05, control vs the acidotic or combined group). The apparent additive inhibitory effect in thrombin generation rate in the combined group was not statistically significant.

In TEG measurements, R time (representing the latency time before initial clot is formed) was prolonged from 3.8 ± 0.7 min to 5.4 ±1.0 min in the hypothermia (p<0.05) and from 3.0 ± 0.5 to 4.3 ± 0.6 in combined groups (p<0.05), with no change in R in the acidotic group. Angle (α, reflecting the rate of fibrin build up and cross-linking) was decreased from 72.4 ± 1.7 to 64.8 ± 2.6 in the acidotic group (p<0.05) and from 72.6 ± 1.5 to 56.8 ± 2.2 in combined group (p<0.05), with no change in the hypothermic group.

Figure 2: Changes of splenic bleeding time in experimental groups. * p <0.05, pre vs post within each group.
Figure 3: Thrombin generation kinetics in blood samples obtained with special acquisition procedure from femoral artery measured as thrombin-antithrombin III complex concentration ([TAT]). [TAT] was measured in sample aliquots at time 0 (sample withdrawal), and at 1 min intervals thereafter to determine thrombin generation with time in each sample using the method of Rand et al. [26].

* p<0.05 different than normal value.

4.0 DISCUSSION

Thrombin generation regulates various biochemical and physiological processes involved in coagulation and inflammation. In blood coagulation, thrombin plays a central role in activating cofactors, platelets, enzymes, and inhibitors and in cleaving fibrinogen to fibrin monomer. This study, for the first time, investigated thrombin generation kinetics under hypothermia and/or acidosis induced in vivo. We found that both hypothermia and acidosis impaired thrombin generation. Further, we identified that hypothermia and acidosis inhibited thrombin generation with different kinetics. These findings were well correlated with an increase in splenic bleeding times and results of TEGs.

Hypothermia primarily caused a delay in the onset of initial thrombin generation, indicating that the inhibition was located primarily in the FVIIa/TF pathway. In acidosis, initial thrombin generation was moderately delayed. After initial thrombin was generated, the thrombin generation rate at the propagation phase was persistently and drastically inhibited by acidosis. Consistently, Meng et al reported that the activities of the FVIIa/TF complex and the FXa/FVa complex on phospholipid surfaces were decreased by 55% and 70%, respectively, at pH 7.0 compared with that at pH 7.4 [24]. Because of the persistent inhibition at the propagation phase and moderate inhibition at the initial phase, acidosis might be more detrimental than hypothermia in the development of coagulopathy. This point is worth emphasizing because acidotic effects on coagulopathy have been under-appreciated. In addition, we found that the acidotic inhibition on the
thrombin generation rate was amplified when hypothermia was present, which correlates with clinical findings of a high mortality rate in trauma patients complicated with acidosis, hypothermia, and coagulopathy [18]. Thus, correcting blood pH should be considered a potentially important strategy in reversing clinical coagulopathies.

In this study, the development of coagulopathy was defined as a statistically significant increase of splenic bleeding time. This measurement provided an overall estimate of coagulation, as it included all of the factors involved in coagulation, such as blood flow and systemic effects. Increases as high as 41%, 57%, and 72% in splenic bleeding time were found in the acidosis, hypothermia, and combined groups, respectively, indicating that acidosis and/or hypothermia caused coagulopathy. However, these detrimental effects were not detected in standard PT measurement (assayed at 37°C), since no changes in PT were found in any experimental group. Our findings confirm the widening appreciation that the standard PT is not a sensitive index of coagulation function in clinical practice. Since current commercially available PT instruments are certified at 37°C, it is important to emphasize that standard PT should be used to assess coagulation factor concentration, but not coagulopathy in hypothermia [12].

The ultimate outcome of the coagulation process is clot formation from precursor fibrinogen. Decreased fibrinogen levels have been described in trauma patients, and the decline of fibrinogen levels have been considered as one of the two most sensitive measures of clinical coagulopathy (the other being platelet counts) [28]. Consistently, we observed decreases of about 20% in fibrinogen concentration shortly after induction of acidosis. This 20% drop can be amplified by hemorrhage and resuscitation, as occurs in patients after a trauma injury or post-surgery. The underlying mechanism of the depletion is not clear. Additional investigation is required to clarify the underlying mechanism.

Our measurement of thrombin generation kinetics is consistent with the coagulation profiles obtained from TEG. Reaction time (R) in TEG is the latency time for initial clot formation. A prolonged R time represents a deficiency or dysfunction in coagulation factors. In this study, R time was found to increase in the hypothermic and combined groups (but not altered in the acidotic group), which was consistent with the prolonged delay of initial thrombin generation found in the hypothermic and combined group. Angle (α) measures the rapidity of fibrin built up and cross-linking. It is affected by the availability of fibrinogen and platelets, but mostly by thrombin activity. In this study, α was found to decrease in the acidosis group and combined group, but did not change in the hypothermic group. The decreases of α in those groups were consistent with the decreased thrombin generation rates, as well as decreased fibrinogen concentration, observed in the acidotic and combined groups.

Recently, recombinant activated factor VII (rFVIIa) has been used as therapy in patients bleeding uncontrollably, with beneficial effects in some of these patients [29-31]. However, rFVIIa has not been effective in some acidotic trauma patients (U. Martinowitz, personal communication). These different clinical outcomes may be explained in relation to the findings from the present study. Since acidosis affects thrombin generation on, both, initial and subsequent propagation steps, supplementation with rFVIIa alone may not release the inhibition of propagation. Thus, a better alternative in acidotic patients may be to supplement rFVIIa in conjunction with pH correction (i.e. bicarbonate infusion). In contrast, improvement can be expected from supplementation with rFVIIa, alone, in hypothermic patients, since hypothermia primarily inhibits the activation of FVIIa/TF complex.
5.0 SUMMARY

In summary, we investigated the independent and combined contributions of hypothermia and acidosis in the development of a clinical coagulopathy. Acidosis and/or hypothermia impair blood coagulation process. As a possible underlying mechanism, acidosis and hypothermia inhibit thrombin generation for clot formation by different kinetics. Further understanding of the mechanisms underlying the development of coagulopathy induced by different means may facilitate hemorrhage control in trauma patients.

6.0 REFERENCES


Pathogen Inactivated Plasma Concentrated: Preparation and Uses*

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ABSTRACT

Background and Significance: Plasma transfusion is a crucial element of casualty care. Simple to perform, plasma transfusions restore the clotting factors, electrolytes, nutrients, immune factors and water that are lost in severe trauma. Unfortunately, two plasma components sharply limit the use of this life-saving fluid: contaminants and excess water. Contamination arises because plasma must be obtained from human donors, and these donors may be infected with a wide variety of pathogens. To reduce the risk posed by these pathogens, advanced screening and testing procedures have been developed. These procedures, however, are not entirely effective even in the civilian blood bank system. For the military, the situation is much worse, particularly for advanced field units operating in third world countries. If their supply lines are cut, such units may be forced to collect plasma from a local population suffering from high infection rates of malaria, HIV, or possibly even unknown pathogens. To reduce the risks of infection from plasma collected from either domestic or foreign sources, an effective means of plasma decontamination is therefore necessary. The second plasma problem, excess water, makes plasma difficult to freeze, transport, store, and thaw. Furthermore, this excess water limits the amount of clotting proteins that can be transfused without overloading the kidneys, and also lengthens the time required for lyophilization and other processing. An effective means of reducing the excess water in plasma is therefore necessary.

Approach: Ultraviolet-C (UVC) irradiation is quite effective at decontaminating blood products. Unfortunately, UVC also has sufficient energy to split dissolved oxygen into radicals, which then severely damage the plasma proteins. The solution to this problem is simply to degas the plasma before exposing it to UVC. Without dissolved oxygen, no radicals can form under UVC exposure, thereby sparing the plasma proteins. As an additional benefit, the degassed liquid is highly susceptible to ozone, another technique that is quite effective at decontamination. This high susceptibility ensures rapid, uniform treatment. The next step is to reduce the excess water in the decontaminated plasma. The limiting factor here is that plasma proteins rapidly clog conventional filtration systems. The solution to this problem is to apply ultrasound to cold plasma. The ultrasound generates pure ice crystals, which are then removed to leave concentrated plasma.

Testing: Porcine parvovirus (PPV) was spiked into the plasma to determine decontamination effectiveness. Factor VIII and fibrinogen concentrations were then measured before and after decontamination and cryoconcentration to determine protein quality.

**Results:** Both UVC and ozone yielded a PPV logarithmic reduction factor (LRF) of 6, for a combined LRF of 12. UVC treatment reduced Factor VIII by 10% and fibrinogen by 7%. Ozone reduced Factor VIII by 12% and fibrinogen by 6%. Cryoconcentration up to a factor of 10 showed no measurable protein damage.

**Conclusions:** UVC and ozone yield high levels of decontamination with minimum protein damage. Cryoconcentration greatly reduces the excess water, without damaging the plasma proteins. The process can be applied to single donor units, thus avoiding the risks of pooled plasma. No additives are necessary.

## 1.0 INTRODUCTION

Blood products are absolutely essential at advanced field locations. Whole blood, red blood cells (rbc’s), platelets, plasma and plasma derivatives all have vital roles in on-site treatment, as well as in patient stabilization for transport to better equipped facilities.

There are three possible sources for these valuable components. First, they can be brought in with the rest of the mobile hospital equipment, but this approach is limited by the available space and the need for refrigeration for many products. When these initial supplies are exhausted, it is necessary to restock them by a supply line. If the supply lines are cut, however, it is then necessary to collect them locally, either from the forces themselves or from the local population. While soldiers donating to their fellow soldiers is a time-honored military tradition, it is inherently limited. The only remaining option is therefore to collect blood components from the indigenous populations, but this approach carries a great risk of dangerous infections, particularly in third world countries.

For an ideal supply of blood products, it is therefore necessary to consider all three of the above situations. In each situation, technologies currently under development at CryoFacets, Inc. can make great improvements over what is currently in use. Many of these advances are summarized below, starting with a single plasma unit with double pathogen inactivation. Next, a device to concentrate this plasma for shipment and direct transfusion is described. Lyophilization options are then described. Finally, a few comments on cellular components are provided. Because a great number of topics are discussed, the material on each topic is necessarily brief. CryoFacets, Inc., however, welcomes questions or other inquiries about all of these technologies.

## 2.0 PLASMA DECONTAMINATION

### 2.1 Need for Decontamination Technology

Plasma is the straw-colored liquid component of blood that remains after the cellular components have been removed. Consisting mainly of water, salts, nutrients, immune globulins and clotting factors, plasma is often transfused in cases of severe blood loss. Unfortunately, plasma can also contain a wide variety of pathogens, including parasites, bacteria, yeasts, viruses and possibly even prions.

The net result is that plasma is a high demand item, but plasma transfusion itself poses the risk of transmitting a deadly disease. In the case of domestically sourced plasma, these risks are minimal because of donor screening and extensive laboratory testing for pathogens such as HIV and hepatitis. Even then, there is still some risk due to screening failures, laboratory mistakes, the lack of specific tests for all pathogens, and the “window period” that exists between the time of infection and the generation of sufficient pathogens or antibodies to be detected.
A much more serious problem, however, occurs if it is necessary to collect plasma from an indigenous population. In this case, sophisticated laboratory testing will likely not be available, and even worse, there may be a locally high prevalence of a known or even unknown pathogens for which no test is available. For example, malaria is quite common in tropical climates, but there is no approved blood test for any of the four distinct parasite species responsible for the various forms of this debilitating disease [1].

2.2 Previous decontamination techniques

For these reasons, several different approaches to decontaminating plasma have been tested. One of these techniques, the use of a solvent-detergent, is the first wide-spread blood product decontamination technology to be marketed; the industry name is Plas+SD [2]. This process functions by attacking the lipid sheathes that surround enveloped viruses: viruses such as HIV cannot function without their envelopes. Of course, such an approach cannot attack non-enveloped viruses, but because the most dangerous currently known viruses have such envelopes, the solvent-detergent process can provide some degree of safety from infection. Unfortunately, the Plas+SD product suffers from high material and process costs and also poses the risk of bulk infection due to the pooling of multiple units of plasma in the manufacturing process. Finally, this product has been withdrawn from most markets because of adverse reactions.

Several other companies have since begun their own decontamination efforts, primarily Baxter/Cerus [3], Gambro/Navigator [4] and the Red Cross [5]. The common feature in all of these groups is the addition of some type of reagent, typically followed by exposure to ultraviolet light, either UVA and/or UVB. The light activated compound then binds the pathogen DNA and/or RNA, thereby decontaminating the product.

Note that it is not necessary to remove the pathogens: the above processes only prevent the pathogens from replicating and thus causing disease. Conversely, it is necessary to remove any toxic additives, and this requirement is a major problem with many of these technologies. It is of course not necessary to remove apparently harmless additives, such as the riboflavin used in the Gambro/Navigator process, but this still leaves questions over possible byproducts.

Unfortunately, all useful decontamination technologies also share the problem of being imperfect: not all pathogens are killed. Beyond the inability of solvent-detergent techniques to treat non-enveloped viruses, this rule holds even for pathogens for which the technique is most effective. The currently accepted standard for effective decontamination is a Logarithmic Reduction Factor (LRF) of 6, which means that one pathogen in a million survives. Note that this value is based only on conjecture, but it is so strict that it is difficult to measure for many pathogens. For comparison, even strong household cleaners claim to eliminate “99% of household germs” which is only an LRF of 2, or 10,000 times less effective than accepted plasma treatments.

Of course, such high levels of decontamination come only at the expense of protein quality: the agents that attack DNA and RNA also attack to some extent the chemically similar plasma proteins, notably the clotting factors that are crucial for hemostasis. The net result is that a balance must be struck between decontamination effectiveness and acceptable protein losses. Fortunately, disrupting the helix in DNA or RNA at even a few points completely destroys the ability of a pathogen to reproduce, while clotting proteins such as fibrinogen can still function well with even several damaged segments.

Under this scenario, many of the above groups have been somewhat successful in developing their respective technologies. On the other hand, what is a “safe” dose of HIV or Ebola? Note that there is some indication that HIV inactivation may require an LRF of 8, which requires 100 times the removal of an LRF 6 technology [6]. Furthermore, a unit collected shortly after infection may be at peak viral loads because no antibody
response has yet been established. Such cases could therefore overwhelm a process with only LRF 6 capability.

For these reasons, the Paul Ehrlich Institute mandates that European products be treated by at least two independent techniques [7]. Under this approach, the pathogens that escape one process may not escape the second, etc. Note that for combined processes, the FDA allows the LRF values to be added, so that 2 processes that each have an LRF of 6 thus have a combined LRF of 12, corresponding to the survival of 1 virus copy in a trillion.

2.3 CryoFacets, Inc. Plasma Decontamination

The CryoFacets, Inc. technology produces just such high levels of decontamination, while still maintaining low levels of protein damage. These unmatched results are obtained by a combination of ultraviolet-C (UVC) light and ozone.

2.3.1 Ultraviolet-C

Ultraviolet light is classified into four different components, according to energy (See Figure 1). The lowest energy band is UVA, followed by UVB. These bands are commonly used in tanning booths, as well as the previous decontamination techniques described above. Because these bands lack sufficient energy to attack the pathogens directly, UVA and UVB systems use the light to activate some chemical additive, which is then actually responsible for providing decontamination. At the opposite energy extreme is vacuum ultraviolet. Slightly less energetic than soft x-rays, vacuum ultraviolet is so readily absorbed that it cannot penetrate the sample deeply enough to perform useful decontamination.

Figure 1: UV Segment of the Electromagnetic Spectrum

Lying between vacuum ultraviolet and UVB is the UVC band. A particularly interesting feature of this band is that light in the 250 to 260 nm light is strongly absorbed by DNA and RNA. This absorption yields cis,syn-cyclobutane pyrimidine dimers (CPD), primarily of thymine but also cytosine, as well as noncyclic pyrimidine(6-4)pyrimidone photoproducts [8,9]. CPD accounts for about 75% of the absorption product, while the (6-4) products comprise the remainder. Because of the formation of these compounds, UVC is highly mutagenic at low exposures. At higher exposures, the DNA and RNA are so severely damaged that they cannot function, which is the goal of effective decontamination.
Fortunately, light in this range is less strongly absorbed by blood proteins, which provides some degree of selectivity. As noted earlier, further selectivity follows from the fact that only a very few “hits” by UVC can inactivate DNA or RNA, but protein molecules, such as fibrinogen, can function quite well even with multiple direct hits.

UVC is thus known to be an extremely effective and selective decontamination technique, and has therefore been used for decades for pathogen inactivation. These applications typically employ mercury discharge lamps. These lamps are preferred because they emit most of their light at 254 nm, which is the middle of the peak DNA and RNA absorption range.

Unfortunately, these lamps are difficult to apply to blood work because 254 nm light also has sufficient energy to split any dissolved oxygen molecules into two free radicals. Although these radicals are uncharged, they still have sufficient energy to “burn” any proteins that they encounter. Furthermore, as they react, they also produce multiple other reactive oxygen species (ROS) that are also extremely damaging to blood proteins.

One way to reduce such damage is to add a chemical “quenching” agent that traps the radicals and other ROS before they can cause excessive damage [10]. Unfortunately, these quenching agents can be expensive, and it is typically necessary to remove them in an expensive, time-consuming process before the treated plasma can be used.

The CryoFacets, Inc. solution to this problem is to apply ultrasound to the plasma under vacuum prior to UVC exposure. The purpose of the ultrasonic vacuum treatment is to remove much of the dissolved gasses, including oxygen, from the plasma. The underlying mechanism is a process called “rectified diffusion” in
which the successive compression and decompression causes bubbles beyond a minimum critical size to grow and then leave the liquid [11]. Without dissolved oxygen, no oxygen radicals can form under UVC illumination, thereby sparing the plasma proteins.

The vacuum chamber for this process is shown in Figure 2. Figure 3 shows the ultrasonic processor, the top of which accommodates the vacuum chamber. The plasma is contained in a bag placed in the vacuum chamber. Under vacuum, the evolved gasses leave the bag through a sterile vent.

![Figure 3. Ultrasonic Vacuum Degassing Unit](image)

Having thus degassed the plasma, the next problem is to illuminate it. There are two problems, however, that must be addressed here. First, UVC is so energetic that it has a quite limited effective depth in plasma. As a result, it is necessary to treat at most a thin film of fluid. As a further enhancement, it is desirable to expose this film from both sides, thereby producing a more uniform exposure.

Unfortunately, thin films also restrict the amount of fluid that can be processed at any one time. Therefore, some type of flow system is necessary to treat practical volumes. One type of flow is the simple laminar case, in which the center of the flowing liquid moves much more quickly than the boundaries. This kind of flow is commonly seen in rivers and streams, where the current in the middle of the flow is much more rapid than the current along the banks. While common, such a flow is undesirable because an exposure sufficient to treat the center of the stream will over-treat the liquid nearer the boundaries.

The conventional means to overcome this problem is to use a turbulent flow. At sufficiently high Reynolds numbers, the flow will make the transfusion from laminar sections to turbulent eddies. These eddies ensure rapid mixing, and thus uniform exposure. Unfortunately, highly turbulent flows can also damage plasma
proteins. Furthermore, the necessarily high velocities also require long flow paths for sufficient residence time, thereby requiring a large piece of equipment, multiple bulbs, and a great deal of disposable materials. For these reasons, high turbulence systems are unacceptable for this application.

The remaining type of flow field is the plug. In this case, the entire fluid mass moves together at one velocity, thereby ensuring uniform exposure. Plug flows, however, while desirable, rapidly convert to laminar flows due to drag along the flow boundary. To prevent this conversion, the CryoFacets, Inc. system utilizes ultrasound to eliminate the wall drag, thus maintaining a plug flow distribution throughout the entire flow field.

The net result is high pathogen decontamination levels with minimum protein damage. To assess the degree of decontamination, porcine parvovirus (PPV) was selected as a model virus; B19 is the form that infects humans. PPV is an interesting pathogen because it is small and non-enveloped, and thus particularly hard to kill. Note that the porcine form is in fact considered to be more robust than the human form. Thus, any technique that is effective against PPV is most likely even more effective against less robust pathogens, such as HIV. The CryoFacets, Inc. technology inactivates PPV to LRF 6 in 0.4 seconds, at a target thickness of 75 microns.

To assess the level of protein damage during the decontamination process, it is necessary to measure the concentrations before and after exposure. Two proteins of particular interest are Factor VIII and fibrinogen. Factor VIII is useful because it degrades readily, and thus provides a sensitive measure of protein damage. Fibrinogen is useful because this protein is critical for strong clots, such as those made from fibrin glues. Fibrinogen testing thus provides a measure of the expected functionality of the treated products. Factor VIII losses at LRF 6 decontamination levels were only 10%, compared to the 30 to 40% levels often seen with other techniques [12, 13].

![Figure 4. Fibrinogen Degradation as a Function of Exposure Time and Oxygen Concentration](image)

Figure 4 shows the degree of fibrinogen damage associated with this level of decontamination for different dissolved oxygen concentrations. The overall trend is less damage with progressively less residual oxygen. Note that the highest concentration curve (11 ppm) intersects the next highest curve (7 ppm) because (7 ppm) is the saturation concentration. Thus, the intersection occurs when the supersaturated curve spontaneously approaches saturation.
Even though these results are quite favorable, they are at the limit of the measurable effectiveness of UVC. Specifically, it may be that UVC in this configuration is even better than LRF 6, but the PPV doping is at its technical testing limit at this point. Alternatively, it may be that UVC is at its practical limit at this point, and any further treatment would only damage the proteins. In either case, another technique is necessary to reach LRF 12 and thus meet the requirements of the Paul Ehrlich Institute.

2.3.2 Ozone

Ozone is one such alternative. Ozone is the triatomic oxygen molecule, formed when the conventional diatomic form is split and one atom bounds to another diatomic molecule. Ultraviolet light, electric arcs, and some electrolytic processes can be used to provide the initial split. Because all of these processes are high in energy, the resulting ozone molecule is also high in energy, and is in fact the second strongest oxidizing agent, following only hydrogen fluoride.

This high reactivity has a number of practical applications, including decontamination. The most common such work is water treatment, including waste disposal, municipal water supplies and even expensive bottled water. Ozone is also used in a variety of food and pharmaceutical processes, mainly because it is effective across a wide variety of pathogens, and it leaves no residue to contaminate the treated product.

The common feature in all of these applications is the ability of ozone to work by multiple mechanisms. One of these mechanisms is the direct oxidation of the pathogens on contact. This mechanism is effective against all pathogens, but it is particularly effective against enveloped viruses. Another mechanism is the splitting of lipids at the site of unsaturated bonds [14, 15]. The resulting aldehydes are effective across a broad range of pathogens. A wide variety of less energetic reactions can also occur, depending on the details of the local chemistry, the type of pathogens, etc. The net result of these multiple processes is thus a quite high level of effectiveness.

In terms of selectivity, the essential element of this technology is that, like the UVC process, the pathogens can survive only a few “hits,” while the proteins can function quite well even with multiple hits. Even with this quite high level of selectivity, however, ozone applications in the blood industry have been somewhat limited to date. CryoFacets, Inc. has therefore developed an ozone processor that achieves high levels of plasma decontamination without excessive protein damage.

Like all other ozone systems that treat liquids, the first consideration in the CryoFacets, Inc. technology is to guarantee a thorough mixing of the gas with the plasma to be treated. Noting that the strong contact oxidation reactions cannot occur unless the gas touches the pathogens, it follows that the ideal situation is a uniform distribution of dissolved ozone throughout the entire treatment volume. Conventional systems, however, employ large bubbles that provide a high ozone concentration only at their surfaces.

Conversely, the CryoFacets, Inc. mixing chamber consists of a tube with multiple small holes. This tube, in turn, is attached to an ultrasonic driver. The ozone gas enters along the axis of the tube. As the gas escapes through the small holes, the motion of the tube shears the bubbles while they are still quite small. Specifically, the bubbles are sheared before they reach the critical size needed for ultrasonically assisted growth [11]. Thus, the bubbles are driven into solution, as desired. The driver is shown in Figure 5.
While this approach works well for any gas and liquid combination, note that the plasma from the previous UVC process is degassed. As such, this liquid has a great deal of empty space to absorb gasses, and the ozone uptake is thus extremely rapid. As a further improvement in gas uptake, the system can be operated at elevated pressures, and at low temperatures, as shown in Figure 6; both higher pressures and reduced temperatures improve the gas solubility.

Finally, many conventional ozone systems try to treat the entire product volume in one container. The net result is that the material near the gas inlet is over-treated, while the material in the more stagnant zones is
under-treated. To ensure uniform treatment in the CryoFacets, Inc. technology, the plasma is withdrawn from a starting bag, passed through the ultrasonic mixing nozzle, and then collected in a second bag. The net result is that all of the plasma is treated quite uniformly.

![Porcine Parvovirus Inactivation with Ozone](image)

**Figure 7. Ozone Decontamination Effectiveness as a Function of Pressure for 1 and 2 Degassing Cycles**

Figure 7 shows the ozone decontamination of plasma spiked with PPV. Note the increasing effectiveness with pressure, as expected from Henry’s law. The corresponding Factor VIII loss was 12% and the fibrinogen loss was 6%.

### 3.0 PLASMA CONCENTRATION

#### 3.1 Need for Plasma Concentrates

Plasma is mostly water. Because plasma must be stored and shipped frozen, most of this effort amounts to storing and shipping just water. Furthermore, water has a high heat of fusion and a high specific heat. Freezing, thawing, and warming all of this water is thus expensive. Even worse, the prolonged time for thawing and warming can be deadly if the plasma is needed quickly, as it often is.

The obvious answer to this problem is to concentrate the plasma. Unfortunately, conventional concentration techniques, such as evaporation and filtration, are not effective for plasma and can even damage the plasma proteins.

#### 3.2 Cryoconcentration

The CryoFacets, Inc. solution to this problem is based on a technique called cryoconcentration. The underlying principles of this technology follow immediately from the phenomena of thawing and freezing. Specifically, when pure ice is warmed, the temperature gradually increases until the melting point is reached, which is 0 °C. The temperature remains at this point until all of the ice melts. The temperature of the liquid
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Water then continues to rise as more heat is added. Conversely, the freezing process is the reverse procedure, except that liquid water often still exists below the melting point. This phenomenon is called supercooling. This supercooled state is maintained until ice crystal formation starts at a surface irregularity or due to agitation; the initiation process is called nucleation.

For biological materials such as plasma, however, the situation is much more complicated. In this case, the salts make ice formation much more difficult, so the freezing point is proportionally lower. Then, as the ice crystals begin to form, they are quite pure and thus displace the salts and other solutes. As a result, the freezing point is depressed even further. This process continues until the displaced salts and remaining liquid solidify together at the eutectic point.

In the CryoFacets, Inc. technology, the region of interest extends from the point of freezing, up to concentrations of a maximum of about 10:1. The first concern here is that extensive supercooling leads to nearly instantaneous bulk freezing when nuclei are finally provided. A common example of this phenomenon is opening a bottle of beer that has been left in a freezer too long. In this case, the rapidly advancing freeze front entraps all of the solids, thereby bypassing much of the concentration process.

To prevent this undesirable result, the CryoFacets, Inc. technology begins with the application of 20-50 kHz ultrasound to initiate the freezing process at the melting point, thereby avoiding any significant supercooling. Furthermore, because the resulting nuclei are distributed throughout the freezing volume, the process proceeds uniformly.

The next concern is that the advancing freeze front invariably develops a branched, or dendritic, form (Figure 8). These dendrites are undesirable because they entrap the increasingly concentrated solution. In the CryoFacets, Inc. technology, the ultrasound sources are kept on to break these dendrites, thus freeing the concentrated pools. As an additional benefit, the dendrite fragments thus become additional nucleation sites. Ultrasound also has the additional benefit of aiding heat transfer and diffusion, thereby further accelerating the overall process.

As these processes continue, however, the salt concentrations will eventually become so great that they will either damage the proteins or cause them to precipitate out of the solution. Salt reduction techniques, such as
dialysis, tangential flow ultrafiltration, or salt exchange columns, must therefore be used. The ice crystals can be removed before, during, or after this salt reduction. All that is required is a special collection bag and centrifuge rotor.

The cryoconcentration process is thus rapid and effective. The resulting product is salt balanced, with little or no protein damage, even at concentrations of up to 10:1.

### 3.3 Uses of Cryoconcentrated Plasma

Plasma cryoconcentrates are obviously of some value in terms of logistics: after all, even a 3:1 concentrate would reduce the number of freezers, transports, etc., by 2/3. On the other hand, the cryoconcentration process itself requires some effort as well, thus diminishing this advantage. Logistically a 10:1 concentrate is obviously much better than a 3:1 concentrate. Unfortunately, such high concentrates are quite labor and time intensive. As such, cryoconcentrates have some clear advantages in terms of logistics, but the advantages may not be worth the effort in some cases.

Another interesting use of cryoconcentration is rapid thawing. For comparison, even the best commercial plasma thawing equipment requires several minutes to thaw a single unit. Additional time is then necessary to raise the thawed material up to transfusion temperature. Conversely, adding 50 °C water to frozen plasma at dry ice temperature yields a unit of plasma at transfusion temperature in less than 1 minute. Because all that is required is a bottle of warm water, the whole procedure is thus quite fast and simple.

Incidentally, plasma concentrates thawed under this approach will sometimes have unmixed protein aggregates that resist mixing; a similar problem is commonly observed with lyophilized products. A simple solution to this problem is to apply an ultrasonic transducer directly to the bag surface. In several seconds the ultrasound will disrupt the aggregates and mix the freed proteins rapidly throughout the entire volume.

The net result is that rapid thawing is helpful, but because not all cases require such rapid processing, the advantages are again not always worth the effort.

Cryoconcentrated plasma, however, has significant clinical advantages that are well beyond just logistics and thawing improvements. These advantages follow directly from the reason that plasma is transfused in the first place. Of course, one benefit of a plasma transfusion is to restore volume and salts, but this requirement is preferably met with just saline solution. Instead, in the case of trauma, the transfused plasma also restores some of the clotting proteins required for hemostasis.

In normal plasma, however, clotting proteins comprise only a small part of the total volume. For example, fibrinogen concentrations are typically in the range of the low hundreds of milligrams per deciliter, so the entire circulating mass is about ten or so grams. Such a small amount is rapidly depleted in the event of the loss of a limb due to a mine, or the impact of a high velocity round. In such cases, transfusing a unit of plasma augments the body’s limited reserve of clotting proteins, but even these additional materials can be lost rapidly.

As a further complication, compression and other techniques are commonly used to prevent massive fluid loss in such cases. Additional plasma transfusions, without reduced fluid loss, thus rapidly exceed the ability of the body to process the excess water. At this point of fluid overload, no further transfusions can be given, and without adequate clotting proteins, the patient is in danger of severe haemorrhage.
The immediate solution to this solution is to transfuse a concentrated plasma, not one that has been diluted back to its original state. Under this approach, the patient can receive large amounts of clotting proteins without the risk of hypervolemia. For example, a 3:1 concentrate provides the proteins of 3 units of plasma, but only the water volume of one unit.

Of course, increasing the protein concentration also increases the viscosity. In practice, viscosity increases can be either good or bad, and there are many factors to consider. First, the increase in viscosity is not directly proportional to the concentration factor. For example, the normal viscosity of plasma is about 1.5 cP, but a 3:1 concentrate has a viscosity of only about 3.1, not 4.5 cP. The reason for this apparent discrepancy is that water at body temperature has a viscosity of about 0.7, so each increase in concentration factor raises the viscosity about 0.8 cP, within reasonable limits.

The second factor to be considered is that even this elevated viscosity decreases over time as the clotting proteins are lost from circulation. Notably, the decrease in free fibrinogen markedly decreases the effective viscosity.

The third factor to be considered is that the total blood viscosity depends strongly on the presence of the cellular blood components. Quantitatively, whole blood has a viscosity of about 4.5 cP at moderate flow rates, and up to about 20 cP at low flow rates, versus 1.5 cP for plasma. Thus, as the red cells decrease due to haemorrhage, the viscosity drops rapidly.

The fourth factor to be considered is that the body can withstand elevated viscosities for at least a short time without adverse effects. Even extremely high viscosities, about 8 to 9 cP, or twice the normal level, are commonly seen in hyperviscosity syndrome. Note, however, that prolonged high viscosity conditions are associated with the risk of thromboembolism, retina damage, and other problems.

Fifth and most interesting, higher viscosity fluids are currently being studied for resuscitation fluids. This approach is in direct contrast to the long-held assumption that low viscosity fluids are preferable over high viscosity fluids for such purposes. The rationale has been that because lower viscosity fluids can more easily flow through the confined spaces of capillary beds, etc., saline solutions would be preferable over a fluid with the consistency of motor oil. On the contrary, Tsai and Intaglietta [16] have shown numerous that high viscosity blood substitutes have many benefits at the microcirculation level. Likewise, Bertuglia and Giusti [17] have shown the benefits of reduced reactive oxygen species damage with high viscosity fluids.

The net result here is that the higher viscosity of plasma concentrates is not only tolerable, but actually quite helpful. Research is therefore currently underway to determine the ideal concentration, given the above constraints. Several options are emerging in this effort. First, it may be preferable to formulate a very high concentration. Under this approach, the most severely injured patients would receive the maximum possible dose, with the understanding that preserving the life of the patient justifies the risks. As a further justification, the peak viscosity would soon drop in such cases anyway due to rapid loss of blood cells and clotting proteins. Incidentally, such an approach would also provide the maximum benefit from the improved logistics and thawing properties of high concentrates.

As an extension of this approach, the next option is to dilute the highly concentrated plasma with variable amounts of saline. Still more concentrated than normal plasma, the partially reconstituted material would have the clinical advantages described above in proportion to the degree of dilution. This variability would allow the product to be fine-tuned to individual needs if desired in a hospital setting; alternatively, the broad range of safety would allow a given dilution, such as an endpoint of 3:1, to be used for all intermediate patients near the field.
As a further extension of a “one size fits all” approach, another option is to pool small numbers of concentrates. The main advantage of such an approach is that the product would be more uniform on the basis of simple averaging. Thus, the effects of the transfusion would be more predictable. Also, if one donor happened to be extremely high or low in any given component, any consequences would be greatly diminished. Of course, these benefits would have to be carefully weighed against the increased risk of infection, but the use of the two decontamination processes described above largely offset this risk.

Finally, high concentration plasma could also be merged with the various blood substitutes currently under development. Although the ideal formulation would have to be determined for each product, the net result would be an extremely powerful resuscitation fluid.

4.0 LYOPHILIZATION

As noted above, one of the advantages of concentrates is the ability to reduce the number of storage facilities and transport operations. Even the highest concentrations of plasma, however, still require refrigeration, and this requirement can be a problem in isolated areas. An obvious solution to this problem is to lyophilize the plasma, and thus reduce or even eliminate the need for refrigeration.

For this reason, CryoFacets, Inc. began a series of experiments on the lyophilization of human plasma. The underlying concept was that the reduced water component would provide more rapid freezing and more rapid sublimation, thereby greatly accelerating the overall lyophilization process. These experiments have had mixed results.

First, in terms of speed, the ideal situation would first appear to be the maximum possible concentration, i.e., the maximum possible removal of water as ice in the concentration step. Unfortunately, such an approach also yields a quite thick liquid, which amounts to a paste. Freezing this material leaves few channels for the escape of water vapor, thus slowing the overall lyophilization process.

To overcome this problem, it is possible to use a vacuum process that essentially produces a foam. Because the pores in this foam produce large channels for the escape of water vapor, the overall lyophilization process is thus greatly accelerated, as desired. Unfortunately, subsequent legal work has revealed that another group had recently patented the process, so this effort has been discontinued.

Alternatively, it is possible to lyophilize plasma at lower concentrations, but this involves adding a separate step to the lyophilization process. This approach is thus not of great economic value over conventional lyophilization.

Despite these setbacks, CryoFacets, Inc. still has some interest in lyophilization, in particular the ability to handle individual plasma units. As noted above, the overall goal is to avoid pooling; furthermore, it is necessary to maintain the plasma in a closed system for regulatory reasons. In keeping with this goal, the lyophilization process must be completed in a “sealed” bag as well. One option is a gas permeable bag, but this approach is inherently limited because no bag material is capable of providing a reasonable processing speed.

CryoFacets, Inc. has therefore developed a unique segmented bag and coupling device that provides the necessary speed and meets the closed system requirement. This system is significantly faster than even conventional lyophilization devices. Planned improvements should make the system faster still, even without any prior concentration at all.
On the other hand, CryoFacets, Inc. has conducted extensive testing on one lyophilized plasma component: fibrinogen. The reason for these tests is that fibrinogen is the main component of “fibrin glue,” a surgical adhesive.

The results are shown in the following figure, where the strength required to rupture the glue is plotted as a function of the fibrinogen concentration. The overall result is that the glue strength follows roughly the square of the fibrinogen concentration, which is intuitively reasonable because the strength depends on the available surface area, which is a squared term by definition. Note that other researchers have reported a linear dependence. Their findings are actually in agreement with the square law, however, as long as only limited segments are considered.

Not shown on this graph is the data for Tisseel, a commercial fibrin glue. According to the manufacturer, Tisseel has a fibrinogen concentration in excess of 10,000 mg/dl. As such, the clot strength should be (***) according to the square law. Instead, the observed strength is only (****), which is less than the strength of the (***) concentration.

The reason behind this apparent discrepancy is that the Tisseel product is provided in lyophilized form, while the points on the curve are for glues that have not been lyophilized. These results are important because the strength of the clot is a measure of the function of the treated protein. Conversely, simply measuring the fibrinogen concentration would not have predicted these results.

Because similar results are expected for other proteins, and because there have been no technological breakthroughs in the lyophilization process that might change these results, it has been necessary to re-evaluate the overall lyophilization strategy. From this re-evaluation, it appears at this time that a two-tier approach may be required. Specifically, non-lyophilized products would be used if possible due to their greater efficacy. Lyophilized products would thus be used only as “last resorts” if non-lyophilized products were not available.

Under this scenario, there is limited incentive to continue with plasma lyophilization efforts. Some of the technology, however, is of interest in ongoing cellular product efforts.

### 5.0 CELLULAR PRODUCTS

Finally, CryoFacets, Inc. is currently applying the above processes to the treatment of cellular blood products, specifically platelets and red blood cells (rbc’s). Like plasma, the concern here is that the supply lines to advanced field units could be cut. Unlike plasma, however, cellular products are typically stored in liquids, and thus have a quite limited shelf life. Even a brief interruption in supplies would therefore require collection of cellular blood products from the local population. The net result is that while plasma decontamination is important, the ability to decontaminate cellular products is absolutely crucial.

Unfortunately, decontaminating cells is much more difficult than decontaminating plasma. The underlying problem is that cells are complex, fragile, and they have membranes that separate them from the surrounding plasma. It is therefore difficult to introduce any decontaminating agents into the cells, difficult to attack the pathogens without also attacking the cell contents, and difficult to remove the waste products after the decontamination reactions are finished.

To resolve these problems, the above ozone and UVC plasma decontamination processes have been essentially re-engineered. The overall approach is to use ozone primarily as an extracellular agent, although...
there is some research that indicates some intracellular effects may also be occurring. Conversely, UVC is used for both extracellular and intracellular treatments.

Of the two processes, ozone has more difficult to develop because previous work shows that red blood cells are prone to Heinz body formation [18] and ozone is not selective against pathogens versus cells [19]. A new disposable and its associated process have eliminated both of these problems, and have likely reduced the incidence of Transfusion Related Acute Lung Injury (TRALI) as well.

Likewise, the UVC modifications also requires a new disposable and associated process.

The system is still under development, but preliminary results indicate combined LRF’s of 12 or more, with only a few percent cell damage. As a spin-off application, the disposables can also be used to perform a conventional cell wash in 15 minutes, in a closed system, automatically, and with less than 1% cell loss.

Additional work is underway to refine these technologies. If successful, they will then be used as a basis for the development of decontaminated, lyophilized cellular products.

6.0 CONCLUSIONS

The CryoFacets, Inc. technology thus consists not of new processes or procedures per se. Instead, the technology consists of applying a combination of known pieces from a variety of disciplines to make new products. This use of known components has greatly reduced the development costs and time, and has ensured success at each step. Thus, CryoFacets, Inc. has two world leading plasma decontamination processes, with a combined LRF of 12 and protein losses less than 25% even for Factor VIII. The technology also includes a unique cryoconcentration process, capable of concentration up to a factor of 10:1, as well as lower concentrations that could be transfused directly as high viscosity resuscitation fluids. This concentrated plasma can also be used in a unique lyophilization system, although protein losses may be unacceptable. Finally, the decontamination processes are being modified to form the basis of a cellular product decontamination technology. If successful, this approach will avoid the use of the additives that have limited all other cellular decontamination attempts.

7.0 REFERENCES

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Hemostasis and Coagulation Following Uncontrolled Hemorrhage and Resuscitation with Polymerized Hemoglobin Based Oxygen Carrier (HBOC-201) in Swine

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ABSTRACT

INTRODUCTION: Coagulopathy are common complications of hemorrhagic shock (HS). Recently HBOC-201, a novel fluid with oxygen carrying capacity, has been proposed for hypotensive resuscitation and stabilization of HS patients. Coagulation and hemostasis have been studied in a swine uncontrolled hemorrhage model comparing HBOC-201 to standard resuscitation fluids. METHODS: Yucatan mini-pigs (n=24) underwent uncontrolled hemorrhage by laceration and crush injury of a liver lobe. These animals were either non-resuscitated or resuscitated with HBOC-201 or buffered hydroxyethyl starch (HEX) during the 4 hr period following HS, after which they received full hospital care up to 72 hr. In addition to in-vivo parameters (blood loss and in vivo bleeding time (BT)), changes in hemostasis were evaluated by laboratory assays (coagulation (PT, PTT, fibrinogen), thromboelastography (TEG), and closure (in vitro bleeding) time (PFA-CT). Lung histopathology was evaluated for evidence of adverse microvascular thrombogenic pathologic change RESULTS: Hemodynamic parameters were restored more effectively in HBOC-201-resuscitated pigs than in HEX- or non-resuscitated pigs. BT and blood loss were not different in these groups.

although the fluid requirement for the 4 hour resuscitation was significantly reduced in the HBOC-201 group as compared to HEX (respectively 23 vs 12 ml/kg/min). HBOC-201 did not significantly change platelet function or coagulation parameters in the 4hr following trauma. HEX resuscitation did, however, reduce the maximum amplitude on TEG, indicating platelet inhibition. PFA-CT was elevated in both resuscitated groups. Over the 4 hours following the induction of uncontrolled hemorrhage, PT remained stable and PTT decreased by 25% in both treated groups. After 24 hours, most hematological parameters returned to normal, with the exception of PT and fibrinogen; PT remained mildly prolonged and fibrinogen severely in both HBOC-201 and HEX groups. In post mortem histopathologic examination of lung sections, alveolar edema and fibrin deposition were rare in all groups. None of the sections showed evidence of platelets mixed with fibrin or other signs of thrombi or disseminated intravascular coagulation.

**CONCLUSIONS:** Evidence of clinically significant coagulopathic effects of HBOC-201 was not found. The absence of such effects makes HBOC-201 an adequate “bridging” resuscitation fluid during evacuation of HS casualties without exacerbation of coagulopathy.

**1.0 INTRODUCTION**

Coagulopathy develops in patients with hemorrhagic shock [1,2]. Eventually, in severe hemorrhage, coagulation factors in both extrinsic and intrinsic systems are exhausted. Under these circumstances, mortality and morbidity are substantially increased and abnormal coagulation indices (PT above 14 sec and PTT above 35 sec) are commonly observed [3]. It has been reported that early severe coagulopathy correlates with mortality using both Glasgow Coma Scale and Injury Severity Score (GCS and ISS) [4,5]. Coagulopathy commonly occurs soon after injury and subsequently, hypovolemia results in progressive splanchnic hypoperfusion, transient hypercoagulability and ultimately hypoocoagulability. These changes are due to accelerated depletion of platelets, as well as excessive consumption of fibrinogen and intrinsic coagulation factors. As hepatic dysfunction becomes prominent, the ability to synthesize fibrinogen and intrinsic coagulation factors is reduced while consumption is maximal [6]. These changes, in the presence of hypoperfusion, will lead to disseminated intravascular coagulation (DIC), triggered by the diffuse vascular and tissue bed injury. Multiple organ system failure (MSOF) ensues [3]. Early transfusions of whole blood, platelets, fresh frozen plasma and cryoprecipitate, used in trauma management practice, have been life-saving in anticipation of surgical control of hemorrhage. Deployment of blood products in military prehospital environments is rarely feasible. Indeed, on the battlefield, or at the civilian site of events inducing hemorrhagic shock, the administration of resuscitation fluids (saline or hydroxy-ethyl starch (HEX)) provides immediate restoration of intravascular volume and provisional vital organ perfusion. However, these common resuscitative practices also cause hemodilution, and aggravate anemia, thereby diminishing per volume O2 carrying capacity, and enhancing hypocoagulability, with dilution of platelets and circulating coagulation factors [7]. This is especially evident where evacuation of traumatic casualties is delayed, as is often the case in military actions [8]. In these circumstances, the ability to use a lower volume resuscitation strategy and a fluid with oxygen carrying capacity will protect intrinsic coagulation mechanisms, and protect vital organs against damage.

HBOC-201, a bovine polymerized hemoglobin with oxygen carrying capacity (Biopure Corporation, Cambridge, MA) has been shown to increase survival rates in animal models of controlled and uncontrolled hemorrhage [9]; experiments in our laboratory have confirmed this finding [10,11]. Bovine hemoglobin oxygen carriers have been reported to increase bleeding [19], in contrast to human O-raffinose crossed linked hemoglobin in which decreased bleeding was demonstrated. [12]. Studies in our laboratory are evaluating extensively the efficacy and safety of HBOC-201 as the preferred resuscitation fluid for trauma casualties. As part of this effort, the comprehensive assessment of the hemostatic and coagulation effects of HBOC-201 and HEX in uncontrolled hemorrhage are ongoing. In order to mimic battlefield casualties, we have developed a
swine model of abdominal penetration, liver crush and laceration. Our results are reported here with special attention to recovery of coagulation abnormalities following resuscitation.

2.0 MATERIAL AND METHODS

2.1 Animal hemorrhagic shock

2.1.1 Model

Twenty-four (24) anesthetized, intubated Yucatan mini-pigs (23.0 ± 8.5 kg) were used in a HS model simulating uncontrolled bleeding for the battlefield. The icrush and laceration of a liver lobe through an abdominal wound, resulted in a bleeding rate of 1.80 ml/kg/minute within the first 30 min from the damaged organ.

Time 0 designated initiation of the liver lobe crush and laceration. A prehospital phase simulated an “evacuation delay” period during which blood transfusions and surgical stabilization are not available, in common in military operations. Animals were not immediately resuscitated after injury. After 15 minutes (T15) they were infused with 10 ml/kg of resuscitation fluid at over 10 minutes. Subsequent 5 ml/kg infusions were administered at 30, 60, 120 and 180 minutes, if prospectively defined criteria were met (i.e., mean arterial pressure (MAP) < 60 mm Hg or heart rate (HR) increased in any value above baseline. Animals were intensively monitored for a total of 4 hours, but received only respiratory support and fluid resuscitation. After this 4 hour prehospital phase, hospital care was simulated by surgical repair of the liver and access to blood transfusion. Animals received either crossmatched banked whole blood transfusions or normal saline at 4, 24, and 48 hours, for a prospectively defined threshold value of Hb < 7 g/dL at each of these hospital care phase time points. All swine were euthanized at 72 hours.

2.1.2 Fluid resuscitation

Swine were randomly allocated to one of three resuscitation study groups (n=8 in each): No-resuscitation (NON), HBOC-201- and HEX- resuscitation. HBOC-201 is purified and ultrafiltered bovine stroma free Hb that is heat-treated and gluteraldehyde-crosslinked to form polymers with MW up to 500KD, prepared in a 50:50 racemic D and Lactated Ringers (LR) solution [13]. HEX, the standard fluid for battlefield resuscitation presently employed [8], is a 6% hydroxy-ethyl starch (MW=670Kd) prepared in balanced LR solution (Hextend, Abbott Laboratories, Abbott Park, IL).

In vivo monitoring: Vital signs and physiologic monitoring were performed as described elsewhere [10]. Bleeding time measurements were taken at Time 0 and 4 hours after injury. Bleeding time was performed by an ear incision with a scalpel blade # 11 on the ear edge to create a reproducible 5 mm anterior incision. The time for the bleeding to stop was recorded by the paper blotting method (Whatman paper #1).

2.2 In vitro monitoring

2.2.1 Blood Collection

Blood specimens were collected in vacutainer tubes at Times 0, 30, and 60 min, as well as at 3 and 4 hours after injury. Animals were maintained under anesthesia for 4 hours, and subsequently were recovered and extubated. Blood samples were obtained at 24, 48, and 72 hours during the hospital phase. (BD vacutainer, Palo Alto, CA).

2.2.2 Assays. Laboratory studies included: complete blood count (CBC) (Pentra 60C+ cell counter, ABX Diagnostics, Irvine, CA), thromboelastography (TEG), ADP-collagen capillary closure time (PFA-CT),
standard coagulation parameters (prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), anti-thrombin (ATIII), and fibrinogen.

2.2.2.1 **TEG** [14,15]: Thromboelastography (TEG) evaluates clot formation dynamics (TEG, Haemostasis Analyzer, Haemoscope Corp, Skokie, IL). 20 µl of 0.25 mM CaCl₂ and 340 µl of whole blood were pipetted into an oscillating cup. A pin connected to a torsion wire transmits the motion signal generated by clot retraction; this is integrated into a digitally based score. The reaction time (TEG-R) corresponds with initiation of fibrin formation and depends mainly on plasma factors. TEG-K and TEG-α are measurements of the kinetics of clot formation and reflect platelet adhesion on newly formed fibrin and rate of fibrin polymerization, respectively. TEG-MA measures maximal clot strength and shear modulus, and is dependent on platelet number and function, as well as plasma proteins to a lesser extent [14]. TEG-Ly (done at T 30 minutes) measures fibrinolysis due to tissue plasminogen activator (t-PA) activity, and is indicative of the presence of fibrin degradation products (FDP). Computed indices such as TEG-G (the clot firmness) and CI (the coagulation index were also reported. CI was defined as CI = 0.0184 * TEG-K + 0.1655 * TEG-MA – 0.0241 * TEG-α - 0.2454 * TEG-R - 5.022. [15]. TEG-G was derived as TEG-G= 5000*TEG-MA/(100-TEG-MA),

2.2.2.2 **PFA-CT**: The platelet function analyzer (PFA-100, Dade Behring, Fl) measures capillary closure time [CT] and corresponds to *in vitro* bleeding time [16]. 800 µ of whole blood is vacuum aspirated through a 100 µm diameter capillary membrane coated with collagen and adenosine diphosphate (ADP). This promotes platelet adhesion and aggregation. Once a platelet plug has formed, blood flow ceases. This time to aperture occlusion by the platelet plug is referred to as the *closure time* (CT). CT is increased by low hematocrit, low platelet count, and low von Willbrand Factor (vWF) levels; it is unaffected by coagulation factor deficiencies and hypofibrinogenemia [16]. Thus CT is uniquely suited to measure coagulability in traumatic uncontrolled hemorrhage.

2.2.2.3 **Coagulation assays**: Coagulation assays were performed on a Stat Compact (Diagnostica Stago, Parsippany, NJ). This is a fully automated instrument using both mechanical magnetic ball (PT, PTT, TT, and fibrinogen) and colorimetric principles (ATIII).

2.2.2.4 **Histology**: Detection of microthrombi and fibrin deposition was performed by electron microscopy (EM) on lung sections obtained at necropsy. Sections were processed as previously described. Briefly, lungs were fixed in 4F1G fixative (4% paraformaldehyde, 1% gluteraldehyde), post-fixed in 2% osmium tetroxide, dehydrated in graded alcohols and Epon-embedded. Thick block sections were examined by light microscopy, and thin sections were stained with lead citrate and uranyl acetate, and examined in an LEO 912 AB electron microscope.

2.2.2.5 **Statistics**: Results, data and figures, are presented as means, and standard deviation or as otherwise stated. Data were analysed with a two-tailed paired Student’s t-test, or equal variance Student’s t-test. Statistical significance was considered for p<0.05.

### 3.0 RESULTS

#### 3.1 In Vivo

3.1.1 **Physiology** [10]: MAP at baseline was comparable in all three groups and significantly decreased from 69.6±3.2 to 27.6±2.9 mmHg after hemorrhage (p<0.01) at T 30 min. MAP was restored more rapidly
with HBOC-201 resuscitation at 30 minutes than with HEX. In the HBOC-201 group, 7 animals (87.5%) survived compared with only 1 (12.5%) with HEX and NON resuscitation (p<0.01 (Fisher exact)). The 15 animals that did not survive, died between 30 and 300 min after onset of hemorrhage.

3.1.2 Fluid requirement [20]: All animals in each group received resuscitation fluid infusion at 10 ml/kg at T 15 minutes. Using the vital sign criteria described above, all required second (5 ml/kg) infusions as well. At 60 minutes after injury, there was no significant difference between the required infusion volume in HBOC-201 or HEX resuscitated animals (17.0±4.0 and 19.6±2.0 ml/kg respectively). Since a large proportion of the HEX resuscitated animals died during the prehospital phase, the required fluid volume infusion was normalized to the survival time in order to enable comparison at later time points. After T 60 min, animals in the HBOC-201 group received significantly less fluid compared to HEX, (12±4 vs 23±14 ml/kg/min, respectively). At 4 hr (simulated “hospital arrival”) none (0/8) of HBOC-201- animals received blood transfusion whereas 100% (3/3) of HEX-resuscitated pigs were transfused on the basis of Hb < 7 gm/dl.

3.1.3 Blood loss [20]: At 60 min post-resuscitation blood loss in this model was not significantly different among the three groups. At 4 hr blood loss was 49±12 and 58±10 ml/kg (p>0.05) for HBOC-201, and HEX groups, respectively.

3.1.4 In vivo bleeding time (BT): BT was measured at 0 and 4 hours post-hemorrhage. Only one non-resuscitated pig survived at 4 hr and BT was elevated compared to time 0. There were no statistically significant differences in BT values found between 0 and 4 hrs in the fluid-resuscitated groups. Neither were there any detectable differences between the HEX and HBOC-201 groups at each time point (p>0.05) (Figure 1).

![Bleeding time for Liver Injury](image)

Figure 1: In vivo bleeding time (BT) at Times 0 (filled bar) and 4 hours (open bars), in non- (NONE) and HBOC-201-, HEX- (hetastarch) resuscitated animals. Mean and standard deviation

3.2 In vitro results

3.2.1 Hematology: At Time 4 hours, hematocrit (Hct) decreased similarly by ~14% from time zero (~29%) in both fluid-resuscitated groups, likely due to relative hemodilution. This is in contrast with NON animals, where Hct increased by 7%. As expected, hemoglobin concentration (Hb) paralleled the Hct in HEX- and
NON-pigs, but was higher in HBOC-201- than HEX-pigs due to the delivery of hemoglobin by HBOC-201. The mean hemoglobin load in HBOC-201 infusions was 3.1±0.8 g/kg, resulting in a mean peak plasma concentration of 5.3±1.0 g/dl at 4 hours. Platelets decreased similarly in the two treatment groups, from ~420 x 10^6/ml at Time 0 to less than 180 x 10^6/ml at Time 4 hours, probably from hemodilution. We noticed a trend toward a similar platelet reduction in the non resuscitated group at 60 min and 3 hr, probably reflecting consumption.

Figure 2: Hematology data for the 3 groups studied: HBOC-201 (square), HEX (triangle) and NON (diamond). Hemoglobin concentration (Hb) and platelet concentration. Note the hemoglobin hemoconcentration during the prehospital phase for NON, hemodilution for HEX and compensation by HBOC-201 addition for HBOC. Mean and standard deviations are shown.

3.2.2. Ex vivo bleeding time: PFA-CT decreased slowly over the first three hours in the NON group and gradually increased back to time 0 values between 4 and 24h (Figure 3). Both HEX and HBOC-201 groups showed a marked increase in closure time between 3h and 24h. Although not statistically significant in this sample, the data suggest a difference between HBOC-201 and HEX groups at 24 hr. HBOC-201 may have a longer lasting effect on PFA-CT than HEX.

Figure 3: PFA-CT for the 3 groups studied: HBOC-201 (square), HEX (triangle) and NON (diamond). In vitro bleeding time as evaluated by the closure time (PFA-CT) in an ADP/collagen coated capillary increased for HEX and HBOC-201 compared to the NON. Mean and standard deviation ranges shown.
3.2.3 **Thromboeleastography:** Neither TEG-R, nor TEG-MA for non resuscitated animals did change with time in the prehospital phase, as shown in the Figure 4 graphs. TEG-R also did not change for the treated group in the prehospital phase. TEG-K, TEG-alpha indicated no difference compared with time 0 (data not shown). After 24 hr, the HBOC-201 group showed a significant change (p<0.01), characterized by an increase in reaction time. This was difficult to compare to the HEX group, as only one animal survived to this timed data point. At 4 hrs, TEG-MA was significantly higher in NON than in HBOC-201. As well, HBOC-201 was higher than in HEX.

![TEG graphs](image)

*Figure 4: Thromboelastography for the 3 groups studied: HBOC-201 (square), HEX (triangle) and NON (diamond). Measure of the reaction time (TEG-R) and the maximum amplitude (TEG-MA). Mean and standard deviation are shown.*

3.2.4 **Coagulation parameters:** PT remained stable in NON and HBOC-201 groups after injury. However, in HEX treated animals, PT increased steadily, and was significantly different (p<0.01) at 4h. PTT decreased slightly in the treated animals compared with NON, and remained stable for 4 hr; this difference did not reach statistical significance. After 24 hours PTT remains elevated in all groups, probably indicative of the presence of intrinsically produced heparin, unopposed by hepatic coagulation factors.

![Coagulation graphs](image)

*Figure 5: Measure of coagulation indices (PT and PTT) for all the 3 groups studied: HBOC-201 (square), HEX (triangle) and NON (diamond). Mean and standard deviation*
3.2.5 **Histopathological lung sections:** Electron micrographs of lung tissues taken at day 3 showed modest intraalveolar fibrin deposition and edema in 62% of HBOC-201, and 37% of HEX treated animals. This difference was not statistically significant, and may be caused by the fact that HEX treated animals died at earlier time points. No platelet aggregates or microthrombi were found in pulmonary blood vessels.

4.0 **DISCUSSION**

The coagulation status in patients with uncontrolled hemorrhage is difficult to evaluate because fluid administration can significantly affect the monitored parameters [2]. The experimental model presented here allows us to study the coagulation profile under 3 different conditions up to 3 days with and without resuscitation. Hemoconcentration and hemodilution strongly influence coagulation indices and distinguish the groups presented here. Obviously, HS induces consumptive coagulation; as well, plasma factors are further reduced during HBOC-201 or HEX resuscitation. As expected, hemoconcentration occurred during hemorrhage in the NON animals as evidenced by the increase in Hct. In contrast, hemodilution develops after both HBOC-201- and HEX- resuscitation, and changes is Hct and cell count confirm this. The decline of total platelets as seen in the NON group indicates a consumptive mechanism. The contribution of platelets to wound stasis in this liver injury model, is demonstrated by a small decrease in platelet activation (PFA-CT). PFA-CT (as well as BT) is known to be prolonged by a number of factors. These include reduction of platelet number, decreased levels of vWF factor, and low hematocrit. The remarkable increase of PFA-CT after fluid resuscitation, is likely due to the hemodilution by HBOC-201 or HEX between 20 min and 3 hrs, most likely related to the relative thrombocytopenic state induced by dilution with both resuscitation fluids. This effect becomes fully apparent at 4h, and suggests that there may be a delayed action of both fluids on platelet function. However, there is an important difference between HBOC-201 and HEX; PFA-CT and TEG-MA are both lower than with HEX (also seen in the controlled HS model) [11] suggesting that HBOC-201 may stimulate platelet function more than HEX. The elevated PT at 4 h underlines the stronger coagulopathic effect of HEX compared to HBOC-201, probably due to hepatic dysfunction. The origin of this difference is not clear in this data set, and awaits further study. Intrinsic coagulation pathway activity, as reflected by PTT decline, in both resuscitation fluid groups is indicative of the relative physiologic importance of the intrinsic system in this model. The coagulopathic effect of HBOC-201 seems to be most evident at 24 h as TEG-R and PT remain elevated. All these effects are mild and transient, resolving by 72 hours.

HS induces hypercoagulation which is followed by hypocoagulation after fluid resuscitation. The change in the indices after HEX infusion is comparable to the data presented by Ledgerwood et al [2]. Recent reports of NO scavenging activity in HBOC-21 experiments [18] might imply that platelet activation would be expected. The absence of microvascular thrombosis noted at necropsy do not support this hypothesis and suggests that NO scavenging is not a significant procoagulant force in vivo.[19]. Lee et al. [12], using O-raffinose as hemoglobin based carrier, showed shortened BT and enhanced clot formation, whereas we did not see significant changes in BT although we observed a significant hypocoagulation. Lee’s model is significantly different than ours, and this may explain the conflicting results.

5.0 **CONCLUSIONS**

In summary, TEG data from the controlled hemorrhage model suggest that HBOC-201-resuscitation may induce a mild change in clot dynamics particularly at 24 hr after injury in this model of uncontrolled hemorrhage. This phase is transient and the indices returned to baseline at 72 hr. These data suggest that HBOC-201 could be an adequate “bridging” resuscitation fluid during evacuation to hospital, and that the effects of HBOC-201- on hemostasis do not impair survival, and are unlikely to be clinically significant.
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The opinions contained herein are the ones of the authors and are not to be construed as official or reflecting the views of the Navy department, or Department of Defense, or the U.S. Government.

The experiments reported herein were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals”, Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the WRAIR/NMRC Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the American Association for Accreditation for Laboratory Animal Care (AALAC).

7.0 REFERENCES


Hemostasis and Coagulation Following Uncontrolled Hemorrhage and Resuscitation with Polymerized Hemoglobin Based Oxygen Carrier (HBOC-201) in Swine


Novel Non-Intrusive Trans-Dermal Remote Wireless Micro-Fluidic Monitoring System Applied to Continuous Glucose and Lactate Assays for Casualty Care and Combat Readiness Assessment

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Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3), AR 360.5.

ABSTRACT

We present results on both the bio-medical engineering development of a non-intrusive, wireless, micro-fluidic physiological monitoring and remote transmission field system, and its performance measuring continuous glucose and lactate concentrations in the interstitial fluid of rats subjected both to glucose-stress tests, and to hemorrhagic shock. These directly address the two symposium topics of "Monitoring of physiological parameters and measurement of vital signs in the field" and of "Medical surveillance in a command environment, remote monitoring and guidance of field personnel".

1.0 INTRODUCTION

1.1 Relevance to the Symposium

Glucose and lactate concentration variations have been documented in the compensatory and de-compensatory phases of shock. A detailed, continuous, and precise knowledge of these concentrations in an individual can provide many advantages to combat casualty care personnel and emergency medical surgeons. These include individual healthy baselines, information for triage, and monitoring the physiological evolution of shock so as to pinpoint optimal stabilization and resuscitative treatment strategies. The prototype system we have created provides this advanced diagnostic aid and decision support using the most recent technology for remote wireless electronics and novel non-intrusive sampling based on micro-fluidics.

1.2 Description of Methods Employed and Results Obtained

With DARPA support, we have fabricated a prototype micro-fluidic platform system, called BFIT (Bio-Fluidic Integrated Transdermal Microsystem), for the non-intrusive, sequential, real-time trans-dermal sampling & analysis of molecules that ordinarily do not diffuse across the skin, such as polar molecules. The BFIT provides an individual, personal baseline while being both disposable and sterile. Our approach employs the non-invasive sampling of living tissue, in a unique and minimally disruptive fashion, retrieving an

Insignificant volume of interstitial fluid (<<10 ng). The monitoring is time controlled and can cover short- or long-term time periods (days-months). It is an absolute measurement with no need for an external or on-board glucose reference. Finally, it can be generalized to monitoring almost any moderately hydrophilic/soluble biomolecule of molecular weight less than 60k Daltons, as are commonly found in measurable concentrations in the interstitial fluid just under the skin’s stratum corneum. Biologically, we sample, analyze and record the concentration of glucose and of lactate in the interstitial fluid as a stress and shock/trauma physiological indicator. We have proven both optical and electrical implementations of the transduction technology. Finally, we have adapted wireless control circuitry (RRAPDS), currently used in the field to monitor Army missiles and munitions, to the control and remote reporting of the BFIT’s glucose and lactate assays. Here we present physiological data obtained from controlled animal experiments on healthy rats, and on rats in shock, comparing our data with periodic blood-gas assays obtained from whole shed blood.

1.2 Military Significance (Resuscitative Medicine / Casualty Care)

The potential to be successfully resuscitated from severe traumatic hemorrhagic shock is time-critical for both combat casualties and civilian trauma victims with traumatic exsanguinations. There are a number of critical care medicine research areas in which nonintrusive quasi continuous field deployable monitors would greatly benefit successful resuscitation. First, for both triage and effective treatment, there is the pin-pointing exactly the moment in the shock time course the casualty is found by medics. Work by Pearce et al. documents the typical hematocrit, plasma glucose and lactate values observed during the hemorrhagic shock and identifies four progressive phases: i) early compensatory (homeostatic mechanisms), ii) maximal compensatory, iii) early decompensatory (during blood re-infusion, close to irreversibility) and iv) late decompensatory (leading to death). If resuscitative fluids can be administered before the late compensatory phase when organs such as kidneys and the liver are ischemic, and there is severe acidosis, then survivability is high. There is evidence that small volumes of glucose infusions (or iso- or hyper-tonic saline, or hespan, not blood) can both moderate systemic acidosis as well as delay the onset of the fatal decompensation phases. From a research point of view, a quasi-continuous monitor (e.g. a reading every 1 - 2 minutes) would also shed light on the efficacies of both the volume and composition of the resuscitation fluid employed. Thirdly, to know even more precisely where one individual is situated on the compensatory/decompensatory time course, one must have a “baseline” prior to injury of that individual’s plasma glucose and lactate (even alcohol) concentrations which can be significantly altered from normal rest values by extreme stress and exertion as encountered in combat. In this context, nonintrusive monitoring of glucose and lactate, of each individual warfighter with an unobtrusive band-aid like device throughout combat, from rest to exertion and possible injury, is highly desirable. It is only the frequency of sampling which changes from infrequent, to frequent, to quasi-continuous in the case of casualty when the monitoring device then serves as both a triage and a critical care instrument. Combined with other physiological instruments under development in the Warfighter Physiological Status Monitoring suite, a complete picture is available for a commander, a medic or a field surgeon.

2.0 THE BFIT MICROSYSTEM

2.1 System Components

The microsystem consists of four major components, each of which have been developed separately and are now being demonstrated in an integrated fashion. Referring to the lettered features of the photographs in Figure 1 below, we see A) Flexible disposable micro fluidic electro-chemical chip with multiple sensor cells, B) Flexible disposable micro fluidic optical chip, C) Flexible cable and connector, D) Wireless control and
messaging unit (modified US Army, 100 m range), E) Wireless Integrator computer, or node of Land Warrior’s computer; F) ultimate integration goal for A, C & D on an equipment band or strap (‘05). We discuss some of the unique engineering that went into the BFIT sampling chip below.

**BFIT MICROSYSTEM: some selected details**

**Figure 1.** Photographs of the demonstration BFIT microsystem. See the text above for identification of the lettered features.

### 2.2 BFIT Sampling Chip

The BFIT sampling chip is a flexible patch-like chip with a multilayer polymeric metal laminate structure and was fabricated using SU-8 as a structural layer, Teflon-AF release layer, polymethylmethacrylate (PMMA), polypyrrole (PPy) and glucose oxidase (GOD). The BFIT fabrication process uses SU8 as a principal structural material consisting of five steps (Figure 2). This process is a subset of an earlier technology developed for the polymer material PDMS. The first step was the deposition of a Teflon release layer on a glass substrate, which allowed the multi-layered multi polymeric devices to be removed easily from the glass after fabrication. A thin layer of SU8 was formed by spin coating and acted as a base layer (10 μ) for the rest of the device and provided adhesion to the Teflon. The third step in the fabrication process consisted of spin coating a thick (150 μ) SU8 layer. This thick layer provided the structural support for the device. Chromium/gold electrode/heater metallization (0.5 μ) was sputtered deposited and patterned on top of the thick SU8 (150 μ) layer. 10μ PMMA was then spun coated as a protective layer for the selective deposition of PPy and enzyme. In order to prevent electrode pads getting covered by PMMA, scotch tape was applied on the electrode pads prior to PMMA spin was removed before PMMA baking process. PMMA layer was further selectively plasma etched in such a way that only one of the electrodes was exposed and the other electrode was covered (Figure 3). The metals were patterned using positive photo-resist and wet-chemical etching. Before the sputter deposition, a plasma surface treatment was employed to improve the adhesion between the SU8 and the metal layers. Releasing the device from the glass substrate using a razor blade was the next step. The release layer was formed by spin coating a solution of amorphous fluoropolymers (Teflon) diluted with perfluorinated solvent.

Glucose oxidase (GOD), the current enzyme prototype, was adsorbed electrochemically onto a polypyrrole (PPy) layer using a potentiostat together with an electrolyte solution consisting of 0.1 M, each, of PPy and KCl at 0.8 V for 2 minutes. 0.1 M Ferricyanide and 8001 units/ml of GOD (18 μl GOD and 48 μl K3FeCN6 in 10 ml phosphate buffer solution) were further added in the electrolyte solution for the deposition of GOD. Selective deposition of Ppy + GOD was then done on one of the exposed electrodes of the B-IT cell (Figure 3). Chronoamperometric dose responses were recorded and the results revealed that the sensor had a good linearity from 0 to 10 mM glucose with the sensitivity of 2.9 mA/mM. For our lactate sensor chips we use the same process except we substitute lactate oxidase for the GOD.
2.2 Connector/Cabling

We modified the design of our glucose and lactate chips to make them compatible with a new generation of zero-insertion force (ZIF) connectors made by Kyocera. Chip thicknesses were adjusted to be 150µm +/- 10 µm for optimal insertion reproducibility and the connector pad pin-outs were drawn to meet with the 300-pitch staggered connector positions. Most important is the ability to make “bottom” chip contacts allowing a flat connector and chip surface that can be pressed to the body of the subject. That is, there is no step in level at the connector body that prevents a good flat contact with the skin. The body of the connector is 1.8 mm high in a surface mountable dual in-line package and is equipped with a ZIF slider mechanism that locks the chip into place once correctly inserted. We have seen that this allows us to change BFIT sensor chips reproducibly in a minute, yet be study enough to accept multiple insertions and to resist forces that would withdraw the chip from the socket due to the normal movement of the animal under experimental study. The part is made in different widths corresponding to a range of 17 to 91 pin contacts. We have used the ones for 31 and 61 pins in our development work. The connector photographed in Figures 1, 7D, 8G, and 8H is the 61 pin version soldered to a wire cable. We have also used in with flexible multi-conductor kapton tapes as shown in Figure 1C. In the integrated device shown in Figure 1F this connector is rigidly connected to the body of the control electronics package. The connector is available in both tape and reel-to-reel packages for economic automated assembly and manufacture.

2.3 Control Electronics

The control electronics used in this study have been made by Holeman Scientific Corporation16 of Huntsville Alabama with support from the DARPA/ARO grant (Ref.1). It is a modification of electronics developed by the Redstone Arsenal’s RDEC used to monitor the readiness and functioning of missiles and of munitions. The electronics consists of two parts. The first is a computer-interfaced wireless data collection system that is capable of addressing up to 64 remote sensor nodes managing the identification of each one as well as interrogating each one for the contents of its memory buffers. The second part is the sensor node. It is made up of five functional parts: RF communication (with unique identification), a microprocessor controller, a multiplexer, analog circuit sources and A/D converters, and multiple sensor input. The modifications made by Holeman concern principally programming the microprocessor, and adapting the analog circuits, multiplexer and input lines to our device. The details of this electrical engineering will be given elsewhere. Of importance is that the range of communication is about 300 m to allow communication between a command center and members of a platoon. Each soldier can be monitored individually for glucose and lactate concentrations. If the soldier is healthy, an individual rested baseline can be measured and stored. As the soldier exerts himself the blood glucose and lactate levels can be monitored. Extreme exerting can be seen in hypoglycemia and
elevated lactate levels. This physiological state affects the soldier’s ability to perform in battle or subsequent situations of high exertion. If the soldier suffers a casualty, the monitors can be activated to measure quasi-continuously as shown in the studies below, and the micro system behaves as a critical care and triage instrument.

2.4 Overview of System Specifications for BFIT and CGMS Systems used in this Study

<table>
<thead>
<tr>
<th>Sensor Component</th>
<th>BFIT Performance Specifications</th>
<th>MINIMED CGMS Performance Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Sensor</td>
<td>NONINTRUSIVE: OK for &gt; 7 days. There is no inflammation. Micro-pores heal in hours.</td>
<td>Designed to be worn by the patient up to 3 days; storage lifetime dry &gt; 4 months</td>
</tr>
<tr>
<td></td>
<td>Accuracy: &lt;20%</td>
<td>Accuracy &lt;20%</td>
</tr>
<tr>
<td></td>
<td>Drift: &lt;0.2%/h</td>
<td>Drift: sometimes a problem; replace</td>
</tr>
<tr>
<td></td>
<td>Calibration: by batch</td>
<td>Calibration: multiple finger sticks</td>
</tr>
<tr>
<td>Lactate Sensor</td>
<td>In development</td>
<td>none</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monitor Component</th>
<th>BFIT Performance Specifications</th>
<th>MINIMED CGMS Performance Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Measurement Range</td>
<td>20-1000 mg/dl;</td>
<td>40-400 mg/dl</td>
</tr>
<tr>
<td>Typical Operating Range</td>
<td>&gt;1200 ft. (300 m)</td>
<td>6 ft. (2 m)</td>
</tr>
<tr>
<td>Display Window Length</td>
<td>PC-based currently: to screen resolution</td>
<td>1.40 in : 0.70 in</td>
</tr>
<tr>
<td>Dimensions Length</td>
<td>breadboard prototype 3 in : 3 in : 1 in</td>
<td>3.56 in : 2.77 in : 0.08 in</td>
</tr>
<tr>
<td>Weight</td>
<td>Prototype: 2.8 oz; miniaturizing to 0.5 oz.</td>
<td>4 oz (114 grams)</td>
</tr>
<tr>
<td>Warranty</td>
<td>prototype; Electronics MILSPEC reliable &gt;10 yrs in US Army Missile Command</td>
<td>1 year</td>
</tr>
<tr>
<td>System Memory</td>
<td>Monitor stores 1k readings to RF-download</td>
<td>Stores up to 21 days of data</td>
</tr>
<tr>
<td>Alarms Audible</td>
<td>PC-based currently, using PC speakers (50 decibels @ 1 meter) Optional Vibrate Mode</td>
<td></td>
</tr>
<tr>
<td>Batteries</td>
<td>Prototype Transmitter Monitor uses (2) AAA alkaline batteries. Battery life &gt;1 mo. Miniaturizing to 3 V Li cell</td>
<td>The Monitor uses (2) AAA alkaline batteries. Battery life exceeds 1 mo under normal use</td>
</tr>
<tr>
<td>Frequency</td>
<td>1 measurements: 30 seconds; variable intervals</td>
<td>1 measurement per 5 min (300 sec); fixed interval</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transmitter Component</th>
<th>BFIT Performance Specifications</th>
<th>MINIMED CGMS Performance Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Compatibility</td>
<td>NONINTRUSIVE</td>
<td>Complies with ISO 10993-1 long-term contact</td>
</tr>
<tr>
<td>Transmitter Life</td>
<td>prototype; Electronics reliable &gt;10 yrs</td>
<td>1 year under anticipated normal use conditions</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrochemical Detection Component</th>
<th>BFIT Performance Specifications</th>
<th>MINIMED CGMS Performance Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOD Enzyme anchor</td>
<td>electrochemically deposited polypyrrole</td>
<td>optically exposed hydrogel</td>
</tr>
<tr>
<td>Response Time [s]</td>
<td>10; time averaged to 300</td>
<td></td>
</tr>
<tr>
<td>Electrode Area [mm²]</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Stability</td>
<td>Drift: &lt;0.2%/h</td>
<td>sometimes 100%/h</td>
</tr>
</tbody>
</table>

Table 1. BFIT and CGMS comparative system specifications.

2.5 System Operation Overview

Each measuring chip currently has 25 individual measuring cells that are addressed individually and sequentially by the control electronics. The number 25 was chosen arbitrarily for demonstration purposes. These cells are designed into a 5x5 array. We could practically integrate 100 in a 10x10 array with little technical challenge, and perhaps ultimately as many as 1600 in a 1cm² area. Each cell has a heater element
that is used to ablate the stratum corneum, and then is fused to create an open circuit. The microprocessor of the sensor node electronics selects one cell with the multiplexer and then applies a train of controlled electrical pulses of precise energy and voltage to perform the ablation and fuse the heater wire. One of the arms of the conductive gold metal paths leading to the heater has been selectively deposited with a polymer matrix containing either GOD or LOD enzymes. The other arm is left bare. Together they form a two-electrode electrochemical cell selectively sensitive to glucose of lactate. The sensor node electronics then applies a voltage pulse of 0.2 V for duration of up to 30 s and precisely measures the current flowing at a programmable time following the application. We have waited 10 s before measuring the current to ensure that the current transients have all attenuated. This current measurement can be repeated indefinitely until the cell no longer registers a current. As we will see below this cell lifetime varies from a few minutes to a few hours. We are working to increase this lifetime to a day or more. When one cell no longer responds the next one in the sequence can be opened. With some program modifications we have been able to open and follow up to three cells that are open at the same time to check in real time for reproducibility in the measured response. More on the technical engineering details will be published elsewhere.

3.0 IN-VITRO EXPERIMENTS, CALIBRATION

After fabrication, BFIT sensor chips are calibrated for sensitivity in composition controlled solutions. Figure 4 below shows the results of chronoamperometric calibrations over an hour of one typical cell of the 25 on a sensor chip as the ambient glucose concentration is increased in 10 mmol steps every 10 min from 0 to 50 mM. The calibrations are performed using a 50 cc vial containing the sensor chip under test bathing in isotonic phosphate-buffered 5% saline solution. In each case we add measured volumes of concentrated glucose and mix with a small stirring bar located in the bottom of the vial for 8 min. When agitation stops we perform an I-t sweep over a 30 s period, first grounding the electrodes, then applying a 30 s 0.2 V potential between them and record the electrochemical current. The observed current pulse reaches a peak value in milliseconds and relaxes exponentially to an equilibrium value of 0.1 – 1.0 mA for the electrode areas of 0.07 mm² we employ. At any instant the current is proportional to the glucose concentration. Practically, we wait 10 sec from the beginning of the voltage pulse before recording the current. In Figure 6, we plot this current as a function of the precise ambient glucose concentration as a calibration curve in the physiologically sensitive 1-10 mM range. Note that our devices have a linear calibration curve, saturating in concentrations far above 50 mM.
We employ a similar calibration procedure with our lactate chips. They too are linear in the physiologically important concentration range of 0.1 – 20 mM.

4.0 IN-VIVO EXPERIMENTS

4.1 Animal Experiment Overview

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition.

Animal experiments were all performed under approved hemorrhagic shock protocols at the Walter Reed Army Institute of Research as amendments to ongoing research programs. Some of the details of the protocols as well as the results of those experiments appear in the subsequent papers in these proceedings. The modifications permitted us to add i) our nonintrusive glucose and lactate monitoring microsystem developed for diabetics (MiniMed-CGMS), ii) a commercial intrusive, subcutaneous glucose monitor, iii) either IP injection or arterial artery infusion of glucose for glucose stress testing, iv) a laser-Doppler probe to measure circulation in the immediate vicinity of the BFIT placement throughout the hemorrhagic shock, and v) to measure 1 ml of the frequently drawn arterial blood samples in a precision glucose and lactate assay system (YSI-2700). The animals used were both Sprague-Dawley rats (both male and female, 250-350g) and male pigs (65-80 kg).

Both rats and pigs are good animal choices to demonstrate our monitoring micro-system, and in particular the BFIT sampling chip. The pig’s skin and physiology are very close to those of the human, although the stratum corneum is rougher and thicker than human’s. Despite the fur and the lack of sweating, the rat’s skin too is a useful model to living human skin since the stratum corneum is smooth and the same thickness as humans. Rat fur must be carefully shaved-away on its abdomen using a micro-screen razor so that no cutting edge scrapes and inflames the skin or disrupts the stratum corneum. The same technique was used to trim and remove the sparse but wiry hairs on the pig’s inner thighs where we placed the BFIT sensors.

At the time of writing, we have completed three pairs of experiments. In the first, we performed two hemorrhagic shock experiments with glucose stress-testing on rats. One involved an arterial glucose injection following resuscitation of a female rat (R16 – these numbers refer to animal numbers used in the protocols of Baranyi and Lee), the other involved two glucose arterial injections prior to controlled hemorrhagic shock, and a third following resuscitation of a male rat (R226). The second pair of experiments was performed on two male rats who were administered 0.1 mg of theophylline intra-arterially 10 min prior to beginning the hemorrhagic shock. The third pair of experiments was performed on two male pigs who were subjected to two hemorrhagic shock cycles with resuscitation using isotonic saline.

In all experiments glucose concentrations were monitored with the BFIT transdermal microsystem, with the commercial CGMS unit and arterial blood draws. The BFIT was placed on carefully shaved skin as described above, and pressed onto the skin by a glass slide. The BFIT chip was visible through the slide and available for microscopic observation and filming during the ablation and assay phases of its operation. Since the BFIT chip is transparent any presence of interstitial fluid at the ablation site could be seen and recorded. For rats, 20 mJ pulses of 2.3 V were sufficient to ablate the stratum corneum and allow interstitial fluid to emerge, wetting the electrodes, but not damage in any way the underlying viable epidermis. These were the parameters that we designed for human use, and tested previously on living human graft skin. For pigs with their thicker epidermis, we found that 25 mJ of energy and 2.7 V were required. In the case of pigs we also washed and
patted dry the skin to remove any traces of soiling. The laser Doppler probe is an optical fiber in a plastic pedestal type mount that can be glued, or pushed onto the skin leaving the fiber pressed onto the skin perpendicular to the skin surface. The foot of the pedestal is about 6 cm in diameter, and it was placed as closely as possible to both the BFIT and CGMS sensors. It can be seen as the black device in Figure 8G and 8H below. Its role was to measure fluid velocities in the arteries and veins just under the skin’s surface as a check that during hemorrhage circulation does not shut down to the tissues in which we are measuring the glucose concentrations present in interstitial fluid. Loss of circulation would mean that arterial concentrations no longer are in contact with these tissues and we would then expect no further equilibration between concentrations found in the arteries with those in interstitial fluid. For ease in BFIT micro-fabrication we chose to place two BFIT chips side by side, one with 25 glucose-sensitive cells and the other with 25 lactate-sensitive cells in the experiments when the two concentrations were logged simultaneously.

The CGMS sensor unit was placed according to the manufacturer’s instructions. We disinfected the site, and used sterile gloves. Skin, including the fatty tissues beneath it, was pinched-up and the sensor’s 2 cm-long stainless steel syringe together with the coaxial hollow plastic syringe containing the glucose-sensitive electrochemical electrodes was inserted at a glancing angle so as to be below the skin in the fatty tissues but not in the smooth muscle layers. Once in the desired location, the stainless steel syringe was removed, any blood or fluid swabbed away and the sensor was taped in place. The manufacturer recommends that the CGMS electrode be allowed to stabilize for one hour after implantation however we found that sensors can require much longer to stop drifting and providing fluctuating current values. After the first 60 minutes, a known blood glucose concentration was obtained by immediate (<5 min) analysis of freshly drawn arterial blood. This value was entered into the CGMS controller as a calibration point. The CGMS unit updates its measurements of currents every 10s, and records a time-averaged value in its memory along with a calculated glucose value every 300s, or 30 measurement cycles. One of the main interests in measuring glucose with the CGMS is that it is the only commercial system currently available, of which we are aware, that measures glucose using interstitial fluid. It has obtained FDA 510K approval for implanted use in humans for up to three days, and has been found to be “indicative” of blood glucose levels.

Finally, we employed the YSI 2700 analyzer beside the animal in surgery to assay with high (<5% error) precision the concentrations of glucose and lactate in arterial blood samples. This machine was preferred to point of care analytic tools such as the i-Stat hand-held analyzers or the Radiometer ABL 700 series blood gas machines for several reasons. Firstly, the latter analyzers are designed for use with whole human blood, and we are dealing with rats and pigs. The calibrations may be as much as 5% different. Secondly, the intended use and calibrations of the latter analyzers is for blood whose hemocrit is close to normal (30-50%). During resuscitation the subject animal’s hemocrits can dip as low as 10%, far from calibrated ranges. We avoided these sources of error by measuring glucose and lactate concentrations in plasma as follows: Fifty μL samples of blood was taken from larger draws (1-5cc) immediately before any separation or clotting could occur, and were centrifuged to separate out the blood cells. Hemocrits were measured manually and immediately 12.5 μL blood plasma samples were introduced into the YSI analyzer. We strove in this way to keep any error in both blood glucose and blood lactate assays to less than 5%.

4.2 Non-Intrusiveness

Some of the tests we have performed on rats to prove the nonintrusiveness of the sampling technology are summarized in the images of Figure 6. In the sequence of four micrographs on the left we show pictures taken every 30 ms during a 30 ms 20 mJ pulse that ablates the stratum corneum. In the middle of each micrograph we see the serpentine structure of the heater (called TPS – thermal perforation system) in the 50 micron gap between gold conductor strips. The photograph is taken by a shallow depth of field optical microscope.
through nearly 1 mm of living human graft skin. One can make out the outlines of the stratum corneum cells touching the plane of the BFIT chip surface, with a flattened slightly oval shape of about 50 microns in diameter. In the first picture of the sequence we see first the skin and heater before power is applied. Power is applied in the second picture, and some thermal expansion of the serpentine structure is visible as it heats, ultimately to 140° C. In the third picture we captured the first stratum corneum cell that ablates. Thirty milliseconds later, interstitial fluid is observed to wet the vicinity of the opening. In the middle photograph we zoom-out by a magnification factor 20X to see the full 5x5 array of individually addressed measurement cells. To establish the scales we show a 25-gauge syringe tip touching one of the sampling cells. Finally, in the pictures at the right of Figure 6 we show a stereo-zoom micrograph of the 50-micron diameter opening in the stratum corneum created by the BFIT visible once the chip is removed from the rat’s skin surface. By way of comparison we show a picture of the rat’s skin where a syringe has penetrated the skin. A cut of ½ mm is seen where the syringe cut into the skin, as well as a small amount of dried blood in the corner of the skin flap, and generalized swelling and bruising with a ½ mm diameter, at depth in tissues.

4.3 Two Hemorrhagic Shock Experiments with Glucose Stress Testing on Rats (R16, R226)

We refer to the multivariable graphs and photo of Figure 7 below to give the details of these experiments. First looking at the photo of Figure 7D we see Rat 16 lying on its back with a BFIT pressed onto its shaved abdomen by a glass slide. Above it is the barrel of a microscope zoom lens and the tip of an optical fiber illuminator. The BFIT device is inserted into its ZIF micro-connector and a flexible 66-wire cable communicates to the microsystem controller, delivering current bursts for ablation and collecting electrochemical currents from the 25 separate glucose sensitive cells located on the device. To the right of the photo one sees the closed incisions in which are placed catheters to the femoral artery and vein. Carotid catheters are not visible, but one can see some of the electrical probes placed for measuring EKG and EEG. Some of the details of the hemorrhagic shock are given in the time chart of Figure 7A. The green line gives the mean arterial blood pressure (MBP [mmHg]) as a function of the time of day on which the experiment was conducted. The blue line provides a measurement of the shed blood volume (SBV) by measuring the weight of shed blood on a digital microbalance in grams as a function of time. The red line called marker identifies with its ticks the interesting physiological points of progress in the hemorrhage. From left to right, the first tick gives the moment when blood begins to be with drawn and the MBP begins its decline. The next three ticks correspond to the moments the SBV reach half maximum, maximum and half maximum again as blood...
is first withdrawn then pumped-back into the body. The pumping of blood is performed so as to keep the MBP at 40 during the controlled hemorrhage. About 40 minutes after resuscitation, at the final group of ticks, two ml of 300 mM glucose in isotonic saline is administered through the femoral arterial catheter. About 30 min later the rat dies. The blood glucose assays [mM] of the YSI analyzer are plotted together with the electrochemical currents registered from BFIT cells interrogated during the shock experiment. The YSI curve shows a slow increase from 3 to 4 mM before shedding blood, and the usual peak shape during the course of blood shedding and restoration, rising to 9 mM and declining to 7 mM, and then 3 mM again as glucose is delivered to the circulating system principally from the liver, metabolized and eliminated by the kidneys. At the point of glucose injection the concentration rises rapidly to 18 mM, falling to 8 mM at the animal’s death. The first BFIT sensor G9 only operated for 15 min in interstitial fluid and followed the decline proportionately until a twitch from the rat moved the sensor and broke contact to the point of ablation. It took several minutes to open two new channels through the stratum corneum. G15 and G10 once again track proportionately (with the exception of the final reading from cell G10) the blood glucose concentration until the animal dies.

Figure 7C shows the CGMS currents in blue reported during the same time course. The sensor appears to drift to lower currents from the time it is implanted until the animal’s death. Along the way there are some rises in current above the general decline. However, the peak in these rises does not correlate with either the time or magnitude of the arterial glucose concentrations. This sensor appears to fail to work correctly at all.

A second experiment was performed in a male rat R226. Prior to hemorrhage, two glucose injections were given, and after hemorrhage, like R16, another glucose injection was given. We will see that as we expect, before hemorrhage, the rat clears quickly in a few minutes the excess blood glucose and that this is simply slowed-down in the resuscitated rat. Figure 7E gives the measured MBP and SBV for the duration of the experiment. Looking at the MBP, we see that it rises, as does the base blood glucose during the first four hours of surgery, most likely due to a sympathetic response. The ticks on the marker line identify the instants that the three glucose injections are made as well as the moments of initial, half-maximum and maximum shed blood volume. In Figure 7F, the arterial blood concentrations and the BFIT electrochemical currents are presented. Focusing on the event of the first injection, the YSI measures a rise from 10 mM to 20 mM within a minute followed by a decrease to near 10 mM after 8 min, then a rise to 15 for an hour. The BFIT cell G1 began to track the rise, and then became an open circuit. We immediately switched to cell G5. Its current rose and declined simultaneously with the blood glucose level following the first injection, albeit at an attenuated level. That is the peak to valley change in current increases about 30% while the blood glucose level increases 100%. A second rise in glucose concentration is indicated by the BFIT G5 cell, but there are not sufficient blood analyses during that period to confirm that. Clearly by the sec, the G% cell no longer responds, its current remains flat up to and through the hemorrhagic shock. Following the shock, three cells were opened on a new chip, numbers G1, G2, and G3. No change was observed in any of the three cells at, or following the third injection up until death during a period when the blood glucose rises from 8 to 17 and falls back to 2 at death. With no response in three separate cells it appears that the interstitial fluid no longer communicates with the circulating blood. This is also seen in the lactate signal discussed below.

Figure 7G shows both the blood lactate assay concentrations and the current of lactate cell L13. These show very good correlation up until a moment following the second hemorrhage when the current becomes constant, exactly like the glucose concentrations in Figure 7F. The observed current changes by the same proportions as the blood lactate concentration. The final curve in Figure 7G shows the CGMS current together with the blood glucose concentration. The CGMS signal responds simultaneously with the first glucose injection and in direct proportionality, rising again, noisily after the blood glucose returns to normal. At this point the CGMS begins to drift downwards, misses the second and third injection blood glucose response. This is difficult to understand given that the sensor is intended to follow blood glucose for three days...
following implantation. Yet it does correlate qualitatively with the observed BFIT signals. If the CGMS currents are accurate, then there are very significant changes in interstitial fluid concentrations compared to blood levels following major physiological changes like hemorrhagic shock.

4.4 Two Hemorrhagic Shock Experiments on Pigs (P21, P22)

The next two experiments involve single and double controlled hemorrhages in pigs with no glucose or drug injection. The results of the first experiment on pig 21 appear in Figure 8. Figures 8G and 8H show two views of the placement of the three sensors: BFIT, CGMS and Laser-Doppler. In Figure 8A shows the MBP and SBV traces. Looking at the sequence of ticks on the marker line from left to right, one sees the beginning, half maximum and maximum shed volume points of two successive controlled hemorrhages with minimum blood pressures maintained at 40mmHg. The laser-Doppler signal we logged scaled directly with the mean blood pressure signal. This indicates that blood continued to flow into the area in which our BFIT and CGMS sensors were placed throughout the experiment up until death. It also confirms that the surgical incision and placement of the catheter visible next to the BFIT in Figure 8H. In Figure 8B we show the measured currents in three BFIT cells actuated sequentially: G6, G21 and G25. Taken one after the other, they track well the YSI blood glucose assays. Blood would have to be drawn much more frequently to shed light on whether the BFIT current peaks in current follow blood glucose peaks. Referring back to the experiment with Rat 226, when blood was drawn every minute or two following a glucose injection, there was no lag between the two signals. This contrasts to the graph in Figure 8C of the CGMS current throughout the experiment where there is no correlation observed to the blood glucose curve.

The experiment with Pig 22 was longer that the previous one and the animal survived a second resuscitation. This is evident in Figure 8D where there are two shed blood volume peaks and a final blood draw until death. The tick marks on the marker line indicate sequentially the points of starting the bleed, half maximum volume, maximum volume, and finally when the shed blood volume is replaced by isotonic saline. After the first resuscitation the MBP slowly climbs back to 90 mmHg, its pre-hemorrhagic value. After the second resuscitation, the MBP reaches 70 mmHg. In the final and fatal hemorrhage, the MBP drops to zero. The blood glucose concentration during this time rises slowly from 4.5 to 6, with very slight increases during the shedding of blood. Three BFIT sensors operated during the experiment. None of the three track completely the blood glucose curve. This is in part because of the infrequency of blood draws. The G16 current curve shows a small increase in glucose during the first controlled hemorrhage, but does not decrease in the same way as the blood glucose. The cell G13 shows a flat response as does the blood glucose except for the abrupt step in current observed in the first few minutes. Finally, cell G6 begins by tracking a small decrease in glucose concentration following the second bleed. The shape of that curve is similar to that of G16 in which the decrease is very gradual. At the end of the fatal hemorrhage, the glucose current fell abruptly, similarly to the current drop of sensor G25 in the previous experiment in Figure 8E. The final graph of Figure 8G shows the measured CGMS currents throughout the experiment. That sensor seemed to drift continuously from 22 nA down to 17 nA while the blood glucose concentration increased continually. During the fatal hemorrhage, the CGMS signal dropped sharply by 65% as did the BFIT cell G6.
Figure 7: Two hemorrhagic shock experiments on rats.
Figure 8. Two hemorrhagic shock experiments on pigs.
4.5 Two Hemorrhagic Shock Experiments with Adenosine A1 Receptor Inhibitor in Rats (R224, R225)

Figure 9. Two hemorrhagic shock experiments on rats.
This pair of hemorrhagic shock experiments is similar to the first pair, except for two changes: i) 10 min prior to the controlled hemorrhage, a 0.1 mg dose of theophylline is administered by arterial infusion, and ii) there is no resuscitation, the rat is maintained by controlled bleeding and infusion of heparinized blood at a MBP of 40 mmHg until death. One effect of this drug is to constrict blood vessels. One hypothesis is that vasoconstrictors can reduce the amount of resuscitation fluid required and improve survivability. We can not comment on this hypothesis and our observations are strictly limited to the ability of the BFIT and CGMS devices monitoring glucose in interstitial fluid to track blood. From this stand-point the drug treatment produces two new desirable effects: i) blood glucose and lactate concentrations increase dramatically – a factor of two higher than in previous experiments, and ii) the vasoconstriction effectively isolates the interstitial fluid from the blood for a period of about an hour until, near death, the constriction disappears and the blood and interstitial fluid concentrations can equilibrate. These represent new measuring challenges for a monitor both in terms of response-amplitude and frequency.

The first experiment on rat 224 is summarized in Figure 8A. Following the ticks on the marker line from left to right we see the following sequence of events: begin recording, drug injection, starting hemorrhage, half-maximum, and then maximum shed blood volume followed by half maximum and maximum resuscitation volumes. The latter event occurs just prior to death. Figure 8B gives the arterial blood glucose concentration and the BFIT glucose currents measured. Note that the cells G1 and G2 both track the blood glucose until the drug is administered. Then the blood concentration rises rapidly to 30 mM and drops-back to 4 mM. Both cells stopped recording just prior to death. But the glucose concentration in the interstitial fluid continues to decline very slightly: appearing to be cut-off from the concentrations in the blood. Figure 8C gives the corresponding CGMS currents measured. They appear to be quite noisy throughout the whole experiment. Time-averaging the currents leads to a response similar to the one recorded by the BFIT sensors in Figure 8B.

The final experiment on rat 225 repeats the same hemorrhage sequence as rat 224. In Figure 8D, we see that the MBP and SBV signals are smoother, indicating a more robust subject. Also the monitors were all kept recording for several minutes following death to explore novel effects in glucose concentration. Note the MBP is followed down to 0. Looking at Figure 8E we see that once again the blood glucose peaks sharply then drops to hypoglycemic levels. The BFIT sensors vary by about 10% one from another but reproduce the very slowly declining glucose current seen in Figure 8B above. Note that the cell G-22 suddenly decreases and tracks the blood glucose. This is confirmed by the tracking of lactate cell L4 as discussed below. The two other cells G12 and G24 have currents that begin to drift-up slightly.

Figure 8F gives the blood lactate concentration as well as the currents observed in three BFIT lactate cells. The blood lactate suddenly increases from a constant concentration following the drug injection then saturates at 14 mM. The BFIT cells L1, L4, and L8 track the blood lactate until drug injection when they begin to drift lower, likely being sealed-off from blood concentrations by the vasoconstriction. Shortly after drug injection, the rat began to move and cells L1 and L8 ceased to register current. Cell L4 continued to work and initially tracked the lactate rise, then relaxed downwards – again in an apparent isolation from the blood. However, at the precise moment when glucose cell G22 began to track the blood glucose, lactate cell L4 begins to track the blood lactate concentration.

The final curve, Figure 8G shows the electrochemical current response of the CGMS sensor. It appears to track neither the blood glucose, not any of the BFIT sensors.
5.0 CONCLUSIONS

We have demonstrated in vitro with human graft skin and in vivo with rats a prototype fieldable remote non-intrusive monitoring system for the assay of glucose and lactate in interstitial fluid. It has the flexibility to monitor almost any moderately hydrophilic/soluble bio-molecule of molecular weight less than 60k Daltons, as are commonly found in measurable concentrations in the interstitial fluid just under the skin’s stratum corneum and in other body fluids such as saliva.

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Evaluation of a Capacitively-Coupled, Non-Contact (through Clothing) Electrode or ECG Monitoring and Life Signs Detection for the Objective Force Warfighter

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ABSTRACT

A new device that measures ECG inter-beat intervals through clothing is described and compared to a resistive contact electrode. The capacitively coupled non-contact electrode (CCNE) underwent a 40 person human trial at the Walter Reed Army Institute of Research this past year. This sensor can detect ECG and respiratory signals thru clothing and is being considered by the US Army as a physiological monitoring detection sensor on the Objective WarFighter uniform of the future. In this study, three CCNE sensors were compared to an FDA-approved monitor (3-lead) using contact electrodes to determine if the R-R inter-beat intervals of the two methods were “the same” or not. Results of the “at rest in supine position” for determining heart rate based on inter-beat intervals of the ECG are presented. The test results indicate that, relative to the ECG contact electrode, the CCNE sensors work for determining R-R inter-beat intervals reliably. Test subject variability in the different weight categories indicates similarity between the two types of electrodes and statistics for comparison are presented. The CCNE sensor gives "unbiased" estimates (116 out of 117 difference signals gave "unbiased" estimates). The CCNE sensor gave close estimates of the inter-beat intervals in 30 out of 39 test subjects with less than 14 ms differences. The CCNE difference signals give statistically "similar" results within each test subject (37 out of 39 test subjects had statistically similar results). Females showed more variability than males for each weight class. Males and females in weight class 5 had the largest measures of variability.

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3), AR 360.5.

INTRODUCTION

Biopotential sensors can generally be categorized as invasive or non-invasive. Invasive sensors are implanted surgically and are used for isolation of specific potential sources in the brain or the peripheral nervous system. Non-invasive sensors are referred to as surface, skin, or scalp electrodes or sensors, and are applied to the skin surface. To ensure a good resistive contact to the test subject, such electrodes typically utilize a conducting electrolyte or gel and are hence often referred to as wet electrodes. Such electrodes are the standard method used in clinical and research applications.

Various attempts have been made to overcome the limitations of wet electrode technology for measuring bioelectric signals such as ECG and EEG on the human body. Advances include one class of surface electrodes that does not use electrolytes. These electrodes are referred to as active electrodes and employ an impedance transformation at the sensing site via active electronics. Active electrodes are subdivided into two types—dry electrodes, which rely on a metallic surface in direct contact to the test subject that uses a combination of resistive and capacitive coupling to the local skin potential, and insulated electrodes, which utilize only capacitive coupling.

Detection of human body bioelectric signals using purely capacitive coupling was first reported in 1968 [1]. A schematic for a capacitive electrode system, comprised of two conducting plates placed close to the body and connected to the input of a differential amplifier, is shown in Figure 1. The plates have a capacitance, $C_b$, to the region of the body in the immediate vicinity of the plates, and a capacitance $C_f$, to the free space electric potential. $C_f$ represents the capacitance to the source via all other paths except the paths close to the plate.

![Figure 1: Approximate Equivalent Circuit for Capacitive Coupling to the Human Body](image)

For prior capacitive electrodes, $C_b$ had to be high—typically 1 nF to 100 nF. $C_b$ is approximately given by the standard expression for a parallel plate capacitor, and is thus inversely proportional to the spacing between the electrode and the body [2,3]. This dependence is so sensitive that if the electrode moves from being on the surface of the skin to just 100 µm away, $C_b$ changes by approximately a factor of 10. Thus, even though they do not require direct resistive contact to the body, traditional capacitive (insulated) bioelectrodes are very susceptible to displacement.

In 2001, researchers at Quantum Applied Science and Research (QUASAR) developed a new class of sensor that measures the electric potential in free space, i.e. without physical contact to any object. It was observed that the sensors were able to measure the ECG of a fully clothed person standing within a range of about 10 inches. (Patent Pending) In 2002 QUASAR developed a compact version of the sensor, termed the capacitively-coupled noncontact electrode (CCNE), specifically to measure ECG through clothing (See Figure 2) This first version of the ECG electrode, including all amplification electronics, is approximately 1 inch square with thickness of 0.35 inches.

This report describes the results of the first human trial of this new electrode technology. The goal was to compare the CCNE operating through regular clothing with a conventional 3-lead ECG using resistive electrodes for the purpose of measuring interbeat intervals.
The principal application envisioned for the technology is continuous readout of ECG of military personnel as part of the Objective Force Warrior (OFW) soldier uniform. OFW is the Army’s flagship Science and Technology initiative to develop and demonstrate revolutionary capabilities for Objective Force soldier systems. Including physiological monitoring such as ECG in the Objective Force Warrior and Land Warrior systems is of great interest to the Army. It is widely known that there are many problems associated with contact electrodes for long-term ECG monitoring, including loss of contact to the test subject due to drying of application glue or environmental factors (e.g., rain) and test subject resistance to wearing the electrodes due to discomfort caused by factors such as skin irritation. Therefore, a non-contact system would be of great benefit to the Army.

It has been observed that 90% of combat fatalities during conventional warfare occur forward of the battalion aid station (BAS), the first organized medical treatment facility, and that two-thirds of these fatal injuries involve significant hemorrhage [4]. Furthermore, about 60% of these deaths occur within the first 10 to 15 minutes after injury. These statistics underscore the importance of the Army’s equipping their medics with the capability to rapidly locate, assess, and effectively treat the wounded. It is estimated that about 25% of these casualties might be salvagable with prompt hemostasis, and some degree of fluid resuscitation to sustain them until definitive surgery and resuscitation can be achieved in the later phases of their care [5]. Since the medic carries so little resuscitation fluid (only enough to replace a 15% blood loss) and he has to often deal with many potential casualties at once, he must use his resuscitation resources judiciously, deciding when and how much fluid should be given. The medic will need reliable physiological monitoring that is already on the soldier. A non-contact based system would easily be integrated into the OFW program and provide the foundation for operational and combat medic medicine. The information provided by such a system would be helpful to the combat medic to properly ascertain the level of injury and survival potential of the injured, and provide optimum care in the battlefield.

Methods and Materials:

The WRAIR Clinical Trials Division solicited test subjects for 5 weight groups of 4 test subjects each; one set of the 5 groups was to be females and another set males, for a total of 40 test subjects. The inclusion criteria were that they be healthy males and females with no known heart defects, who ranged in weight from 101-->173 lbs for females, and 124-->220 lbs for males. This sampling represents the majority of the men and women in the Armed Forces today. Table 1 shows the different weight groups is as follows:

Inclusion: Healthy Men/Women with no known cardiac defects, Civilian/Military, ages 18-50, Weight- female: 101-->173 lbs or greater; male: 124-->220 lbs or greater
Table 1

The groups for women are:
101-119 lbs.  4 test subjects
120-137 lbs.  4 test subjects
138-155 lbs.  4 test subjects
156-173 lbs.  4 test subjects
and greater than 173 lbs.  4 test subjects

The groups for men are:
124-147 lbs.  4 test subjects
148-172 lbs.  4 test subjects
173-196 lbs.  4 test subjects
197-220 lbs.  4 test subjects
and greater than 220 lbs.  4 test subjects

A pilot study using two test subjects determined that the best placement for this initial trial was sensors placed 4 inches below the right and left nipples, and one on the left rear shoulder plate transverse with the other two sensors. [6] Because the data acquisition system and test subject were separated by about 6 feet, a ground strap was attached to the right wrist to minimize DC potential differences. This ground strap has been eliminated in the latest iteration of the technology. The three CCNE sensors were held in place with a commercially available Velcro strap (see Figure 3) to hold the sensors to the body over a cotton T-shirt provided by the study. (see Figure 4).

Each sensor detects the local electric potential. As for all such situations, the potential must be measured relative to another voltage. For conventional electrodes the reference voltage is usually obtained by resistive connection to one or more limbs. The goal of the CCNE approach is to operate through clothing without a resistive electrical contact to the test subject. Accordingly, the output of each CCNE electrode was recorded relative to one of the other CCNE electrodes giving a total of three purely capacitive difference signals, i.e.: channel 1 – channel 2 (X0-X1), channel 1 – channel 3 (X0-X2), and channel 2 – channel 3 (X1-X2).

Figure 3: Arrangement of Sensors on Strap
Each test subject was connected to an ECG monitor, pulse oximeter, and blood pressure cuff. An FDA approved clinical ECG monitor BCI Model #3404-001 AutoCorr Plus was used to provide the "gold standard" reference ECG waveforms for all comparisons with the CCNE waveforms. The frontal plane configuration for the 3-lead ECG was used to take resistive measurements [7] with the 3 contact electrodes placed on the right/left chest and lower abdomen. The AutoCorr has an analog output capability to use as comparison to the CCNE. A pulse oximeter was placed on the right hand of the test subject and blood pressure was taken at the beginning of the experiment.

All test subjects were provided with the same make T-shirt to wear during the test in small, medium, large, extra large, and extra-extra large sizes. This shirt was made of 100% cotton to reduce static charge buildup on the body of the test subject. This provided a consistent thickness through which to record all CCNE measurements across the test population.

The test equipment was connected to a data acquisition system using a Measurement Computing PC Card connected to a Laptop computer running Labview. The data were sampled at 500 Hz and stored digitally on the laptop, which was running on battery power. The data were filtered through anti-aliasing band-pass filter amplifiers using 2-35 Hertz. This eliminated interfering radio-frequency interference noise that would have caused trouble for interbeat analysis centered around 17 Hz. Everything was battery powered to provide safety to the test subject. The CCNE provided leakage current resistance to provide protection to the test subject as well. The test subject was put into the supine position and data were taken for 1 minute with normal breathing.

Results:

The data obtained from the study were post-processed using Matlab. A peak-finding algorithm was applied to detect the peak R wave of the ECG in each of the waveforms and the time between two consecutive peaks calculated in milliseconds. The resulting interbeat interval was used to compare the CCNE with the ECG contact electrodes. The raw waveforms for one test subject are shown in Figure 5. This figure indicates that the waveforms are very similar in nature for this individual; this result is typical of most of the test subjects.
The interbeat intervals were calculated using a Matlab Script for peak detection based on the R wave of the ECG for the contact electrodes and the CCNE. A typical result for one test subject using this analysis is in Figure 6. This shows that the interbeat intervals from the CCNE and ECG look identical upon inspection.

Figure 5: CCNE Difference Signals Compared with Contact ECG Electrodes

Figure 6: Comparison of R-R Interbeat Intervals of CCNE and ECG Electrodes for 1 min.
Pearson’s correlation for each test subject was performed between the CCNE difference signals and the interbeat intervals obtained from the ECG contact electrodes. The results from the correlation are shown in Figure 7. We can see from these results that there is a high degree of correlation between the difference signals of a pair of CCNE electrodes and the ECG contact electrodes. The coefficient of variation was calculated and that is shown in Figure 8. This figure indicates that relative variability (measured by the coefficient of variation) for each subject is nearly identical for the CCNE and ECG contact electrode signals.

**Figure 7:** Correlation Between Difference Signals of CCNE with ECG Contact Electrodes

**Figure 8:** Coefficient of Variation for difference signals of CCNE and ECG contact electrodes ((standard deviation/mean) x 100)
Figure 7 shows that there is a high degree of correlation between the CCNE and ECG contact electrode interbeat intervals. The correlation between the CCNE and ECG intervals suggests that the two sets of intervals might be almost identical, but a high correlation does not exclude the possibility that the CCNE intervals are biased (i.e. CCNE intervals might be consistently higher or lower than the corresponding ECG intervals). To determine whether the CCNE intervals were almost identical to the "gold standard" ECG intervals, two properties of the CCNE intervals were examined. The two inspected properties were "bias" and "closeness" (distance of CCNE intervals from the ECG intervals). Difference scores (i.e. difference signal measurement minus the corresponding ECG measurement) were examined. Results showed that two of the three CCNE difference signals, X0-X2 and X1-X2, gave unbiased estimates for all 39 test subjects, and the CCNE difference signal X0-X1 gave unbiased estimates in 38 out of 39 test subjects (test subject 8 was removed from the study due to data acquisition problems with that test subject). It was noted that the only biased estimate came from subjects 24 and 34 (females in weight class 5).

To indicate the closeness of the CCNE difference signal interbeat intervals to the ECG contact electrodes' interbeat intervals, the absolute value of the differences between the difference signal Xi-Xj interbeat interval (ms) and the corresponding ECG contact electrodes’ interbeat interval (in milliseconds) was examined. Results showed that 20 out of 39 test subjects had all CCNE difference signal interbeat intervals within 10 milliseconds of the corresponding ECG contact electrodes’ interbeat interval and 30 out of 39 test subjects had all CCNE difference signal interbeat intervals within 14 milliseconds of the ECG contact electrodes’ interval. It was noted that 3 of the 9 test subjects with absolute differences exceeding 14 milliseconds were females in weight class 5.

To determine if the three CCNE difference signals gave similar results for each test subject, the proportion of positive, negative, and zero difference scores were examined for each test subject. A chi-square test with 4 degrees of freedom for homogeneous proportions for (3-by-3) contingency tables was calculated. Results showed that 37 out of 39 test subjects had difference signals giving similar results. It was noted that the only two test subjects (24 and ) having dissimilar results were both females in weight class 5.

To determine if test subjects in the same gender/weight category had similar results, and to calculate a relative measure of variability among test subjects in each of the 10 gender/weight categories, the proportion of positive, negative, and zero difference scores (defined above) was examined for each of the 10 gender/weight categories (i.e. 10 contingency tables were examined). A chi-square test with 6 degrees of freedom, (except for males in weight class 1 for which the test had 4 degrees of freedom) for homogeneous proportions was calculated. Except for test subjects 24 and 34, the data for each test subject were obtained by averaging over the three difference signals. The pooling of the three difference signals is justified by the results indicated above. Results showed that test subjects in the same gender/weight category gave statistically different results except for males and females in weight classes 1 and 4. Other results shown were: 1) within each weight class, females showed more variability than males, 2) males and females in weight class 5 showed the largest relative measures of variability (i.e. had the largest chi-square values), and 3) within each gender, weight class 1 showed the smallest relative measures of variability.
Discussion / Conclusion:

Although resistive contact electrodes for measurement of ECG are the gold standard [8], new methods are becoming available to accomplish this task. In this study, capacitively-coupled (through clothing) sensors were evaluated against this standard to determine if the R-R interbeat intervals of the two methods were “the same” or not. The test results indicate that, relative to the ECG contact electrode, the CCNE sensors work for determining R-R interbeat intervals reliably. The statistical analysis of the test results between the CCNE and the contact electrode give the following conclusions:

1) The CCNE method gives "unbiased" estimates (116 out of 117 difference signals gave "unbiased" estimates).

2) The CCNE method gives estimates "close" to the ECG measurements (30 out of 39 test subjects had all CCNE intervals within 14 milliseconds of the ECG intervals).

3) The CCNE difference signals give statistically "similar" results within each test subject (37 out of 39 test subjects had statistically similar results).

4) Females showed more variability than males for each weight class.

5) Males and females in weight class 5 had the largest measures of variability.

Part of the pilot study was to choose a location on the body for the sensors that would provide a reasonably good signal and be consistent across the test population. The transverse plane location (see Figure 4) that was chosen worked well for most of the test subjects. As indicated by the statistics, the larger weight group (group 5 for males and females) had the greatest variability. This can be explained in terms of the placement of the sensors relative to the transverse plane at the sternum. The placement in weight class 5 for females and males was the hardest placement due to anatomical differences in the larger stature of this group of test subjects—large body types for the men and fairly large breast sizes for the women. Since the sensors were held onto the test subject with a Velcro strap, these anatomical differences provided more possibility of incorrect placement or misalignment of the sensors compared to the other weight classes. Part of this study was to identify these problems and see how susceptible this technology is to factors created by the difference in body types. As a result of this study, many problems have been identified and corrected in the latest version of the sensor. A new clinical study of the technology with these corrections will be undertaken to see if these problems have been eliminated.

This study has shown that ECG interbeat intervals can be obtained through clothing reliably using the new noncontact capacitively couple electrodes. More work is required to study the fidelity of the waveforms to each other to determine if clinical diagnosis is possible using these electrodes.

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A Wireless Vital Signs System for Combat Casualties

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A WIRELESS VITAL SIGNS SYSTEM FOR COMBAT CASUALTIES

Combat casualty care can be improved if vital signs can be obtained easily. The work presented in this report describes the status of a compact and portable wireless vital signs (WVS) system that provides vital signs obtained in a traditional manner and is seamless from the field to definitive care. This system uses off-the-shelf, FDA-approved technology in a package that is lightweight, portable, and easy to use. WVS equipment is battery-powered and is comprised of a standard inflatable blood pressure cuff, a pulse oximeter, and a two-lead ECG. The information obtained from this unit is transmitted via Bluetooth® technology to a standard off-the-shelf PDA, which can track multiple patients simultaneously. Patient vital signs can be displayed on the PDA or on other devices such as a wearable ‘head-up’ monitor or a pre-positioned stand alone displayer. From the PDA, the information can also be transmitted via Wi-Fi to a laptop server, which then allows the information to be used in a variety of ways. This new WVS system can be deployed in the field at the point of wounding and remain on the patient through various stages of transport and through all echelons of care (e.g., helicopter, ambulance, gurney, trauma bay, surgical suite, ICU). Thus, the currently used monitoring devices will no longer have to be disconnected and reconnected through successive patient encounters and various means of transport. This capability would obviously make the handling of the patient easier and faster. Because the vital signs are obtained using standard FDA-approved devices rather than futuristic promising technology, this new WVS will soon become a reality. Support from the Office of Naval Research has allowed the Navy Trauma Training Center and the Naval Health Research Center to collaborate with NASA Ames Astrobiotics and others to develop the WVS device. First-generation prototypes were delivered in 2003 and the WVS system is currently being evaluated on civilian trauma patients. Experience to date suggests that the wireless vital signs capability described here is very achievable in the near term. Prototype evaluation and the next development cycle will be reported in this symposium. The capability afforded by WVS is expected to have broad application in Emergency Medicine, beyond combat casualty care.

1.0 BACKGROUND

Naval Operational Health Service Support doctrine emphasizes meeting the demands associated with Operational Maneuver From the Sea (OMFTS, 1996; endorsed under the Secretary of the Navy’s, the Chief of Naval Operations’, and the Commandant of the Marine Corps’ Naval Transformation Roadmap, 2003) with the need for enhanced battlefield trauma management. Execution of the OMFTS concept requires a smaller medical footprint because troops are increasingly likely to be inserted into forward ground areas by air from sea-based platforms. Future battlefronts will also have rapidly moving non-linear battlefronts. Combat casualty care and evacuation from point-of-wounding to upper echelons will be more complex and may involve great distances.

Current equipment used for combat casualty care in the battlefield is minimal. At the level of the Battalion Aid Station or Shock Trauma Platoon, the powered vital signs monitors are few and expensive. Thus casualties are triaged as to who will be monitored by the available equipment. In addition, every time casualties are moved, the equipment is not transported with them. If a casualty requires monitoring during transport, the attachments from the monitor are usually switched to the transport monitoring system. With conventional monitoring systems, valuable time is lost in set up of multiple wires, individual sensors, electrodes, etc. that need to be managed or reconnected during transport. If a casualty requires surgery, the monitoring system again needs to be changed on the patient. Every time the patient is transported, the monitoring equipment is switched from the facilities system to the transport system. This is cumbersome and causes delays. Efficient and portable vital signs systems that monitor multiple casualties and are tailored to the battlefront are needed.

2.0 OBJECTIVE

The primary objective of this effort is to develop a system that obtains vital signs from multiple casualties on demand, using currently available technology in an efficient and portable manner. The physiologic parameters measured should be those that are currently used and accepted. This will ensure easy interpretability and acceptance by those who currently treat combat casualties.

The goal of a wearable, wireless vital signs (WVS) monitoring system is to combine medical data sensor and wireless communication technologies to enable continuous monitoring, quicker transport, and better decision making by avoiding lapses in medical data acquisition. The WVS unit is designed to integrate and wirelessly transmit multiple medical modalities from the patient to hand-held computing devices [i.e., personal digital assistant (PDA)] or pre-positioned displayers (see Figure 1 for concept illustration).

The WVS system concept is a blood pressure cuff that also has a pulse oximeter and two lead ECG. Customarily, these are the only vital signs used in trauma care today to make medical decisions. This WVS unit can be placed on the patient by the first responder near point of wounding and will remain on the patient throughout various stages of transport (e.g., helicopter, ambulance, gurney, trauma bay, surgical suite). Thus this eliminates the need to switch monitoring systems and the cumbersome wires and sensors that are integral aspects of current monitors. Patient-transmitted vital signs data will automatically be picked up and displayed for the provider (either on a handheld computing device or a display monitor). While all patients with the WVS system placed on them will be transmitting their vital signs, the monitoring systems will only display the casualty of interest. From WVS display menus, the care provider can easily choose the casualty that he or she would like to see. Because the transmitted vital signs from the patient are continually picked up by the PDA, the switch from one patient to another will be rapid. The current mode of transmission from the casualty to the PDA uses Bluetooth® technology, a low energy communications protocol capable of capturing
information from a casualty up to 100 feet away. Once captured at the PDA, information can be relayed and displayed on a head-up monitoring system or on a static display monitor. This flexibility will facilitate ease of transport while using non-invasive means to continuously collect and transmit vital signs without the logistics of transporting conventional monitors.

When a server is included in the system (preferably a laptop server or other more portable model), vital signs from the PDA will be transmitted to the server via Wi-Fi so that the information can be processed and transmitted to other providers’ PDAs. Therefore, all caregivers with a PDA and WVS software within a local area can have access to all patients wearing the WVS system.

A key advantage of the WVS system is the cost. In comparison to the traditional transport monitor, which can cost up to $18,000 per unit, the WVS system is relatively cheap so that many units can be acquired for the cost of one conventional monitoring system. For example, an off the shelf PDA used with WVS can be purchased for approximately $300 and flat screen displayers are approximately $250. The battery-powered WVS sensor unit, with modular blood pressure cuff, pulse oximeter, and ECG leads, is anticipated to have a cost of approximately $600 per unit.

A planned feature of the WVS system is that it can be incorporated with PDA based medical records which are currently being used in civilian trauma systems. Medical record information along with pictures and voice recordings taken with the PDA can be stored on an inexpensive memory disk and sent with patients as they are transported. Electronic medical records have advantages as they can be transmitted ahead of the casualty and data management information, including trauma registry, can be easily extracted.

A paramount feature of the WVS development effort is to yield a product that achieves the vision described here and that is deliverable in the near term (e.g., 6-month development cycles) at low per unit cost. In contrast to other ongoing efforts aimed at monitoring various physiologic parameters of non-injured combatants with futuristic modalities, the Wireless Vital Signs (WVS) system concentrates on acquiring traditional physiologic parameters with customary equipment used today. Thus the goal is merely repackaging currently available off the shelf technology to a form factor tailored to the battlefield. By leveraging currently available technology, the goal of this project is well feasible.

### 3.0 DEVELOPMENT AND PROGRESS

Staff from NASA Ames Astrophysics program assembled and delivered first-generation WVS prototypes within a 6-month time frame and a budget less than $100,000. Prototype WVS units performed as required and have been universally positively received, attracting various professional interests in further system development. Second-generation WVS prototypes under construction are again expected within a 6-month assembly timeframe. Delivery and initial testing of second-generation WVS prototypes are scheduled this summer, with initial testing to occur between the submission of this paper and the corresponding oral presentation and demonstration at the NATO Human Factors in Medicine symposium at the annual meeting for Advanced Technology Applications in Combat Casualty Care (2004).

### 4.0 APPROACH

WVS prototype development and initial evaluation in emergency room environments are ongoing. Although this technology is intended for combat casualty care in field settings, it presents opportunity for application in a range of military and non-military clinical settings. Emergency room evaluations provide vital short-term
feedback, not dependent upon scheduling of field exercises. This short-term feedback provides a key resource in the “build-test-build” process endorsed by the Naval Transformation Roadmap (p. 33, Sea Trial, Spiral Development, 2003) and supplements field testing in essential ways.

Design and assembly teams for each of the two prototype development efforts have been selected according to a competitive review process. This approach has generated various ideas for achieving required capabilities and worked to control development costs. Capabilities may be adjusted as experience is gained across generations of WVS builds, but overarching requirements for each successive WVS generation adhere to key parameters of minimal weight, size, battery demand, and cost. Another overarching requirement is that WVS must be as simple as possible, operable by novice medical personnel with no access to a user manual and with less than 5 minutes of hands-on supervision.

5.0 PRODUCT COMPARISON / OTHER CAPABILITIES

The WVS system uses visual display to support the on site medical provider in delivering medical care to combat casualties, especially in trauma. A separate project, the Warfighter Physiological Status Monitoring (WPSM) component of the Objective Force Warrior (OFW), will also yield visual display to benefit medical providers, but OFW has other medical and non medical objectives, making the two projects quite distinct. The different approaches and unique capabilities of WVS and OFW are indeed separate but they complement each other well in combat casualty care. In official description of OFW and WPSM, OFW has a focus on a more global battlespace and visual display of remote information. WVS has a focus on the immediate physical vicinity of the medical provider and visual display of proximal information. The different approaches are not mutually exclusive and, in fact, when used together, yield a more robust combat casualty care capability.

A first distinguishing feature between OFW and WVS is the scope of the information network used. The network used by OFW covers a vast area and is rich with medical and non-medical information. OFW, when developed, will enable “remote triage” and processing “according to algorithms” (p. 46, Operational Requirements Document for Land Warrior, 2001). Further, in an operations requirements document, the on site support specified for the field combat medic is network-based “interface with senior medical personnel at the Battalion Aid Station to assist in the treatment of casualties” (ibid.). WVS is a local ad hoc medical network only, that provides information display and relies on the training and expertise of the on site provider.

Another distinguishing feature is the manner in which each system is applied and worn. Part of OFW’s value is that, as a “unit of issue,” it makes information continuously available because the WPSM is continuously worn, whereas the WVS is only applied to and worn by casualties in serious condition, on an as-needed basis. (This means that only casualties appear in the limited WVS network.)

Other distinctions can be drawn. The two described here are readily apparent examples. The two systems, OFW and WVS, used together complement each other well: WVS as a local unit that can be applied when OFW’s network is not accessible or when the WPSM is non functional as a result of damage, loss, or removal for treatment; OFW as a remote sensing unit that extends information access beyond WVS’ limited ad hoc network. As a dedicated medical system, WVS applied by the first responder will support continual awareness of patient conditions for the immediate emergency medical personnel who are able to respond until patients leave the emergency medical system for recovery or return to combat.

The final distinction drawn here is that the simpler, dedicated function of WVS means a simpler design and development process. Emergency room validation of already delivered first generation WVS prototypes is
ongoing and experience to date is positive. For comparison, “the Army Science Board performed a study specifically focused on the Objective Force Warrior. This study identified selected technology opportunities and provided a sample roadmap for technology integration. They also found the greatest opportunities for performance gain were beyond 2012.” (National Security Directorate Oak Ridge National Laboratory, 2001.) WVS can fill a role today and a limited, dedicated medical role in the future. These WVS roles are excellent and valuable complements to OFW and various other grand scale technology development efforts.

Figure 1. WVS System Concept
REFERENCES


Development of a Ballistic Impact Detection System

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ABSTRACT

The Future Force Warrior program is a revolutionary redesign of the warfighter platform that, for the first time, will incorporate soldier-worn physiological monitoring equipment. Part of this physiological system will include a Ballistic Impact Detection System (BIDS), designed to detect potentially injurious impacts to the body. Proof-of-concept data showed a consistent biphasic vibration pattern consisting of low amplitude, high frequency (Avg 656.6, SD 96.3 Hz) segment followed by a higher amplitude low frequency (98 Hz) segment. Low velocity impacts have shown that similar vibration patterns are created in the live swine (490.4 Hz, SD 118.1 Hz), dead swine (Avg 494.9 Hz, SD 123.0 Hz) and live human (Avg 437.1 Hz, SD 78.6 Hz). High velocity impact experiments in swine have been the basis for the design of an analogue/digital circuit that discriminates between normal activity and bullet and blast overpressure and fragment surrogates.

1.0 INTRODUCTION

The future battlefield is projected to be asymmetric, non-contiguous and nonlinear. To meet the challenge of future conflicts, the U.S. Army is changing its paradigm from linear and sequential operations to simultaneous and distributed operations. Sophisticated and adaptive adversaries are making unconventional tactics, such as guerrilla warfare and terrorist attacks, commonplace. In the future as today ground forces will continue to be counted on to win and hold the ground and rebuild the peace. The centrepiece enabler of the Army’s transformation is the Future Force Warrior (FFW). FFW is a revolutionary redesign of the individual warfighter platform from the skin out. FFW is a system of systems – data from sensors on the individual soldier are fused with similar information from other soldiers in the unit of action. As the data is integrated and sent back, the warfighter becomes a sensor node in a bigger network mesh that ultimately allows battlefield commanders to quickly react to critical information. Elements of the individual warfighters health status will be incorporated into the data stream from physiological monitoring devices worn by each soldier. The Warfighter Personal Status Monitor (WPSM) is the overarching medical system that will deliver pertinent information that will keep the soldier in the fight and in the event of becoming a combat casualty, aid the medic in rescue and recovery operations.

The central tenet to the Army’s transformation to FFW is the ability to “see first, understand first, act first and finish decisively” [1].” The underlying foundation for achieving this detect-decide-deliver goal of battlefield tactics will be information technology. Acquiring critical information and delivering it rapidly and correctly will have a profound impact on the tactical, operational and strategic success of future combat missions. In the future, the Army unit of action will conduct operations over larger spaces. This translates into small, disparate fighting groups covering far more territory with a single medic in support. It is quite likely that FFW

warfighters will be out of sight and hailing distance of medics and will rely on a medical information sub-network to achieve adequate levels of medical support. Early notification of a soldier’s need for medical attention can reduce the time to initial treatment and thus may reduce the morbidity and mortality of wounded soldiers. The overall goal of the Ballistic Impact Detection System (BIDS) and WPSM program is to increase survivability of the soldier on the battlefield, and facilitate more rapid triage for the combat medic.

Data from Bellamy’s study of causes of death from the Vietnam War [2] shows that while 66% of combat casualties die within the first 5 minutes of being wounded there is an opportunity to save lives if a medic can get to a soldier quickly. Figure 1 shows the percentage of all combat deaths as a function of the time from the wounding event. A therapeutic window of opportunity exists for those soldiers killed in action (KIA) in the timeframes encompassing 5 minutes to 6 hours. Given Carey’s findings [3] during Operation Desert Storm that the predominant cause of deaths in Corps hospitals was exsanguination from extremity wounds, it is likely that with advances in body armour, extremity wounds will become a large percentage of potentially salvageable casualties on the battlefield. Knowing when a wounding event occurs and the ability to engage other physiological apparatus on the soldier to determine the extent of the casualty can play an important role in the required remote triage capability needed to change battlefield casualty statistics.

![Figure 1](source: Pearce, F.J. from Bellamy, R. F.)
2.0 METHODS

The Ballistic Impact Detection System (BIDS) project began with the hypothesis that acoustic vibrations on the skin created by penetrating ballistic missiles could be sensed and analyzed to determine severity of the wounding event. A proof-of-concept phase was conducted with the acquisition of impact signatures from a swine model used in a non-lethal wounding protocol. During this protocol, a single ‘plastic bullet’ (a 12mm steel bearing ball with a thin plastic coating weighing approximately 16 g) was fired from a gas gun at an anesthetized pig from a distance of 8 feet. Three impact locations were used – lateral chest, sternum, and abdomen. Velocities ranged from 239 to 298 feet/second. Two piezo-film sensor elements were attached using duct tape to the back of the animal, symmetrically about the spine just below the scapulas or symmetrically about the sternum. The voltage response from the sensor elements were digitized at 20,000 samples per second and digitally recorded. Figure 2 shows an impact to the left lateral chest. This image was captured from a high-speed (4500 frames/sec) video recording for animal 65-4.

![Figure 2](image)

Analysis of the impact signatures showed consistent characteristics. Each waveform was made up of two distinct frequency patterns. The first pattern was a low-amplitude, high-frequency section lasting on the order of 20 ms. The second pattern was a high-amplitude low-frequency section. Figure 3 shows the voltage recording for ID 65-4.
Development of a Ballistic Impact Detection System

For our purposes, we will refer to the high frequency section as the shock wave and the lower frequency section as the tissue displacement wave. High-speed photography (Figure 2) shows the two frequency sections - the shock wave as a fast moving slight rippling effect that moves outward from the impact site and the slower developing tissue displacement wave radiating outward tantamount to ripples in a pool. Examination of Figure 3 shows that the shock wave amplitude of the left sensor is greater than the amplitude of the right sensor, confirming that the impact site was the left lateral chest. Examination of the tissue displacement waves also confirms a left side hit. They also show that once formed, they move rapidly as witnessed by the imperceptible delay of the tissue displacement waves between the sensors. Frequency analysis in the form of Fast Fourier Transforms (FFT) was performed on the waveforms. Figure 4 shows the FFT performed on the initial 7.5 ms of the shock wave for ID 65-4. Peak frequencies in the range from 500 to 1000 Hz were typical in all shots. The peak frequencies (frequencies with the highest power) in this range from this type on analysis are tabulated in Table 1. FFTs were also performed on the tissue displacement section of the waveform. In every case the predominant frequency of the tissue displacement wave was 98 Hz ± 19 Hz.
The similarity of the primary frequencies provided a proof-of-concept for ballistic impact detection. The primary frequency range (489 – 822 Hz) of the impact signature is much higher than what is typically generated in the body during routine activity. Running, jumping and even blunt thumps to the body elicit only the typical 100 Hz tissue displacement frequency that was also seen in the ballistic signature analysis.

2.1 Low velocity impact models:
A multi-protocol research plan was developed to compare impact signatures across models with those from humans. For this purpose a commercial paintball rifle was chosen to deliver standardized impacts. Paintballs offered a socially acceptable method of delivering an impact to human volunteers for comparison to similar...
swine and human cadaver impacts. Similarity of low velocity impact signatures with that of humans would build a strong case for the necessary high velocity impacts in that model.

Four swine weighing from 48 to 75 kg were impacted in four locations each (sternum, lateral chest, abdomen and hind leg) with and without body armour while under anaesthesia. Five paintballs were fired at each location for a total of forty shots per pig (4 locations x 5 shots with body armour plus 4 locations x 5 shots without body amour). Eight piezofilm sensors were attached to the pig’s back in two columns of four, symmetrical about the spine. The amour/non-amour portions of the testing were randomized, as was the shot order in each portion. However, all 20 shots were fired before changing into or out of the body amour. Similarly, all five shots per position were fired before changing to a different impact location. The animals were fitted with older versions of aviation flak jackets for these tests. A total of 1280 impacts recordings were acquired (4 pigs x 4 locations x 2 body amour x 5 shots x 8 sensors). Analysis revealed that while impacts were discernable for almost every sensor and every shot, many of the impact recordings were too low in amplitude and not similar to the non-lethal impacts seen in the proof-of-concept work. Load cell analyses of the paintball impacts show forces that are 25 times less than the solid steel balls used in the non-lethal phase. Calculated values for the non-lethal projectiles range from 46 to 66 Joules, at the velocities (250 – 300 ft/sec) used in the protocol. Paintball impacts can be calculated at 8 Joules (using 3 g and 250 ft/sec); however, this calculation does not consider the work expended as the paintball breaks upon impact. Paintball impacts were measured using a load cell at 2 Joules. It is suspected that the difference in the force of the impacts does not cause the characteristic impact signatures of the non-lethal wounding studies. A typical lateral chest with amour signature is shown in Figure 5.

The signature in Figure 5 can be broken into three separate sections: the pre-impact section from 0 to 9 ms, the impact section from 9 to 12 ms and the tissue displacement section from 12 ms on. With no way of determining the actual time of impact of these recordings, we believe that the pre-impact section of the signatures represents the response from the air blast of the paintball rifle when fired. The impact section corresponds to the shock wave section in the non-lethal wounding signatures. Due to the diminished force of impact of the paintball, the impact section duration is shorter than the non-lethal signatures. The diminished force of impact also causes the amplitudes of the tissue displacement wave to be much lower than the non-lethal recordings. It should be noted that a plywood baffle was used in the non-lethal wounding protocol to dissipate the air blast from the gun to prevent the chronographs from prematurely actuating. No indication of the air blast is evident in those recordings.
Figure 6 shows a typical response for all but the closest sensors for shot locations in the abdomen, sternum and hind limb. The tissue displacement phase of the signal is present albeit very low in amplitude, but no discernable shock component of the signal is present. It is not surprising that the high frequency ‘shock’ components are lost over time and distance. The body’s elastic and dampening nature acts to filter higher frequencies faster. Work remains to be done to characterize this phenomenon and use it to determine location of the impact.

![Figure 6](image)

Signals from all sensors were digitally recorded at 50,000 samples per second on each channel with a 10,000 Hz anti-aliasing filter. Analysis of the lateral chest shots of the pig (both amour and non-amour) revealed that the predominant shock frequencies occurred in the range of 300 to 700 Hz. Fast Fourier Transforms were performed on the shock section of each signature. The first 120 points of the shock section was zero-padded to 1024 points. The FFT returned 512 frequency coefficients over 25,000 Hz range for a resolution of 48.8 Hz per coefficient. The top two frequencies were recorded based on amplitude for each FFT. Figure 7 shows the FFT for the shock wave portion of the signal shown in Figure 5. It was quite common to see the double peaks shown in Figure 7. These peaks are considered to be harmonics.

![Figure 7](image)
As stated above, while there were eight (8) sensors used on every pig for each shot, many of the recorded signatures were not of a high enough quality to perform analysis. Ninety signatures were examined for the case with body amour. These data represent signatures from sensors 1, 2, 3 and 4 for all 5 shots on all 4 pigs and sensors 5 and 6 for 5 shots each on 1 pig. Sixty (60) signatures for the unamoured case were examined. In the unamoured case, there are poor quality signals (and thus unanalyzed) on sensors 3 and 4 for pig P379 and sensor 2 for pigs P377 and P378. In the unamoured case, only P337 had 20 analyzable signatures from sensors 1 through 4. In 93% (84 of 90) of the impact signatures, one of the top two frequency peaks fell in the range between 317 to 951 Hz in the armoured cases. In the unamoured cases, 83% (50 of 60) of the signatures had one of the top two frequencies in the range of 316 – 781 Hz. Table 2 shows the averages for each pig at each sensor for the five shots at the lateral chest location while wearing body amour. In the cases where there was no top frequency in the 300 – 1000 Hz range, the most significant peak in that range was selected and the amplitude noted.

<table>
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<tr>
<th>Pig ID</th>
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Table 2

The location of all lateral chest shots was on the right side on the animal approximately equal distance from sensors 1 and 3. While the frequencies are tightly grouped, the amplitude for the sensor 2 shots seems too high. This sensor is on the far side of the animal and logically should be lower in amplitude then sensors 1 and 3. The amplitudes across the pigs vary greatly for sensor 2: P337 and P377 are under 100, while P378 and P379 are over 200. Removing what seems to be an outlier, P378 with amplitude of 531.20 from the average yields a revised amplitude average of 133.7 (St Dev 76.7). Similarly, sensor 1 for P377 has what seems to be
Development of a Ballistic Impact Detection System

abnormally low amplitude given the fact that it is closest to the shot. Removing these shots from the overall average yields a revised sensor 1 average of 222.9 (St Dev 65.3). The coupling of the sensor to the body surface remains the largest variable to overcome. The snug fit of the body armour stabilizes the sensors and their response. The data from the armoured pigs are more consistent than the unarmoured data. However, there seems to be too little coupling in the case of P377 sensor 1 resulting in low amplitude and too much coupling in P378 sensor 2 resulting in high amplitude. The data does show consistency between shots at each sensor location. This consistency is evident in the abnormally coupled shots as well. One question prior to this study concerned the response of the tissue over the course of five shots in the same location. There is no significant deviation in frequency or amplitude over the five shots.

The four animals were sacrificed and kept frozen at -20 °C for a month at which time they were thawed and impacted again. The freeze/thaw cycle was meant to emulate the circumstances that a fresh frozen human cadaver would undergo. After thawing, the animals were impacted using the same format described above. Table 3 shows the results of the lateral chest shots with body armour, comparable to the live animal impacts summarized in Table 2. Immediately noticeable in the cadaver impacts was the lack of analyzable signatures in channels 2 and 4 in some of the animals. These are the sensors on the far side (non-shot side) of the pig. This loss of signal occurred in pig 379 (channel 2) and pig 337 (channel 4) with body armour, but was more prevalent on the non-body armour shots (not shown). Of those eight signatures (4 pigs channel 2 and channel 4), only pig 377 channel 2 was of sufficient signal strength and quality to analyze. Overall the top frequencies are very comparable to those of the live impacts. While the frequencies are remarkably similar, the amplitude of the cadaver signals is dramatically lower across all four sensors. Signal strength in the live animals ranged from 160 to 230 (except for sensor 4), signals in the cadavers were markedly lower: the closest sensor registering an average of 65 and sensors 2 and 3 registering 26 and 24 respectively. Sensor 4 is again understandably lower than the other sensors as it is the farthest from the shot in both scenarios.

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Table 3
The averages for sensors 2 and 3 are very similar that is counterintuitive since sensor 3 is much closer to the impact. This discrepancy can be accounted for since the unanalyzed signal from Pig 379 sensor 4 was not included in the average coupled with the unusually low signal recorded on sensor 3 in Pig 337. This test shows that while the key frequency components are still discernable, allowances for lower amplitudes must be made in the frozen/thawed cadaveric tissue.

Three human test subjects volunteered for the paintball impact testing. Each subject received eight impacts, four with body armour and four without body armour. Subjects received impacts in the abdomen, lateral chest with and without the amour and received two extremity shots (one arm and one thigh) wearing armour and then two more extremity shots (opposite arm and opposite thigh) while not wearing the amour. Sensors were placed on the back of the subjects and fixed with adhesive tape similar to the pigs as shown in Figure 8. Subjects wore jacket-style NATO body armour for this test. In general more of the human impact locations could be analyzed. With the exception of the sensors farthest from the extremity impacts (e.g. sensors 1 and 2 for the leg impacts), FFT data from all eight, impact locations were computed.

Figure 9 shows a representative left lateral chest impact with body amour signal from sensor 2. It should be noted that the small pre-impact waves that were visible on the swine recordings are not visible in the human recordings. As previously stated, these small waves are believed to be artefact from the air blast of the gun. Since the human subjects stand behind protective plywood it seems logical that the air blast is dissipated.
The human impact signature in Figure 9 seems to have shock and tissue deformation components corresponding to the swine recordings. Figure 10 shows the FFT performed on the impact shown in Figure 9. FFT analysis on the shock portion of the recordings similar to those performed on the swine impact recordings for the lateral chest impacts are summarized in Table 4.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Average</th>
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<td>Frequency</td>
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<td>215.4</td>
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<td>259.7</td>
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</table>

Table 4
Development of a Ballistic Impact Detection System

The frequencies noted in Table 4 are all (100%) one of the top two frequency peaks in the FFTs. The lateral chest impacts for Subjects 1 and 2 occurred on the left side, therefore the even numbered sensors (2, 4, 6, and 8) are closest to the impact. This is clearly evident by the amplitudes of the peaks for subject 2; however, not as clearly the case for subject 1 as sensors 4 and 6 have lower amplitudes than what might be expected. In both cases the amplitude for sensor 1 seems lower than expected. The lower amplitudes have caused speculation that the upper two sensors, located directly below the scapulae, are not coupling as well due a shielding effect of the scapula. The protruding scapula may be preventing close contact of the vest and the sensor unit. The impact to Subject 3 was on the right side meaning that the odd-numbered sensors were closest to the impact. Subject 3 has very low amplitudes on the whole indicating either poor coupling between the sensors and the body or better protection from the body armour. In fact, the signals from sensor 2 and 4 were so weak they were not analyzed. Overall to this point, the peak frequencies of the human subject correlate well with the swine frequencies. The average frequency for the human lateral chest shots with body armour is 437.1 Hz with a standard deviation of 78.6 (n=22). The average frequency for the swine lateral chest shots with body armour is 471.1 Hz with a standard deviation of 122.0 (n=90).

2.2 High velocity impact models:

Similarities between the low impact swine and low impact human signatures provided the needed impetus to perform high velocity swine impacts. A protocol was written to perform a limited number of shots using two calibre bullets (7.62 M80 ball and 5.56 M855 ball), four locations (sternum, lateral chest, abdomen and hind limb) and three velocities (2800 ft/sec, 2300 ft/sec and 1300 ft/sec). The impact schedule is shown in Table 5. It was important to test a combination of threats facing the soldier today. Given that resources were limited, certain tradeoffs were made. The velocities were chosen to reflect an AK47 muzzle velocity (2800 ft/sec), an approximate 200-yard rifle engagement (2300 ft/sec) and handgun velocity (1300 ft/sec). The M855 round (5.56 mm) is a standard NATO round and represents the trend of reducing the bullet calibre and total round weight to gain higher velocity and decrease soldier basic ammunition load weight. This smaller calibre provides information about smaller high-velocity fragmentation munitions impacts (e.g. howitzers, mortars, grenades, etc). The chosen locations reflect the desire to maintain consistency with the low velocity protocols. However in this study, body armour was used for all impacts. The targeted population for the BIDS is the frontline combat soldier. Projected warfighter designs call for body armour. It was important in the low velocity impact study to relate back to the proof-of-concept work originally done without body armour. Therefore low impact tests were conducted wearing body armour and without body armour. The high impact tests do not need to relate back to previous low impact tests since results from these high impact tests alone will be the basis for the BIDS circuitry. Interceptor body amour from Point Blank with SAPI and Gamma Plus ceramic plates were used for this study. Sternum shots were fired into the ceramic plates of the vest, abdomen shots were fired into the Kevlar outer tactical vest just below the ceramic plates, lateral chest shots were fired into the Kevlar outer tactical vest and hind limb shots were fired into the unprotected thigh of the animal. The ceramic plates, which are rated to protect against 7.62 rounds at 2800 ft/sec, defeated all rounds at all velocities, although permanent backface deformations of approximately 1 cm were created at the high velocities. The Kevlar outer tactical vest is rated to defeat handgun rounds; however, the high ogive of the rifle rounds allowed all rounds to penetrate the Kevlar. A pilot study was performed to determine if freshly euthanized animals could be used instead of live anesthetized animals. Lateral chest shots using 7.62 rounds at 2800 ft/sec from six live anesthetized animals were compared to animals that were euthanized minutes before the impact. As in previous experiments eight sensors were placed equidistant about the spine in two columns of four. Unlike other experiments, these tests employed six newly design piezofilm sensors as well as two of the older style bone conducting sensors. Unfortunately, the new sensors were not as responsive as the older bone conducting ones and have been left out of the analysis. The results from these tests will be from signatures recorded from the two bone conducting sensors. Figure 11 shows typical impact signatures from the pilot study. The graph on the left is a 2800 ft/sec, 7.62 round, left lateral chest impact from a live, anesthetized animal. The graph on the right is the same parameter from a freshly euthanized animal.
<table>
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<th>Location</th>
<th>Round</th>
<th>Amour</th>
<th>Velocity</th>
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<th>Location</th>
<th>Round</th>
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</table>

Table 5
Similarly Figure 12 shows the frequency spectrum of the above impact signatures: live on the left and freshly euthanized on the right. The FFTs below are 1024 point FFTs using 75 data points (1.5 ms) and zero-padding. The y-axis is always in arbitrary units which can be compared between graphs in which similar processing has been performed. After completing the pilot study, the remaining animals were impacted directly after euthanasia. It should be noted that the sensor used in these recordings has a particular resonance at 17,000 Hz explaining the large frequency response in that area on the graphs in Figure 12. While there was some signal present in that frequency region, care must be used in characterizing the frequency response of the sensors as it affects the analysis of the impact signatures.

Forty pigs ranging in weight from 41.8 – 74.9 kg have been used in the testing to date. In an effort to conserve animals, most animals were shot twice. Shots to the hind limb and lateral chest were executed serially (hind limb first) while the pig was in a recumbent position. Similarly, abdomen and sternum shots were executed will the animal was suspended in an upright position. Because the shot distal to the sensors was carried out
first, it is assumed from visual inspection that damage to the animal was not sufficient enough to negatively impact second shot signature.

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<th>Velocity (ft/sec)</th>
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<th>Ch 5 Amp</th>
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<th>Ch 6 Freq</th>
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Table 6 shows the frequency component analysis of the high velocity impacts on the swine. The table shows the FFT analysis of the first 7.5 ms of the shock wave. While eight sensors were affixed to the animal, this
The table represents the signals from two sensors, channels 5 and 6. For impacts to the lateral chest and the hind limb, channel 5 was closest to the entry wound and channel 6 was closest to the exit wound. For sternum shots and abdomen shots the sensors were equidistant from impacts. As previously mentioned there was no penetration of the ceramic ballistic plates for sternum shots. There were exit wounds for all other shots except some of the 5.56 calibre shots at 1300 ft/sec. In the analysis in Table 6, the frequency between 350 Hz and 1000 Hz with the highest power was recorded in this table. In many cases this was not the highest powered frequency in the spectrum. For channel 5, in 53 of the 69 shots, the peak in this 350-1000 Hz range was not the frequency of largest power. In 8 of the 53 cases the largest frequency peak was higher than 1000 Hz and in the rest of the cases the highest power was below 350 Hz. For channel 6, roughly half of the highest powered frequencies fell into the 350-1000 Hz range (33 out of 69). This discrepancy seems to be due to the shape of the bullet (i.e. the ogive). The high ogive of the rounds, necessary for stability during supersonic flight, penetrates the skin without causing the impact expected for a projectile of such weight and velocity. The spherical shapes used in the proof-of-concept less than lethal tests produced a more distinct shock frequency that in every case produced the highest powered frequency in the 500-1000 Hz range. With rifle bullets, the shock wave epoch is much shorter, on the order of 3 ms. Therefore FFT analysis over 7.5 ms tends to include more of the lower frequency tissue displacement segment resulting in high powered lower frequencies. Also of note is the discrepancy in power between channel 5 and channel 6 in the lateral chest shots. In almost all cases, the channel 6 frequencies are markedly (at least 10 times) higher than their counterparts from channel 5. Only in the 5.56- calibre shots at 1300 ft/sec does this tendency reverse. Almost everyone is familiar with the size difference between the entry and exit wounds from a gunshot. There was no exception to that understanding in these tests. As the bullet passes through the body it tumbles creating a large exit wound. While the entry wound was rarely much larger than the calibre, the exit wound was normally 4+ cm in diameter. This is presumably the cause of the higher powers noticed on the exit side (channel 6). In the cases of the low velocity 5.56 round impacts on the lateral chest there are no exit wounds resulting in a much lower power than the entry side.

2.3 Blast Overpressure Impacts

In July 2002, testing was conducted under an approved human use protocol at the Aberdeen Test Center (ATC) to assess the effectiveness of blast suits against anti-personnel mines. This was a multi-agency/service effort involving ATC, the Medical Research and Materiel Command, the University of Virginia, the Walter Reed Army Medical Center, the Uniformed Services University of the Health Sciences, and the Armed Forces Institute of Pathology, which was funded by the Communications and Electronics Command, Humanitarian Demining effort.

Mine surrogates containing 100 and 200 g of C4 explosive were used against cadavers with and without the blast suit. Sensors were affixed to the cadavers using superglue in the same configuration as Figure 8. Signals were recorded at 50,000 samples per second on each channel. Figure 13 shows the impact signal from a 200-g blast with a blast suit. The subject’s nose was 55 cm (measured radially) from the centre of the mine surrogate. The response from the sensor seems to be that of a second-order system in response to a step function. Figure 13 shows a longer duration event lasting well beyond 400 ms. Examination of the recording indicates higher frequency components for the first 150 ms and slower frequencies after 150 ms. It is likely that part of the slower frequency waves are made up of the tissue deformation waves. An FFT on the first 170 ms of the blast is shown in Figure 14. Much of the response to this type of blast is in the lower frequencies, less than 200 Hz. This seems to indicate that the surface of the body couples with the primary low frequency blast wave. However there are significant components in the frequency range (400-1000 Hz) identified in the impact tests that can be exploited by a detection system. For clarity, a partial spectrum (0-10000 Hz) is shown.
Development of a Ballistic Impact Detection System

Figure 13

Figure 14
2.4 Normal Activity

Data was also acquired for simulated normal activity to determine key characteristics of signals from running, hopping and a significant jolt. Human data was collected while the subject ran and hopped in place. The jolt signature resulted in a jump off a 36-inch table. Figure 15 is the resultant signal from the 36-inch jump. Figure 16 is the frequency domain spectrum produced by the FFT. The frequency spectrum shows that the jolt to the body produces two significant frequencies: a larger amplitude component at 293 Hz and a smaller but significant frequency at 586 Hz. This latter frequency is in the range produced by the bullet. Of the three ‘normal’ recordings, only the big jump proved to contain frequencies in the range of those produced by the bullet impacts.

3.0 BIDS DESIGN

The BIDS design requirements centred on reducing false-positive indications to near zero. It would be approaching impossibility to completely rule out false-positive indications due to the number of ‘normal’ tests on all body types necessary. Because the BIDS was soldier-born, they were always requirements of near-zero cube, weight and power. An analogue based system was designed based on the proof-of-concept data. Testing from the proof-of-concept phase indicated that discrimination could be achieved by isolating frequencies in the 400-1000 Hz band. If these frequencies met a threshold voltage requirement, an impact criterion would be met. While high velocity swine tests corroborated the earlier proof-of-concept tests in terms of the frequency range of interest, it was decided to employ a high-pass filter for the circuit.

The circuitry for BIDS could be purely digital in nature or an analogue-digital hybrid. In its current embodiment, the circuitry is primarily analogue with a digital output that is compatible with computing devices. The low power analogue components allow the system to be ‘on’, in a listening mode, continuously. To meet power requirements, a digital system would have to incorporate sophisticated wake-up circuitry which would allow the microprocessor to reside in a sleep state until a signal of interest required processing. The current embodiment is described below and shown in Figure 17. The BIDS consists of two sensors that couple to the body in such a way as to sense the vibrations in the skin. The sensors are piezo-film mounted on a flexible substrate of Mylar plastic. The vibrations produce a voltage commensurate to the frequency and amplitude of the vibrations. Each sensor signal is processed in similar circuit sections. All sensor signals are ultimately fed into logic circuitry that makes a determination as to the location of the impact. Two circuit sections are shown below, one section will be described here.
The voltage signal is conducted to an input buffer amplifier. The signal then passes through a high pass filter. The current embodiment uses a 3-pole Bessel with a cut-off frequency of 5000 Hz. While much of the work to date indicates that telltale frequencies exist in to 400-1000 Hz range, it is impossible to create an analogy filter with such a narrow band pass. The 5000 Hz filter is 42 dB down at 1000 Hz and 60 dB down at 500 Hz. We’ve found that there is enough frequency information passed by this filter to adequately discriminate the impact signals collected to date. The signal is then conducted to a full-wave rectifier which converts the voltage from bipolar to only positive. The signal then passes through a 3-pole 1000 Hz low pass filter which widens the voltage peaks of interest. The signal is then conducted into a logarithmic amplifier. The output of this amplifier stage is a voltage equal to the log of the input voltage. This stage prevents a large signal from saturating the next stages. Saturation would cause loss of frequency information that could lead to false-positive impact determinations. The next stage is a peak hold circuit which determines and conducts the peak voltage in the signal to the threshold circuit stage. The threshold section compares the peak voltage of the signal to a threshold reference voltage. If the signal voltage is higher than the threshold reference then an impact has occurred. The original signal voltage is passed to the logic that determines location. The location logic compares the amplitudes of all the sensors to make a location determination. In the current two-sensor embodiment, if the amplitude signals from the two sensors are less than a 2:1 ratio and at least one sensor signal meets the threshold voltage requirement, the impact is deemed to have occurred between the sensors, or in a centre location. If the ratio of the signals is greater than 2:1 and the greater signal also meets the threshold voltage requirement, the location is deemed to be distal to the sensor with the greater amplitude signal. Thus in the two sensor embodiment, three locations are possible: centre, right and left. The voltage signals for these location outputs are latched and then available to be read or transmitted to a computer. Once read, the BIDS accepts a reset voltage signal which returns the location outputs to ground. In its current embodiment, the BIDS circuitry can distinguish one impact in each location until a reset is received. The current analogy BIDS circuit measures 1.5 square inches and requires approximately 600 microamperes of current at 3 volts. Bench testing of the BIDS circuit consisted of converting the digital impact signatures in analogy voltages and feeding them through the circuit. The impact threshold settings were set to discriminate between the swine impacts and the normal human movement signatures. Setting the threshold is somewhat arbitrary since the voltage at that point is dependent upon the initial amplification from the input amplifier. More important is the relative voltage levels between the smallest detectable impact and the largest normal movement signal. Figure 18 shows a comparison of two signals that have been filtered using the 3-pole 5000 Hz high pass filter in the BIDS circuit. The blue trace is the time domain signal from a hind limb, 5.56 calibre impact at 1300 ft/sec; the red trace is the time domain signal from the big jump in Figure 15. Figure 18 shows the ability to easily discriminate the weakest bullet impact recorded from the strongest ‘normal’ activity recording.
3.1 Future Work

The current embodiment of BIDS will be integrated into the WPSM and tested for false-positive indications during normal soldier activity. In parallel, validation testing using human surrogates will begin with the low impact paintball model validation studies. If the model is adequate, high velocity impact testing will be initiated.

A further aspect of BIDS is linking the impact detection with the wounding severity to provide the medic with as much triage information remotely as possible. Pathology reports for all the high velocity impacts conducted will be analyzed in conjunction with the impact signatures to establish a relationship between the impact signature and the resulting wound. In the future it is anticipated that the BIDS can be based on a digital signal processor which would sample the incoming analogy voltage from the sensors, perform Fast Fourier Transforms and power analysis on the signals to better determine impacts and locations. It is quite possible that sophisticated algorithms could tell from frequency analysis whether bone has been struck and from power analysis whether an exit wound exists as well as better wound localization. In this digital embodiment, BIDS could track multiple impacts in the same general location, as well as potentially providing indication of wounding severity.
4.0 REFERENCES:


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Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals and experiments involving animals and adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council.

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3), AR 360.5.
Life-Signs Determination Model for Warfighter Physiological Status Monitoring

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ABSTRACT

The U.S. Army is leading an effort for a Warfighter Physiological Status Monitoring (WPSM) system that interprets data from a suite of wearable physiological sensors to infer a soldier’s current health status on the battlefield. The future WPSM system will consist of a body-worn network of biosensors with a central processing control unit whose firmware contains a probabilistic Bayesian Network for assessing the soldier’s physiological status. The Bayesian Network will assess the status of the soldier in terms of Life-Signs Presence, Absence or Unknown (PAU) state. Together with this health status assessment, another goal of the Bayesian Network will be to assess the related level of confidence in the diagnosis as resulting from clinical uncertainty, sensory information patterns and reliability of the hardware. This information will be made available to field medics and others over separate communication channels, in order to help prioritize the urgency of medical assistance and evacuation. This paper describes the current development of the PAU Determination Model, which demonstrates the various techniques that will be adopted in the final version of the Bayesian Network to fulfill the health status assessment goals and highlights the robustness of the approach.

1.0 INTRODUCTION

Perennial objectives of battlefield force health protection include:

- The reduction in mortality and morbidity rates,
- The enhancement of force effectiveness by reducing the likelihood of non-battle injuries (such as heat stroke and acute mountain sickness), and
- The improvement of casualty management in remote situations.

Many technological advances in body-worn sensory devices have made these goals realizable. Since the first hour after injury is crucial [1], the ability to rapidly locate, triage, diagnose, and render appropriate initial
Life-Signs Determination Model for Warfighter Physiological Status Monitoring

treatments are vital to improving the outcomes of battlefield injuries. In the near future, it will be possible to identify in real time the wounded soldiers on the battlefield who require immediate assistance, even when they are greatly dispersed and out-of-site, which could result in a reduction in battlefield morbidity and mortality.

In response to this challenge, the U.S. Army is developing a set of computerized devices as part of future combat systems, comprised in the Warfighter Physiological Status Monitoring (WPSM) system [2]. The system will feature a configurable array of miniaturized and computationally capable sensors. Among them, physiological sensors will monitor heart and breathing rates, metabolic energy expended while working or marching, skin and core temperatures, activity patterns (body positioning: upright lying face-up or face-down), and several other parameters. These sensors will transmit physiologic information to a small central processing control unit carried by the soldier, where appropriate Bayesian Network model will perform higher-level data analysis. The resulting assessment of the soldier’s status will be then made available to the field medic and upper echelons of care, as well as small unit leaders and commanders, as required. To allow quick localization, the uniform will also feature global positioning system capabilities.

Among the assessment capabilities featured by this system are a series of Life Sign Decision Support (LSDS) algorithms. The LSDS algorithms process the sensory data streams and produce meaningful information to help combat medics assess, triage, and manage life-threatening injuries. Specifically, as a primary indicator, a Life-Signs (PAU) status is estimated by these algorithms and transmitted to the field medic or other desired locations as part of the output provided by the WPSM system.

Due to the critical nature of life-signs determination, a key requirement is to reach a high level of confidence in the reliability of the PAU assessment. This means that the system must perform a statistical evaluation of the accuracy of the incoming signals as well as a probabilistic interpretation of the soldier’s physiological state. As appropriate for the PAU status determination, these specific algorithms are not concerned with the future state of a wounded soldier but rather are concerned with the use of the present signal information and the associated uncertainties to determine the most likely current state of the soldier. To achieve this result, the LSDS algorithms perform a temporal analysis of the sensory data, including the arbitration of contradictory information, processing of multiple sensors, and performing non-monotonic reasoning on the collected data. The algorithms take into consideration the various elements of data imprecision, as derived from possible sensor/device faults and data transmission failure. They also assess the reliability of the integrated array of sensors and devices by taking into account the probability of failure of each component as well as the probability of failure of the entire sensor array and data transmission system as a whole. All this information on the data imprecision and system reliability is finally merged as part of a diagnostic model that represents both data imprecision and clinical uncertainty pertinent to remotely determine the life-signs status of a soldier. When the system cannot reach a definite determination, the algorithms will report an Unknown condition.

These diverse results can be achieved using the Bayesian Network (BN) probabilistic modeling method [3]. BNs are used to develop knowledge-based applications in domains that are characterized by inherent uncertainty. BNs provide an organized representation of knowledge resulting from the combination of human expertise and statistical analysis. BNs also accept real-time information that they use with the stored knowledge in order to formulate diagnostic or predictive conclusions.

In the case of LSDS, a set of BN models has been developed to satisfy our diagnostic goals. One set of BNs model the behavior of the sensory system, the influence on the sensors of several external factors (e.g., temperature, vibration) and sensor reliability. These sensor models appropriately analyze the incoming sensory data streams, identify possible inconsistency patterns and evaluate the “health” of each sensor in the WPSM system.
These processed data streams are then forwarded to the PAU Determination Model (PAU-DM). This model incorporated through another set of BN modules the heart of the LSDS algorithms and reproduces the human inference process for PAU determination. A detailed description of the architecture of the PAU-DM subsystem is presented in [4]. In this paper, we provide an overall description of the PAU Determination Model and future research directions.

### 2.0 METHODOLOGY

The PAU-DM will be continually evolved by cycling through stages in an iterative fashion. The first iteration, called “Phase I”, which is featured in this article, was intended to highlight the robustness of our approach and demonstrate various techniques that will be adopted in the second version of the system, called “Phase II”. In Phase I no claim is made about the correctness of the health status assessment performed by the model. In fact, it is understood that the Phase II model, currently under development, will extend the Phase I model by incorporating sound medical expert knowledge of the human physiology.

The Phase I model can generate a series of ancillary assessment conclusions. These are provided to illustrate how the approach can be used to deliver “amber” outcome warnings indicating that critical situations exist that require immediate intervention. We show that, as a by-product of exploiting appropriate steps in the PAU determination process, a set of indicators can be used as alerts for triggering the medic’s attention/intervention. Future releases of the model will include indicators that recognize meaningful situations for use in first-level triage, thus helping the medic establish a weighted priority for providing assistance of the injured soldiers.

The information used for the Phase I PAU-DM was gathered from literature, legislation and consultation with a medical doctor experienced in emergency room trauma situations. A meticulous search was conducted in Phase I for existing procedures or algorithms for determination of death [5] from a remote location. No appropriate models were found. For example, to establish a legal final determination of death, a visual inspection by a physician over a period of time is always required [6]. When determination of brain death is involved, there are also standard procedures that require a medical facility several hours to complete [7]. Since the brain death determination procedure is used to establish death for subjects whose cardio-pulmonary activity has been artificially maintained, it is inappropriate to determining the death of a soldier who has been injured out in the battlefield and not directly helped by a medic. The intent of PAU-DM is not to establish a clinical assessment of death, but rather to be used as a tool in estimating if a wounded soldier has life signs present and thereby helping triage prioritization in operational settings.

The above considerations suggest that it is not possible to establish determination of death with absolute certainty by remote sensor measurements alone, at least from a legal standpoint. In fact, while the lack of both heartbeat and breathing are excellent clues of possible death, they are not conclusive for timely determining the incipience of death. Many other considerations need to be taken into account, including time elapsed, ambient temperature and drug intake among others. Nevertheless, a sensory system can provide reliable information on possible death (absence of life signs) or extreme physical distress that requires immediate attention/medical intervention. In the latter case, it would raise a warning before an irreversible condition is reached.
2.1 Modeling Framework

To capture medical assessment expertise for PAU determination, we made use of the Bayesian Networks probabilistic framework [3]. BNs provide a method to represent interdependencies between variables that represent elementary chunks of knowledge, even if the relationships involve uncertainty, unpredictability or imprecision. The relationships may be learned automatically from data files, constructed from experiments or other data, created by an expert, or developed by a combination of these approaches. BNs are used to develop knowledge-based applications in domains that are characterized by inherent uncertainty. A BN allows us to combine prior knowledge and incoming data with the likelihood of a hypothesis of interest, such as a soldier being dead given his/her physiology and the sensory data-time series.

In a BN, the problem domain is modeled as a set of nodes interconnected with arcs to form a directed acyclic graph. Each node represents a probabilistic variable that can take two or more possible values. The arcs signify the existence of direct influences between the linked variables, and the strength of each influence is quantified by a forward conditional probability. Bayesian Networks do not use “algorithms” in the conventional procedural sense. Rather they give a probabilistic association between an assembly of input variables, as established by experts in the field, as to the relative influence of these variables on the outcome of any given node in the network. The key to this association is the conditional probabilities assigned by the experts to each incoming variable on the state of the receiving node. These conditional probabilities relating the variables to the output state of the node are defined in Conditional Probability Tables (CPTs). Each node in the network has a CPT describing the relative dependence of that node on its parents. Therefore, a CPT is used to define the conditional probability (or likelihood) of a specific value of a variable based on combination of states of the parent variables. A typical BN and some of its CPTs are shown in Figure 1 above.

The time dimension can be handled in a BN by resorting to two strategies. The first strategy is to introduce explicit time variables, starting from the moment a specific condition or set of conditions occur. Examples of this approach in our PAU-DM network are a variable representing time elapsed since the heartbeat stopped and one indicating when breathing has stopped.
The second strategy handles time in an indirect way. This leads to “Dynamic BNs”. In this case, time is
represented by discrete values and a model is created to represent the status of the world at a given time-slice.
Time is implicitly represented by specifying how one set of variables at a given time-slice is affected by
another set of variables from the preceding time-slice. While this is a more elegant approach, it has the
drawback of slowing down the inference process. Care must be taken in order to balance cost and benefits.
An example of this second approach in our PAU-DM network is the modeling of the dynamics of oxygen
saturation. Since we use both strategies to represent time in our model, the mix between the two methods has
been selected so to minimize the interdependence between subsequent time slices in order to improve the
speed of inference.

2.2.1 Why use a BN Framework?
The PAU Determination Model utilizes a BN used to infer the PAU status of a soldier using the information
provided by the various sensory outcomes. The reason for using the BN framework comes from the need for
representing and handling the uncertainty, or level of confidence, in the data streams. The BN framework
naturally handles this issue and allows us to merge data uncertainty with clinical uncertainty in order to derive
a final confidence level in the derived soldier’s health status.

The clinical uncertainty stems from the need for representing medical expertise. Since no procedural
algorithm exists for remote death determination, our goal is to reproduce the reasoning process of physicians
that are expert in trauma and emergency procedures. This kind of knowledge is best expressed through a
probabilistic framework, since the experts themselves are not able to conclude a definite diagnosis for a
combination of sensory data. That is, they are not able to easily classify all the possible combinations of data
streams into three precise classes, i.e. PAU.

To illustrate, suppose that a subject has been experiencing a lack of circulatory activity for two minutes. The
experts we consulted were unable to say whether the subject is either definitely alive or definitely dead, since
several contextual, and often intangible factors are not detected with the remote sensory system that would
influence this conclusion.

Using this approach, the experts in our team agreed on the fact that a subject is 50% likely to be dead after
two minutes of lack of circulatory activity. After another minute, this likelihood may rise to 95%. Finally,
they felt comfortable in stating that the subject is definitely dead (100% likely) after a total of six minutes of
lack of circulatory activity. All these rules are considered valid unless the subject is experiencing
hypothermia. The BN framework has explicitly been devised to capture this kind of non-deterministic
reasoning. As discussed in the previous section, it also provides a good level of flexibility in handling the
time dimension.

Another issue related to clinical uncertainty is that the set of possible combinations of data streams can be
quite large. This makes impractical (and possibly unreliable) to map all the possible combinations into only
three categories such as PAU. On the other hand, the BN approach allows us to break down this classification
into a combination of much simpler processes that reproduce the human reasoning activity in the specific
domain. This has two beneficial effects. The first one is that we need not explicitly encode the full mapping
of all the possible combinations of data streams. In fact, this mapping will automatically emerge as a
combination of the various simpler reasoning processes. In this way, we can greatly simplify the modeling
activity and obtain significant savings in computational resources. The second benefit is that, by reproducing
the human reasoning process, every step of reasoning will have a clear meaning and adjustments can be made
by focusing on narrow aspects of the problem at a time.
The BN framework also offers a natural approach for taking into account the effects of data uncertainty in the diagnostic process. For example, suppose that we are not sure whether the heartbeat actually stopped beating two minutes or three minutes ago, given the received data streams and the reliability of the hardware. In fact, although the heart rate data stream indicates that the heart stopped two minutes ago, we may have clues that immediately prior data were unreliable. Even if in that minute the heart rate was reported as present, it is possible that it was actually absent. We will therefore provide the PAU-DM network with a likelihood distribution for the heart rate being absent. This distribution may indicate that the two-minute absence is 80% likely to be correct, and the three-minute absence is 20% likely. This kind of information, called evidence, will propagate through the PAU-DM network and merge with the domain knowledge that is encoded in the model. This propagation, called inference, will result in an overall likelihood of the subject being alive. This overall likelihood will then take into consideration the various possibilities regarding the absence of heart rate, and substantially weigh the effect of each one on the final assessment.

The advantage of the BN framework is that it provides a principled way for handling the non-deterministic knowledge described above. Furthermore, the inference process is mathematically exact, in the sense that it obeys the laws of probability theory. This ensures that the outcomes of inference will be always coherent, that is, they do not violate common sense logic. Finally, the graphical approach at the basis of the BN framework is also intuitive. This feature greatly helps the task of knowledge elicitation, since the experts can easily understand the basics of the formalism and contribute directly to the development of the networks.

3.0 THE PAU DETERMINATION MODEL

For its health assessment, the PAU-DM network receives measurements from a human subject. Each measurement follows the path shown in Figure 2 before reaching the network. A Human Subject utilizes a physical sensor represented by the Physiological Sensor box. The sensor performs a specific measurement such as heart rate. The resulting measurement is fed into a Pre-Processing module that performs a series of operations such as computing the average heart rate occurring in the last minute, or counting how much time has elapsed since the last heartbeat was present. The Pre-Processor also translates the resulting quantity into a format that can be used by the next step in the chain. Finally, the pre-processed data enter into an appropriate Sensor Model, encoded with a BN. Here the data are analyzed and conditioned by the sensor reliability, and possible sensor failures are detected. The resulting information is then delivered to an appropriate node in the PAU-DM network.

The PAU-DM Bayesian Network is shown Figure 3. It encompasses 4,343 probabilistic rules relating 45 variables. This initial model may not be clinically accurate. It is rather intended to represent a reasonable behavior with the goal of illustrating how a BN can be used to solve our diagnostic problem. A more realistic health state assessment model is now under development using the techniques that we are about to illustrate.
Figure 3: PAU Determination Model.
A general remark on the model is that it represents the subject’s status in a one-minute time window. Several variables in the model refer to a one-minute average. For example, the $\text{HeartRate Mean T1}$ variable at the center top of the figure stands for the average value of heart rate in the last one-minute interval. Other variables refer instead to persistence of conditions that can stretch far beyond the last minute, such as $\text{TimeNoHeartbeat T1}$. This variable in fact represents how long the subject has shown no sign of heartbeat.

With this model, a non-deterministic PAU diagnosis can be performed and simple “amber” outcomes can be synthesized as a by-product. An “amber” outcome is an alert that can be used to warn about a critical situation that requires a medic’s immediate attention and possible intervention. For example, an oxygen saturation level below 50% sustained over a period is deemed no compatible with life. This oxygen saturation level will therefore raise an alert.

4.0 TESTING THE MODEL

In order to test the PAU-DM network with sensory data, we simulated a subject’s physical condition over a period of time. This task was carried on by a Human Physiology Simulator that generates realistic data streams for the input variables in the model. The Human Physiology Simulator attempts to provide realistic physiological behavior during the dying process. It represents the assumed “true” physiology model interrelating the time evolution of a set of physiological parameters. A central simulation manager contains a number of rules that link the various physiological states to provide reasonable cause and effect patterns.

Each simulated parameter features random fluctuations to provide realism and takes into account different possible physiological behaviors as emerging from different subject profiles. Three basic profiles are encoded in the simulator representing individuals with different physical fitness. This, for example, affects the levels at which the heart rate is considered too fast or too slow, or the individual capability of coping with apnea.

Using the Human Physiology Simulator, we generated a set of data streams representing different possible dying processes. We then fed the PAU-DM network with these data streams and analyzed the appropriateness of the assessment conclusion, with the help of a medical doctor specialized in intensive care. We also studied the behavior of the model when the network receives only some of the above data streams, to verify what information is more relevant and how the assessment model degrades in performance. Finally, we corrupted the data streams with several levels of noise in order to analyze the robustness of our approach.

Given that we did not use real-world data and because our Human Physiology Simulator was quite simple, we were not able to precisely quantify the PAU-DM performance in terms of sensitivity and specificity. Instead, our medical consultant analyzed the results in order to identify assessments that were clearly inappropriate and to understand the limits of the model. With this qualitative testing, we proved that we are able to obtain an accurate life-signs PAU determination. This holds also for situations in which the data streams present a consistent level of noise, thanks to the choice of averaging the input quantities over a one-minute interval.

There were no simulations in which the system gave a gross misdiagnosis of the subject’s condition. The main difference between the physician and the PAU-DM assessment of death was normally related to the onset of the condition. Our model tends to slightly delay this determination mainly because the oxygen saturation model was developed for a full-lung voluntary apnea. This is rarely the case for a subject that has suffered a traumatic injury.

We also want to underline that we did not perform any fine-tuning of the set of parameters present in the PAU-DM network before our testing. The results are therefore even more encouraging because the model is
not optimized. The best approach for tuning would use a set of the simulations to calibrate the parameters, and the rest of the simulations as verification baselines. It is expected that by proceeding in this way the assessment performance will greatly improve. We plan to use this approach in the development of the Phase II model.

5.0 CONCLUSIONS AND FUTURE WORK

The PAU-DM network we have discussed here represents the starting point for a new, more accurate life-signs PAU determination model. The techniques introduced in this demonstrative network have proved that with an appropriate mix of dynamic behavior and timers, it is possible to properly handle the uncertainty connected to the time dimension. The BN formalism allowed us to translate medical expertise into simple and intuitive elementary models that can be combined together in order to perform the desired assessments. This separation of a complex model into subparts that can be handled independently and then interrelated, provided a powerful yet simple enough tool to compose a coherent set of over 4,000 probabilistic rules.

The resulting PAU-DM network contains several parameters that can be fine-tuned in order to enhance the health state assessment performance. Other parameters allow us to take into consideration the different individual physiological responses expressed by different individuals.

As a byproduct of the PAU assessment process, we also synthesize information that may be effectively used to signal a serious problem requiring immediate medical assistance, before a complete absence (i.e. death) condition is reached. This result goes beyond the original life-signs presence/absence goal and represents an additional benefit that is worth exploiting in future versions of the model.

Building on the success of this proof of concepts, we are now developing a new PAU-DM that makes use of a larger set of sensory data to infer the subject’s status. This model will reuse the techniques presented here and perfect the medical knowledge to reproduce a more accurate assessment process.

To generate the appropriate physiological data streams, the user will be able to exploit state-of-the-art physiological simulators, data collected on the field and stored in a file, and even real-time data. In fact, in order to validate the final product we intend to use real measurements of humans and/or animals. This will allow the PAU-DM network to assess the outcome that was experienced by a subject in real life. The resulting assessment from the model will then be compared to the actual clinical outcome.

We are also generalizing the system architecture. We are creating a user-friendly development platform using a flexible framework, whose qualifying features will be easy scalability and modularity. A physiologist will be able to select and compose the assessment system through a simple interface, by selecting a pool of sensors, placing them at appropriate locations on the human body and performing a set of simulations. The software will assemble the appropriate simulation and diagnostic algorithms in the background, given the description of the used sensors, their characteristics and possible redundancy, the behavior of their components, and their performance.

The next generation software under development will allow the user to perform sensitivity analysis. The user will be able to select a sensor suite to measure a set of parameters that are meaningful for PAU determination. For each one of the sensors, the user will be able to specify accuracy levels, failure modes and the likelihood of their occurrence. This information will be taken into account to establish the expected level of confidence in PAU determination over a specified mission time. By changing parameters that describe the quality of the sensors, the user will be able to investigate the change in the PAU determination confidence as the sensors...
change in performance. Moreover, the user will be able to compare the impacts of different sensor suites that incorporate different kinds of sensors. Further issues such as the effects of environment, aging of the sensors and their self-diagnostic capabilities will also be taken into account.

Several of the sensors that are appropriate for PAU determination are quickly evolving. Our software will be flexible enough to accommodate changes in the architecture and behavior of those sensors. The software architecture has been designed to accept sensors and features that will likely be available in the foreseeable future. Indeed, the software will be used to provide indications of the desired performance of the sensors in order to achieve a reliable health status assessment. Thanks to these features, our software will constitute a valuable tool during the design of the sensor suite appropriate for the PAU goal.

5.1 Acknowledgements

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5.2 Disclaimer

The opinions and assertions expressed in this paper are those of the authors and do not necessarily express the official views of the U.S. Department of the Army or the U.S. Department of Defense.

6.0 REFERENCES


Use of Near-Infrared Spectroscopy in Early Determination of Irreversible Hemorrhagic Shock

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SUMMARY

Progression to irreversible shock may not be clinically apparent until a patient has been given several liters of fluids as well as multiple units of blood and blood products. In combat situations or in situations in which fluids for resuscitation are limited, resources need to be appropriately allocated. Therefore, early differentiation between patients who will progress to irreversible shock and those who are resuscitatable is important. We investigated whether the use of near-infrared spectroscopy (NIRS) in hemorrhage and resuscitation could assist in early detection of irreversible hemorrhagic shock. An established porcine model of hemorrhagic shock was used for experimentation. Twenty animals were treated with the same protocol including sedation and mechanical ventilation followed by instrumentation with a pulmonary artery catheter, arterial catheter, inferior vena cava (IVC) cannula, and placement of NIRS probes on the liver surface (during laparotomy), stomach (via modified nasogastric tube), and hind limb surface (skeletal muscle monitoring). Hemorrhagic shock was induced by removal of 35% blood volume via the IVC cannula. Animals

remained in shock for 90 minutes after which resuscitation was promptly initiated using lactated Ringer’s solution (20 cc/kg) in a stepwise fashion of four fluid boluses. Hemodynamic and NIRS variables were measured at baseline, every 30 minutes during shock, and after each resuscitative step. NIRS measurements of tissue oxyhemoglobin saturation (StO$_2$) in the liver, stomach, and hind limb were compared at all time points between animals that expired during resuscitation (unresuscitable) and animals that survived all resuscitative steps (resuscitatable). All animals were similar with regard to body weight, volume hemorrhaged, and baseline hemodynamic and NIRS variables. After the first resuscitative step, both stomach and leg StO$_2$ differed significantly between resuscitatable and unresuscitatable animals. Neither global oxygen measurements nor lactate distinguished resuscitatable from unresuscitatable animals. Linear regression analysis revealed that skeletal muscle (leg) StO$_2$ obtained after the first resuscitative step was a significant predictor of death despite resuscitation ($r^2=0.45$) ($p=0.005$). Combined, stomach and skeletal muscle StO$_2$ strongly predicted death despite resuscitation ($r^2=0.74$) ($p=0.003$). In conclusion, non-invasive monitoring of leg and stomach StO$_2$ with near-infrared spectroscopy differentiates resuscitatable from unresuscitatable animals after the initial resuscitative bolus. Use of this potentially pocket-sized, non-invasive spectrometer may help guide appropriate use of resuscitative fluids and has possible point-of-care applications.

1.0 INTRODUCTION

Death from hemorrhagic shock occurs in thousands of patients each year after civilian and military trauma. The initiating event in hemorrhagic shock includes decreased blood flow to tissues due to hypovolemia, resulting in a decrease in tissue oxygen delivery and subsequent cellular dysoxia. Optimal treatment for early hemorrhagic shock includes adequate control of bleeding followed by restoration of tissue oxygen delivery with appropriate resuscitation. Unfortunately, from a military perspective, this optimal strategy may not be available for many patients due to field situations that preclude prompt transport to the appropriate treatment facility [Holcomb 2003]. Thus, patients may reach definitive treatment in the advanced stages of shock, having passed a point beyond which restoration of oxygen delivery cannot overcome the “oxygen debt” despite appropriate resources and the best efforts of medical personnel.

Although decades have elapsed since the description of irreversible hemorrhagic shock by Wiggers [Wiggers 1950] in animal models of hemorrhagic shock, the events that occur at the cellular level are still not completely understood. Accordingly, the ability to determine which patients will progress to irreversibility is not accurate. With evaluation of laboratory measures such as base deficit and lactate, clinicians can gauge the magnitude of oxygen debt [Mooney 1999] [Abramson 1993] [Rutherford 1992] [Siegel 2003]. However, alone these laboratory measures alone do not accurately predict irreversibility and are not available to medics in the field. Therefore, determination of the magnitude of shock using a rapid, non-invasive method may be useful at the point of care in the field not only in military, but also in our urban trauma setting. Such a method would be useful to allow appropriate triage depending on availability of medical resources.

Near-infrared (NIR) spectroscopy is a technique that utilizes fiber-optic light to non-invasively determine the percentage of oxygen saturation of chromophores (e.g. hemoglobin) based on spectrophotometric principles. This technology has been utilized to experimentally determine regional tissue oxygen saturation (StO$_2$) [Mancini 1994] [Beilman 1999] [Beilman 2001] [Taylor 2004] [Cohn 2001] and the content / oxidation state of mitochondrial cytochrome $a$a$_3$ in tissue [Cairns 1997] [Balaban 1996] by monitoring the differential tissue optical absorbance of near-infrared light. Unlike “pulse-oximetry,” NIR spectroscopy measures not only arterial, but also venous oxyhemoglobin saturation at the microcirculatory level. This measurement therefore is a reflection of both oxygen delivery (DO$_2$) and oxygen consumption (VO$_2$) of the tissue bed sampled [Rhee
Non-invasive determination of these parameters using NIR spectroscopy has been described [Beilman 1999] [Beilman 2001] [Taylor 2004] [Cohn 2001] [Rhee 1997] [Simonson 1994].

In light of its non-invasive nature and potential for accurately determining regional tissue oxygen kinetics, we sought to evaluate the utility of NIR spectroscopy for early determination of irreversibility in hemorrhagic shock. A porcine model of hemorrhagic shock was chosen for experimentation in order to appropriately control for hemorrhage volume and timing, amount, and type of resuscitation. We hypothesized that tissue oxyhemoglobin saturation ($\text{StO}_2$), as determined by NIR spectroscopy, may be able to distinguish reversible from irreversible shock early during resuscitation.

2.0 METHODS

2.1 Animal Protocol:
Twenty male Yorkshire-Landrace pigs (Fanning Farms, Howe, Indiana) weighing 13-20 kg underwent an identical hemorrhagic shock/resuscitation protocol after its approval by the University of Minnesota Animal Use Committee. Each animal was maintained without food and with free access to water for 12 hours prior to the experiment. Animals were initially sedated with a single dose of intramuscular ketamine (20 mg/kg) and 2 mL of intravenous althesin (Pitman-Moore Ltd., Middlesex, UK), a steroid anesthetic which has minimal hemodynamic effects as compared to other anesthetic agents [Davis 1984] [Faber 1989]. The animals were endotracheally intubated and then ventilated using a Siemens 900 ventilator with settings adjusted to maintain a $\text{PO}_2$ of 80-120 mmHg and a $\text{PCO}_2$ of 35-45 mmHg. Maintenance anesthesia was an intravenous infusion of althesin (10 mg/kg/hour) and 60% inhaled nitrous oxide. Following anesthesia induction, the animals were splenectomized to avoid autotransfusion. The following lines were placed: Swan-Ganz catheter in the pulmonary artery through the right jugular vein, 12 Fr venous bypass catheter in the inferior vena cava (IVC), cystostomy catheter in the bladder, and an arterial catheter in the right carotid artery.

2.2 Near-infrared spectroscopic methodology/measurements:
Near-infrared spectroscopy probes (Hutchinson Technology, Inc, Hutchinson, Minnesota) were placed directly on the liver at laparotomy, on the surface of the hind limb, and into the stomach via a modified nasogastric tube. These probes contain bundles of multiple silica core optical fibers that transmit optical energy via light emitting diodes (LEDs) to the tissue and detect reflected light from the tissue. The maximum depth of the tissue volume sampled is directly related to the distance between the illumination fibers and the detection fibers, and the mean depth is half the probe spacing [Cui 1991]. The InSpectra™ device used on the hind limb of our animals had a probe spacing of 25 mm. The nasogastric tube, which was modified to incorporate the optical fibers, and the liver probe each possessed a spacing of 3 mm. Reflected light from the tissues returns it to a photosensitive detector that quantifies light absorbance by the tissue (Figure 1). Based on spectrophotometric principles, light absorption is then correlated with the chemical concentration of chromophores (e.g. hemoglobin) in the volume of tissue illuminated by the fiber optic, near-infrared light [Machter 1994]. Percent tissue hemoglobin oxygen saturation ($\text{StO}_2$) is calculated and displayed in real-time.
Use of Near-Infrared Spectroscopy in Early Determination of Irreversible Hemorrhagic Shock

2.3 Hemorrhagic shock protocol:

After baseline NIR and hemodynamic measurements were taken, hemorrhagic shock was induced by a 35% bleed (estimated by weight) into a heparinized blood collection bag via IVC cannula. The systolic blood pressure typically dropped to 40-50 mmHg with this maneuver. The animals remained in shock for 90 minutes at which time, following measurements, they received resuscitation with lactated Ringer’s solution (20 cc/kg body weight) in four boluses (Figure 2). NIR and hemodynamic measurements were obtained every 30 minutes during shock and then after each of the four boluses. Surviving animals were then sacrificed using a bolus of KCl (1-2mg/kg). Hemodynamic and NIR variables of animals that expired during the resuscitative process were compared to those that survived the entire resuscitative process.

2.4 Conventional Oxygen delivery and consumption measurement:

Pulmonary Artery (PA) catheter measurements of cardiac output (CO) were made via thermodilution and obtained at baseline and every 30 minutes during shock and after each resuscitative step in synchrony with NIR spectroscopic measurements. Arterial and mixed venous blood gases as well as lactate, hemoglobin (Instrument Laboratories, Lexington, MA) and other hemodynamic parameters (e.g. mean arterial pressure and heart rate) were obtained along with the PAC measurements. Oxygen delivery (DO$_2$) and oxygen consumption (VO$_2$) were calculated based on the conventional equations (below).

$$DO_2 = [(1.39 \times hgb \times SaO_2) + (0.003 \times PaO_2)] \times CO \times 10$$

$$VO_2 = [(1.39 \times hgb \times (SaO_2 - SvO_2) + 0.003 \times (PaO_2 - PvO_2)] \times CO \times 10$$

<table>
<thead>
<tr>
<th>Hemodynamic measures</th>
<th>Laboratory measures</th>
<th>NIR measures</th>
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<td>BP, HR, temperature, PAP, PCWP, UOP, CO, DO$_2$, VO$_2$</td>
<td>ABG, VBG, lactate, hemoglobin</td>
<td>StO$_2$ of stomach, liver, skeletal muscle (hind limb)</td>
</tr>
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</table>

Table 1 (above): Hemodynamic, laboratory, and NIR spectroscopy measures obtained at all time points. BP: blood pressure; HR: heart rate; PAP: pulmonary artery pressures; PCWP: pulmonary capillary wedge pressure; UOP: urine output; CO: cardiac output; DO$_2$: oxygen delivery; VO$_2$: oxygen consumption; ABG: arterial blood gas; VBG: venous blood gas; StO$_2$: tissue oxyhemoglobin saturation as measured by near-infrared spectroscopy. Significance determined by Mann-Whitney U test.
2.5 Statistical analysis of data:
Hemodynamic and NIRS variables were compared at all time points between the animals that expired during resuscitation from irreversible hemorrhagic shock and those that survived to complete the resuscitation protocol by using a one-way ANOVA and Mann-Whitney U test (SPSS for Macintosh, version 10.0). Variables recorded are shown in Table 1. Correlations between variables were performed using a Spearman correlation. Stepwise linear regression analysis was used to determine the relationship between variables and mortality using survival to the end of resuscitation (resus 4) as the dependent variable. A p value of $< 0.05$ defined significance.

**Figure 2: Hemorrhagic shock protocol timeline**
3.0 RESULTS

Of the twenty animals that underwent the hemorrhagic shock / lactated Ringer’s resuscitation protocol, eighteen animals survived the 90 minutes of hemorrhagic shock to reach resuscitation. Of these eighteen animals, six died from hemorrhagic shock despite receiving at least one fluid bolus. The hemodynamics and NIR spectroscopic measures of these six animals dying of “irreversible” hemorrhagic shock were compared to the other twelve animals that survived to complete all four resuscitative steps. In this study, we defined “irreversible” to indicate death after resuscitation began.

All eighteen animals were similar with respect to body weight and volume hemorrhaged as well as baseline measures of mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), DO₂, VO₂, and plasma lactate (Table 2a). Baseline StO₂ of hind limb, liver, and stomach were similar among all animals as well (Table 2b).

<table>
<thead>
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<tr>
<td>Baseline HR (beats/minute) ± SD</td>
<td>158±27.8</td>
<td>178±40.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Baseline MAP (mmHg) ± SD</td>
<td>86.7±10.8</td>
<td>84.8±13.5</td>
<td>0.74</td>
</tr>
<tr>
<td>Baseline CO (liters/minute) ± SD</td>
<td>2.63±0.8</td>
<td>2.68±0.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Baseline DO₂ (mL O₂/minute)</td>
<td>22.2±5.8</td>
<td>22.7±6.7</td>
<td>0.84</td>
</tr>
<tr>
<td>Baseline VO₂ (mL O₂/minute)</td>
<td>4.2±2.1</td>
<td>5.7±2.4</td>
<td>0.30</td>
</tr>
<tr>
<td>Baseline lactate (mmol/liter)</td>
<td>1.7±0.7</td>
<td>2.9±2.6</td>
<td>0.48</td>
</tr>
<tr>
<td>Volume hemorrhaged (cc/kg) ± SD</td>
<td>507.3±101.0</td>
<td>468.6±108.3</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table 2a: Comparison of baseline weight, heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), oxygen delivery (DO₂), oxygen consumption (VO₂), lactate, and volume hemorrhaged for animals with irreversible and reversible shock. Significance determined by Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Baseline StO₂ values</th>
<th>Irreversible (n=6)</th>
<th>Reversible (n=12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle (leg) StO₂ %saturation ± SD</td>
<td>66.0±10.6%</td>
<td>66.7±16.5%</td>
<td>0.96</td>
</tr>
<tr>
<td>Liver StO₂ %saturation ± SD</td>
<td>79.3±9.1%</td>
<td>80.4±11.5%</td>
<td>0.51</td>
</tr>
<tr>
<td>Stomach StO₂ %saturation ± SD</td>
<td>65.0±35.2%</td>
<td>79.4±14.0%</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 2b: Comparison of baseline NIR spectroscopic measurements of tissue oxyhemoglobin saturation in the leg, liver, and stomach for animals with irreversible versus reversible hemorrhagic shock. Significance determined by Mann-Whitney U test.
Expectedly, all animals experienced a decrease in CO (Figure 3a) and DO₂ and an increase in lactate (Figure 3b) during hemorrhagic shock. Animals also experienced a drop in StO₂ in skeletal muscle, liver, and stomach during shock (Figures 4a, 4b, 4c).

![Cardiac Output](image1)

**Figure 3a:** Cardiac output (liters/minute) during hemorrhagic shock and resuscitation for animals with irreversible versus reversible hemorrhagic shock. No significant differences found at any time points as determined by Mann-Whitney U test and ANOVA.

![Serum Lactate](image2)

**Figure 3b:** Serum lactate levels (mmol/liter) during hemorrhagic shock and resuscitation for animals with irreversible versus reversible hemorrhagic shock. No significant differences found at any time points as determined by Mann-Whitney U test and ANOVA.

Time from the onset of shock to resuscitation was significantly shorter in the animals that did not survive resuscitation when compared to those surviving resuscitation (Table 3). Two animals that survived shock expired within four minutes of receiving the first resuscitative fluid bolus. Because these animals were pre-morbid at the time of resuscitation, they were excluded from analysis of NIR and hemodynamic
measurements to avoid falsely skewing the results. With exclusion of these two animals, the median time from the onset of resuscitation to death in animals dying of irreversible hemorrhagic shock was 34 minutes (range 11-59 minutes), with a mean of 34.5 ± 22 minutes. The average time for delivery of a fluid bolus was 2.6 ± 0.5 minutes. The average time between fluid boluses was 25.2 ± 6.7 minutes.

<table>
<thead>
<tr>
<th></th>
<th>Median time from shock to resuscitation (range)</th>
<th>Mean time from shock to resuscitation ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irreversible shock (n=6)</td>
<td>99.5 (92-115)</td>
<td>101.0±8.72*</td>
</tr>
<tr>
<td>Reversible shock (n=12)</td>
<td>112.5 (106-126)</td>
<td>114.3±8.21</td>
</tr>
</tbody>
</table>

Table 3: Times in minutes from onset of shock to onset of resuscitation. *p=0.02 when comparing means between animals with irreversible versus reversible hemorrhagic shock. SD: standard deviation.

Figure 4a: Leg (skeletal muscle) StO2 in animals with irreversible and reversible hemorrhagic shock. *p<0.05

Figure 4b: Liver StO2 in animals with irreversible and reversible hemorrhagic shock. *p=0.046
By 90 minutes of shock, the drop in skeletal muscle StO2 during shock was significantly greater in the four animals not surviving resuscitation when compared to those that survived to complete resuscitation (p=0.050) (Figure 4a). This disparity at 90 minutes of shock was not observed in the liver or stomach NIR measurements (Figures 4b and 4c). Skeletal muscle (leg) NIRS measurements after the first resuscitative bolus (resus 1) were significantly lower in animals dying of irreversible hemorrhagic shock than in those surviving to complete resuscitation (p=0.019). This difference persisted after the second resuscitative bolus (p=0.03). A significant difference was observed in stomach NIR measurements after resus 1 (p=0.031). However, an equipment malfunction with the modified nasogastric tube for one animal did not allow for enough stomach NIR readings to reach a statistically significant difference at the second resuscitative step. In the liver, the only NIR measurement that was significantly different between survivors and non-survivors was after the second resuscitative bolus (resus 2) (p=0.03).

There were no significant differences in cardiac output (Figure 3a), oxygen delivery, oxygen consumption, or lactate (Figure 3b) between survivors and non-survivors throughout shock or resuscitation. Pulmonary artery catheter measures of global DO2 did not significantly correlate with regional NIRS measures of StO2 during shock (Table 4).

![Figure 4c: Stomach StO2 in animals with irreversible and reversible hemorrhagic shock. *p=0.034](image)

Table 4: Correlations between global oxygen delivery (DO2) and regional StO2 as measured by NIR spectroscopy for all animals during shock. Results given as Spearman correlation coefficient (p value). Stepwise linear regression analysis revealed that, by itself, the skeletal muscle (leg) NIR measurement obtained after the first resuscitative fluid bolus (resus 1) was a significant predictor of death despite resuscitation ($r^2=0.45$) (p=0.005). Combined, both the stomach and leg NIR measurements obtained after the first resuscitative step were particularly predictive of death despite resuscitation ($r^2=0.74$) (p=0.003).
4.0 DISCUSSION:

In this study, we demonstrated that non-invasive NIR spectroscopic monitoring of skeletal muscle (leg) StO$_2$ provides a means for early differentiation between resuscitatable and non-resuscitatable animals after a period of severe hemorrhagic shock. Despite identical shock protocols and similar baseline weights and hemodynamics, one third of animals undergoing shock were unresuscitatable. This indicates the severity of the model and underscores the basic pathophysiology of decompensated hemorrhagic shock, that a point exists beyond which resumption of oxygen delivery cannot overcome the preceding cellular energy deficit.

As expected, cardiac output and oxygen delivery decreased and lactate increased upon induction of shock in all animals. However, these global measures did not significantly differ between animals that went on to die during resuscitation and those that survived. Furthermore, these global measures were unable to predict which animals would or would not survive resuscitation. Lactate, DO$_2$, and base deficit have been shown to correlate with global oxygen debt [Moomey 1999] [Abramson 1993] [Rutherford 1992] and have been evaluated as potential predictors of outcome [Ivatury 1995] [Ivatury 1996] [Sauaia 1994] [Mikulaschek 1996]. Although each has its limitations, utilization of these values as markers of oxygen debt may be useful to clinicians in the hospital to determine the overall status of a patient and as measures to follow during resuscitation.

Even if such measurements are available to the clinician, a drawback of using such global values is that they provide only a general picture of the patient’s oxygen debt, not the variations in oxygen debt seen at the regional tissue level. For example, the global DO$_2$ derived from a PA catheter may falsely indicate improvement in status despite deterioration of individual organs. Lactate traditionally “lags” due to its dependence on hepatic clearance, making it less of an accurate, dynamic marker of status. Also, it has been well-documented that epinephrine, produced abundantly during physiologic stress, stimulates lactate production by well-oxygenated skeletal muscle [Luchette 1999] [Luchette 2002]. Due to these limitations, an elevated lactate level may not translate to a DO$_2$ deficiency. In these experiments, lactate continued to increase upon initial resuscitation for both survivors and in non-survivors as its hepatic clearance capacity was exceeded. We also found that early in resuscitation, neither lactate, oxygen delivery, nor cardiac output could distinguish between resuscitatable and unresuscitatable animals. This dichotomy is depicted in Figures 3a, 3b and Figures 4a, 4c. Figures 3a and 3b show the non-significant differences in cardiac output and lactate for both survivors and non-survivors during shock and resuscitation. In figure 4a and 4c, however, a significant disparity in regional StO$_2$ was observed both late during shock and with the onset of resuscitation (resus 1) between those animals that went on to die of irreversible shock and those that survived resuscitation. These differences were not observed in the liver until late in resuscitation when death was imminent (resus 2), potentially reflecting the dual blood supply and unique metabolism of the liver.

We undertook this study to determine whether NIR-measured StO$_2$ could provide early differentiation between resuscitatable and unresuscitatable animals after a period of severe hemorrhagic shock. In evaluating our data, we did not wish to falsely skew our interpretation by including results from animals that were premorbid upon beginning resuscitation. Therefore, we assessed how much time elapsed between the onset of resuscitation and death in animals that received at least one fluid bolus. Two of the six animals were deemed “premorbid” as death occurred within two and four minutes of completing the first fluid bolus. We chose to eliminate the hemodynamic, laboratory and NIR measurements of these two animals in order to accurately evaluate variables in a setting of active, but futile, resuscitation. Even after elimination of these premorbid animals from analysis, a significant decrease in skeletal muscle StO$_2$ in unresuscitatable animals was present after 90 minutes of shock and during resuscitation. Early during resuscitation (resus 1), this value was significantly predictive of irreversible shock. Gastric StO$_2$ measurements early in resuscitation also
differentiated resuscitatable from non-resuscitatable animals, and, when combined, both stomach and skeletal muscle StO2 were very significantly predictive of irreversible shock despite resuscitation.

Monitoring skeletal muscle StO2 with NIR spectroscopy avoids the inherent limitations of following lactate and global DO2 values. While lactate may be elevated even in well-oxygenated skeletal muscle, StO2 may more accurately reflect oxygen delivery. Additionally, because the StO2 value reflects a component of VO2 (StO2 ~ k • DO2/VO2), it may represent the metabolic state of the tissue more appropriately than other oxygen indices. Finally, while global measurements of oxygen debt are inherently impractical for use in a point-of-care setting because they require equipment not routinely available to medics in the field, NIR spectroscopy has the potential to become an easily-portable and straightforward modality.

The field of NIR spectroscopy continues to branch and blossom as the technology and its interpretation improves. The probes used for skeletal muscle measurements in these experiments are applied easily, securely, and non-invasively with associated adhesive backing, similar to an EKG pad. The receiving device for these experiments was associated with a laptop computer. However, prototype hand-held devices are in development and may soon be available for future use. With these smaller devices, it is foreseeable that its use in traumatically-injured patients in the field would be appealing. Use of the skeletal muscle probe may be more feasible for use in the point-of-care setting than the modified nasogastric tube used to obtain gastric StO2 measurements as this value may be corrupted by food particles and gastric juices in trauma patients. However, compared to other tissue beds, the potentially greater sensitivity of gastric perfusion to resuscitation adequacy [Ivatury 1995] [Ivatury 1996] warrants further research into this avenue.

Weaknesses of the study include the use of a controlled hemorrhage model. The animals used in these experiments were undergoing evaluation of the effects of resuscitation at the tissue level. Therefore we needed to be able to compare animals that were at a similar severity of shock. Additionally, our method of resuscitation using stepwise boluses differs from clinical practice during which fluid resuscitation is generally performed in a continuous fashion.

In conclusion, we have demonstrated that NIR spectroscopic measurement of skeletal muscle with or without measurement of stomach StO2 may be useful in early determination of irreversible hemorrhagic shock. Although its limitations are incumbent, NIR spectroscopic devices have the potential to become an essential tool for the field medic in the point-of-care setting in order to appropriately allocate medical resources.

5.0 REFERENCES:


Use of Near-Infrared Spectroscopy in Early Determination of Irreversible Hemorrhagic Shock


Photons for Therapy: Targeted Photodynamic Therapy for Infected and Contaminated Wounds

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ABSTRACT

Introduction: Battlefield wounds are frequently contaminated with pathogenic microorganisms present on uniforms and skin. Although the development of serious infections can often be prevented by antibiotics, the rise in worldwide incidence of multiply antibiotic-resistant bacteria necessitates the discovery of alternative methods. In addition, traumatic wounds and burns may contain non-perfused tissue where antibiotics cannot penetrate efficiently. The possibility also exists of the use of biological weapons with unknown antibiotic susceptibility.

Rationale: Previously workers have used photodynamic therapy to kill bacteria in vitro, but the use of this approach has seldom been reported in vivo in animal models of infection. We report on the use of a targeted polycationic photosensitizer conjugate between poly-L-lysine and chlorin(e6) that can penetrate the Gram (-) outer membrane together with harmless red laser light to kill Escherichia coli and Pseudomonas aeruginosa infecting excisional wounds in mice.

Methods and Results: We used genetically engineered luminescent bacteria that allowed the infection to be imaged in mouse wounds using a sensitive CCD camera. Wounds were infected with 5x10^6 bacteria, followed by application of the conjugate in solution and illumination with red light. There was a light-dose dependent loss of luminescence as measured by image analysis in the wound treated with conjugate and light, not seen in control wounds. This strain of E. coli is non-invasive and the infection in untreated wounds spontaneously resolved in a few days and all wounds healed equally well showing the photodynamic treatment did not damage the host tissue. P. aeruginosa is highly invasive and mice with photosensitizer alone, light alone or untreated infected wounds all died while 90% of PDT treated mice survived. Wounds treated with PDT healed significantly better than those treated with an alternative antimicrobial (silver nitrate). We then treated mice with an infection caused by Staphylococcus aureus that had been allowed to grow in abscesses below the skin. Conjugate injected into the infected area together with surface illumination successfully killed the bacteria.

Conclusions: In view of the development of cheap portable light sources PDT may have a role to play in preventing and treating infection in combat wounds.

1.0 INTRODUCTION

Photodynamic therapy (PDT) is a therapy for cancer and other diseases that has received regulatory approvals for several indications in many countries [1]. Its use as a cancer treatment is based on the observation that
Photons for Therapy: Targeted Photodynamic Therapy for Infected and Contaminated Wounds

certain non-toxic dyes known as photosensitizers, (PS) of which haematoporphyrin derivative (HPD, also known as Photofrin) is the best known example, accumulate preferentially in malignant tissues [2]. Therapy involves delivering visible light of the appropriate wavelength to excite the PS molecule to the excited singlet state. This excited state may then undergo intersystem crossing to the slightly lower energy but longer-lived triplet state, which may then react further by one or both of two pathways known as Type I and Type II photoprocesses, both of which require oxygen [3]. The Type I pathway involves electron transfer reactions from the PS triplet state with the participation of a substrate to produce radical ions which can then react with oxygen to produce cytotoxic species such as superoxide, hydroxyl and lipid derived radicals. The Type II pathway involves energy transfer from the PS triplet state to ground state molecular oxygen (triplet) to produce the excited state singlet oxygen, which can then oxidize many biological molecules such as proteins, nucleic acids and lipids, and lead to cytotoxicity. PDT has the advantage over other therapies of dual selectivity: not only is the PS targeted to the tumor or other lesion, but the light can also be accurately delivered to the affected tissue. Although originally developed as a cancer treatment, the most successful PDT application to date (that recently received FDA approval) has been in ophthalmology, as a treatment for age-related macular degeneration [4]. Other non-oncological applications of PDT at a less developed stage include treatments for psoriasis, arthritis, Barrett’s esophagus, atherosclerosis and restenosis.

Bacterial contamination of wounds is a significant cause of morbidity, delayed wound healing and increased hospital stay [5]. In combat conditions, contamination of wounds is sometimes unavoidable, and by the time the wound is treated the bacteria may have penetrated sufficiently into the tissue to be resistant to topically applied antimicrobials. Although wound infections are treated with topical and systemic antibiotics, the rapid emergence of multi-antibiotic resistant strains of bacteria is of considerable concern [6]. In addition bacteria in traumatized or non-perfused tissues are not easily affected by oral or intravenous antibiotics [7]. Photodynamic destruction of bacteria in wounds may be an effective means of killing the bacteria while simultaneously stimulating the host immune system. In order for this treatment to be effective it is necessary to establish the factors which govern the susceptibility of various bacterial strains to photodynamic inactivation [8].

It is now accepted that Gram positive bacteria are relatively easy to kill by PDT with standard photosensitizers while Gram negative bacteria are remarkably resistant [9]. It has been shown that cationic photosensitizers, or anionic photosensitizers in the presence of exogenous naturally occurring polycationic peptides, could kill Gram negative bacteria [10-12]. It is supposed that the effect of these polycationic peptides is to permeabilize the outer membrane of gram negative bacteria allowing the sensitizer to gain access to more sensitive locations inside the bacterium.

In 1997 our laboratory formed the hypothesis that by covalently conjugating a suitable PS to a pL chain a bacterial-targeted PS delivery vehicle could be constructed that would efficiently inactivate both Gram (+) and Gram (-) species. Because the resulting polycationic entity is a macromolecule, it would be taken up by mammalian cells by the time-dependent process of endocytosis, thus giving temporal selectivity for bacteria, into which the polycation could penetrate rapidly. This was demonstrated [13] by the preparation of a conjugate between one molecule of chlorin(e6) and a pL chain of 20 lysine residues that, after 1 minute incubation and illumination with red light, killed >99% of the oral pathogens Gram (+) (Actinomyces viscosus) and Gram (-) (Porphyromonas gingivalis) while sparing an oral epithelial cell line (HCPC-1). A similar construct was subsequently used by another group (composed of one c6 molecule and a 5 amino-acid lysine chain) to kill several oral pathogens in the presence of 25% whole blood [14]. Polo and coworkers used conjugates between pL and porphycenes with a significant phototoxic activity against Gram (-) bacteria [15]. In a subsequent report [16] these authors showed that several strains responsible for periodontal disease were efficiently inactivated by visible light irradiation in the presence of porphycene–polylysine conjugates.
Repeated photosensitization of surviving cells did not induce the selection of resistant bacterial strains nor modify their sensitivity to antibiotic treatment.

We recently compared [17] the effectiveness of pL-ce6 conjugates with chain lengths of either 8 or 37 lysines attached to precisely one ce6 molecule for bacterial PDI, and found the 37-lysine conjugate was able to efficiently mediate the photodestruction of both Gram (+) and Gram (-) species, while the 8-lysine conjugate or free ce6 were only effective against Gram (+) bacteria.

2.0 BIOLUMINESCENCE IMAGING

In order to explore the efficiency of PDT to kill bacteria in tissues of living animals we have developed the use of bioluminescence imaging [18-20]. We used genetically engineered bacteria that emit luminescence together with a sensitive low-light imaging camera [21-24]. These bacteria have been transfected with a plasmid containing the *Photobacterium luminiscens* lux operon (luxABCDE) that encodes for not only the luciferase enzyme, but also the biosynthetic enzymes necessary for biosynthesis of the luciferase substrate. Hence in the presence of flavin mononucleotide from the bacterium and external oxygen these bacteria will glow in the dark. The rate of luciferase enzyme turnover in the presence of substrate allows for real-time measurements, and the enzyme is active at the body temperature of mammals. An image captured by the camera of a living mouse gives information about the intensity and spatial spread of the infection, and each animal can be followed longitudinally dramatically reducing the numbers of animals needed to study treatment of infections. This method is an improvement on the traditional use of survival or body fluid sampling and subsequent plating and colony counting. Rocchetta et al studied the growth of bioluminescent *E. coli* in the neutropenic mouse-thigh abscess model of infection [25]. They found that the number of CFU extracted from the thighs of sacrificed animals closely paralleled the luminescence signal at several timepoints after inoculation and during the period of action of antibiotics.

![Figure 1A shows the relationship between luminescence and bacterial number obtained with luminescent *E coli* DH5a over four logs of bacterial numbers as measured by tube luminometer. Bacterial cfu were routinely determined by streaking out a set of serial dilutions onto agar plates. Figure 1B shows an example of bioluminescence imaging of the agar plates.](image)

We first ascertained that the luminescence signal measured in the luminometer was linearly proportional to bacterial CFU (as determined by serial dilution and plating) from 10^3-10^7 organisms (Figure 1A). The signal saturated at large bacterial numbers i.e. > 10^7. In addition to counting the colonies it is possible to image the plates using the luminescence camera and this is shown in Fig 1B.
We then carried out in vitro PDI experiments in order to verify that killing the bacteria as demonstrated by loss of colony forming units correlated with loss of luminescence. The loss of viability curves as measured by CFU and by loss of luminescence, as a function of light-dose, for bacteria incubated with 3, 6, 12 and 18 µM pL-ce6 conjugate are shown in Figs 2A and 2B. The CFU assay had a limit of sensitivity of six orders of magnitude in reduction of viability, while the bioluminescence assay had a limit of three orders of magnitude. Loss of luminescence showed the same dose-response curves as loss of CFU but the absolute reductions were 1 to 3 logs less. The reasons for this are twofold. The limits of sensitivity of the luminescence assay with the plate reader is a 3 log reduction in signal, while the CFU assay can measure a 6 log reduction in viability. Secondly it appears that the cytotoxic insult to the bacteria causes loss of viability more readily than loss of luminescence. The mechanism by which luminescence decreases after photoinactivation (PDI) is uncertain, but may be due to exhaustion of FMN supplies from the bacteria (needed for the luciferase enzyme to make luminescence) and which cannot be replenished if the cells are fatally damaged.

![Graph A: colony forming units](image1)

![Graph B: bioluminescence](image2)

**Figure 2. Phototoxicity as determined by A, CFU; and B, luminescence assays. Bacteria were incubated with stated concentration of conjugate as above, then washed and illuminated with stated fluence of 665-nm light with removal of aliquots of bacterial suspension at intervals (100, 200 and 400 seconds respectively) and serial dilution and plating or luminescence measurement in 96-well plates. Data points are means of triplicate determinations and two separate experiments and bars are SD.**

### 3.0 PDT OF *E. coli* INFECTED EXCISIONAL WOUNDS IN MICE.

We initially sought to establish the animal model of infection by inoculating *E coli* into an excisional wound on the mouse [18]. Five million cfu from a mid-log culture in 50 µl gave a sufficiently bright luminescence signal from the wound to allow at least two logs of signal reduction to be accurately followed. When this bacterial inoculum was placed into a wound (12.5 X 8 mm) made on the back of a freshly euthanized mouse, the luminescence started to fade rapidly and was totally gone by the time the 50 µl inoculum had dried (< 60 minutes) (data not shown). Untreated infected wounds in living mice showed only a slight loss of luminescence over a period of four hours. We interpret these findings to mean that the living mouse wound
provides nutrients and moisture to the bacteria, and thus is a reasonable model of wound infection. The next day however, control infected wounds in living mice had lost on average 90% of the original luminescence signal, but with considerable inter-animal variability (data not shown).

Since the wound infection with *E coli* DH5a was found to be self-limiting, i.e. this particular strain of *E coli* is non-invasive [26], it allowed the use of each mouse as its own control to follow wound healing with four wounds per mouse. The effect of topical application of pL-ce6 conjugate and successive applications of 660-nm light is presented in a series of overlaid luminescence (false-color) and gray-scale reference images (Fig. 3). These data were obtained from a mouse in which bacteria were inoculated in all wounds, conjugate was added to wounds 1 and 4, and wounds 3 and 4 were illuminated with red light. Therefore wound 1 was the dark control with conjugate, wound 2 was the absolute control, wound 3 was the light alone control, and wound 4 was PDT treated. Topical application of a targeted polycationic PS conjugate followed by illumination led to a 99% reduction in luminescence as measured by imaging software on the luminescence images. There was a semi-logarithmic light dose-dependent reduction in luminescence from the PDT treated wound not seen with any of the control wounds, as would be expected from a standard PDT experiment (Fig. 4). There was an initial modest decrease in luminescence from wounds that received conjugate without light due to the dark toxicity of the conjugate, but the luminescence did not decrease further during the course of the experiment.

![Figure 3. Successive overlaid luminescence false color images and monochrome LED images of a mouse with four excisional wounds infected with equal numbers of *E coli* (A). Wounds 1 (nearest tail) and 4 (nearest head) received topical application of conjugate (B). Wounds 1 and 2 (two nearest tail) were then illuminated with successive fluences (45 - 165 J/cm^-2) of 665-nm light (C-D).](image-url)
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3.1 Wound healing. The assumption that considerable amounts of conjugate bound to the tissue in the wound suggested that illumination might have caused damage to the host cells, blood vessels or extracellular matrix in the wound. However we observed that PDT of infected wounds did not lead to any inhibition of wound healing as seen in Fig 5. There was an indication that the PDT treated wounds actually healed somewhat faster relative to the other control wounds but this was not statistically significant. The lack of host tissue phototoxicity may have been due to the necessity for a macromolecular species such as pL-ce6 (molecular weight approximately 18,500) to be taken up into mammalian cells by the time dependent process of endocytosis. In the present experiments the absence of wound healing inhibition may be explained by a combination of the topical delivery method together with the large conjugate size and the relatively short incubation time. The fact that treated wounds healed as well as control wounds suggests that PDI may have advantages over topical anti-microbial products that have been reported to cause tissue damage or have other undesirable side-effects.

Figure 4. Mean pixel values of luminescence signals from defined areas of wounds measuring 1200 pixels determined by image analysis. Data points are means of values from the corresponding wound on six mice per group and bars are SD.

Figure 5. Mean areas of wounds from six mice per group treated as above. Wounds were measured daily in two dimensions and areas calculated. Bars are SD.
4.0 PDT OF LETHAL PSEUDOMONAS AERUGINOSA WOUND INFECTIONS IN MICE

The previous experiments were a proof-of-principle study using a relatively non-pathogenic strain of *E. coli*, DH5alpha that lacks virulence factors necessary to cause invasive infections [26]. In order to test PDT in a more clinically relevant infection model, we used optical techniques (bioluminescence imaging and targeted PDT) to monitor and cure mice of an otherwise fatal *P. aeruginosa* wound infection [19]. Mice with wounds infected with 5 X 10⁶ CFU of *P. aeruginosa* quickly developed an illness consistent with systemic sepsis. They lost weight, had ruffled coats, and developed progressive inactivity leading to a moribund condition and death occurred between 24-60 hours after infection. When the effect of varying the initial bacterial inoculum was studied, it was found that the LD50 was approximately 200,000 CFU. Mice infected with the lower numbers of bacteria that did not die suffered the same infection as the mice that died, but in a less severe form and recovered between days 6-8. We nevertheless decided to use a bacterial challenge for the PDT experiments that was 25 times higher than the LD50 to provide a robust test of the ability of the technology to prevent death from a fatal wound infection.

Mice were given a single dorsal excisional wound with an area of 100 mm², infected with 5 x 10⁶ CFU of *P. aeruginosa* suspended in 50 µL of PBS. This inoculum gave a sufficiently bright luminescence signal from
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the wound to allow at least two logs of signal reduction to be accurately followed. The bacteria quickly attached to the tissue surface of the panniculus carnosus as evidenced by the failure of an attempt to wash them off by irrigation with saline 30-min after infection, and as quantitated by luminescence imaging. The pLce6 conjugate was added as 50-µL of a 200-µM ce6 equivalent concentration as preliminary experiments had shown lower concentrations to be less effective. This volume was sufficient to spread evenly throughout the surface of the wound and was retained by the edges of the wound to prevent the liquid running off. It was necessary to give the conjugate at least 30-min to bind to, and penetrate the bacteria in order to see effective loss of luminescence after illumination with 660-nm light. As can be seen from a set of luminescence images from a representative mouse shown in Fig 6A-6G, PDT produced a fluence-dependent loss of luminescence until only a trace remained after 240 J/cm2 had been delivered. When the mouse was imaged the next day all traces of luminescence had gone (panel 6H). There was a drop in luminescence seen shortly after applying the conjugate in the dark (Fig 6B, 6J), but this did not decrease further after 30 min incubation (Fig 6C, 6K) or indeed after 60 min incubation (Fig 2L, approximately equal to the time for illumination of the PDT wounds). Infected wounds left untreated or treated with illumination alone showed a rise in luminescence signal (up to 2-fold, Fig 7A) presumably due to growth of the bacteria in the nutrient rich medium of the wound. There was significant luminescence present in control wounds until death occurred 2-4 days later (Fig 6M). The mean luminescence values determined from the infections in the wounds of all the mice in the four groups were calculated using the ARGUS software. The resulting curves are plotted in Fig 7A. The PDT treated group shows a semi-logarithmic relationship between bacterial luminescence and delivered fluence, until 99% of the luminescence has disappeared after 240 J/cm2. There is a significant difference between the luminescence found from the conjugate in the dark group, compared with that in the light alone and untreated control groups. This is due to two factors; firstly to a degree of dark toxicity of the conjugate towards *P. aeruginosa*, and secondly to the ability of the bacteria in the untreated and light alone control wounds to continue to grow.

![Figure 7A](image-url)  
*Figure 7A. Mean pixel values of luminescence signals from defined areas measuring 1200 pixels covering infected wounds determined by image analysis. The four groups comprise untreated control, light alone control, dark conjugate control, and PDT treated. Data points are means of values from the wounds on ten mice per group and bars are SD. Figure 7B. Kaplan-Meier survival plot for the four groups of mice described in Figure 7A.*
All the mice in the three control groups (untreated, light alone and dark conjugate) died within a period of five days infection. By contrast 90% of the mice treated with PDT survived as seen in Fig 7B. These mice appeared to suffer from some symptoms of bacterial infection (weight loss, ruffled fur and inactivity) similar to those mice who received a sub-lethal dose of bacteria described above. They recovered quickly however and by five days after infection were regaining weight and moving normally.

5.0 PDT OF ESTABLISHED SOFT-TISSUE INFECTIONS

The previous experiments were carried on animals whose wounds were recently contaminated with relatively large numbers of CFU. It is unlikely that patients or combat personnel would present for treatment under these circumstances. A more realistic and clinically relevant model would consist of inoculation of a smaller number of bacteria and then allowing the infection to grow and become established in tissue over time. A second major consideration was that the previous experiments used an infection model where the bacteria were relatively near the surface of the tissue in an excisional wound. In real life the bacteria could be beneath the surface of the skin or tissue either because they had already invaded or because they were on skin or clothing that had been forcibly introduced into a penetrating wound such as those caused by gunshot or shrapnel. Since the penetration of visible light (even red light) into tissue is limited we asked whether PDT could be used to treat an infection where the bacteria had been allowed to multiply several hundred-fold and were some distance beneath the skin surface.

We used the injection of 1 million log-phase \textit{S. aureus} CFU (mouse pathogenic strain 8325-4) into the mouse thigh muscle (2-mm deep). The mice had previously been rendered temporarily neutropenic by pre-treatment with cyclophosphamide. We used a model in which the mice developed two equivalent infections, one in each hind thigh in order to allow each mouse to have a PDT treated infection and a control untreated infection and act as its own control [20]. Twenty-four hours after infection the bioluminescence had increased dramatically as the bacteria had multiplied within the infected tissue (Figure 8). The pL-ce6 conjugate (50-µL) at a concentration of 1-mM ce6 equivalent was injected into the infected area resulting in a visible green coloration noticeable beneath the skin. This allowed a judgment to be made about the uniformity of the PS distribution within the infection. Although these mice were neutropenic, and the infection did not accumulate the considerable quantities of pus expected from immunocompetent mice, there was still some matter present in the infection. We had previously suspected that the conjugate might diffuse relatively rapidly through the tissue, however this did not prove to be the case. The green coloration remained in place for some time (several hours) especially in unilluminated mice. Light (660-nm) was delivered to the infection as a spot on the skin about 8-mm in diameter centered on the infected area.

There was a slight reduction in bacterial bioluminescence observed immediately after the conjugate was injected into the infection. Luminescence was further reduced after the 30-minute incubation period in the dark. When illumination was commenced there was a light-dose dependent decrease in luminescence after each 40 J/cm² increment of red light (Figs 8). A typical mouse treated with injection of conjugate into the right infected thigh, followed by illumination of this thigh as described previously is shown in Fig 8, together with the mean bioluminescence values from both legs of 5 mice in Fig 9. After 160 J/cm² had been delivered the bioluminescence of the treated infected legs had been reduced by >99% compared to the untreated contralateral legs. However 4 out of 5 of these treated legs suffered a recurrence of the bioluminescence on succeeding days (data not shown).
Figure 8. Series of bioluminescence images (captured at a bit range of 2-4) from a neutropenic mouse infected on day 1 in both thighs, and treated on day 2 with injection of pL-ce6 into the right thigh, followed after 30 min by illumination of the right thigh with 660-nm light at a fluence rate of 100 mW/cm².

Figure 9. Mean total normalized bioluminescence values from left (untreated) and right (PDT treated) thighs of 5 mice infected in both thighs. Bars are SEM.
6.0. CONCLUSIONS AND FUTURE WORK

The use of a polycationic conjugate between the PS ce6 and a poly-L-lysine chain delivers the PS in an efficient manner to bacteria infecting wounds and tissue, and in addition, the delivery of light a short time after the PS gives temporal selectivity for the bacteria over the host cells as the conjugate binds quickly to bacteria but only slowly to host cells. The use of stably transduced bioluminescent bacteria together with a low-light imaging camera provides an efficient and versatile method of monitoring the progress of the infection in experimental animals in real time and in a longitudinal fashion allows response to therapy to be followed. Due to the widespread occurrence of antibiotic resistance amongst pathogenic bacteria and the relatively slow response to antibiotics when bacteria are infecting traumatized tissue, alternative methods of killing bacteria in wounds are being sought. The recent development of portable and low-cost light sources such as light emitting diodes that are battery-powered may make possible the deployment of systems that can be used for wound decontamination. Our data demonstrating that PDT can also be used for established infections further extends its possible applications.

In future work we are studying PDT for infections in thermal and chemical burns and in other medically and militarily important localized infections. These include fungal and parasitic infections of the skin (dermatophytosis and Leishmaniasis), bacterial keratitis, sinusitis, periodontitis, gastric *Helicobacter pylori* infection, bacterial cystitis etc. The local delivery of the PS and the ability of fiber optics to deliver light anywhere within the body suggest that PDT can treat infections with many hollow organs. We are also working on second generation PS-conjugates with even higher activities and selectivities for bacteria than the pL-ce6 conjugates described.

7.0. ACKNOWLEDGEMENTS.

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8.0. REFERENCES.


Trauma Induced Pain and Wound Management in Emergency Environment by Low Energy Photonic Therapy

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ABSTRACT

Low Energy Photonic Therapy (LEPT) is a new non-drug, non-invasive treatment modality for acute trauma and wound healing acceleration that utilizes monochromatic light. Various monochromatic optical sources (lasers, laser diodes and light emitting diodes) are used for LEPT. LEPT can be applied immediately after trauma. LEPT is administered with a short-term goal to achieve fast resolution of symptoms (pain, swelling, and inflammation) and function improvement. In a long run LEPT is expected to result in faster quality healing and function recovery (after trauma or surgery). A number of clinical studies on LEPT efficacy for wound healing, pain relief, and musculoskeletal conditions were performed using LEP2000 multi-modality therapeutic system for LEPT (IMI Inc., Toronto).

A large body of cellular and animal studies suggests that monochromatic light can activate phenomena vital for body healing. The effects induced by monochromatic light in cells could be of substantial magnitude, e.g., a 1.9-fold increase in cellular ATP or 3-fold increase in percentage of dividing fibroblasts and keratinocytes. Animal (rat, porcine) models confirm substantial (by 25-70%) acceleration of wound healing by LEPT. In a porcine wound model we discovered immunomodulation phenomena that resulted in faster resolution of systemic inflammation and wound healing in wounded (140 wounds) animal treated by LEPT. Animal studies

demonstrating acceleration and improved quality of healing by LEPT in various tissues (skin, muscles, tendons, bones, and nerves) will be presented. Recovery of median nerve function with LEPT as measured by nerve conduction test was demonstrated in a clinical trial in patients with carpal tunnel syndrome. Double blind study (DBS) on LEPT for chronic wounds confirmed 3.4-fold acceleration of wound healing in the LEPT-treated group. Another controlled clinical trial confirmed 1.79-fold acceleration of wound healing in LEPT-treated group that was accompanied by substantial pain relief.

Two double blind clinical studies and a number of open protocol studies demonstrated fast and substantial (by 30-100%) pain relief using LEPT. In a recent DBS involving 72 subjects with post-traumatic pain around joints LEPT resulted in a 4-fold greater pain relief as compared to therapeutic ultrasound. Anecdotal clinical evidence suggests that if LEPT is applied within 4 hours after injury, the recovery is extremely fast for injuries of ISS<9. If LEPT is applied within 72 hours after injury, a 2-3-fold acceleration in recovery is possible. No adverse effects were reported from using LEPT.

LEPT could be self administered by injured personnel. A portable device for LEPT delivery in a field environment is described. Visual materials of fast healing with LEPT will be presented. In summary, presented data suggest that LEPT induces fast pain relief, function and mobility improvement in operational environment after injuries of ISS<9, and accelerated quality rehabilitation to bring the soldier back to duties after injury.

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1. BASIC CONCEPTS AND ADVANTAGES OF LOW ENERGY PHOTONIC THERAPY FOR ACUTE TRAUMA

1.1 State Of The Art In Pain And Acute Trauma Management

Despite recent breakthrough in basic research in medicine, there are no effective treatments for musculoskeletal pain available. The conclusion of a recent review on pharmacological pain treatment for musculoskeletal disorders was that “the effectiveness of currently available drugs in the treatment of musculoskeletal pain conditions is disappointing”.

Authors of a recent review on the efficacy of current non-pharmacological treatments for musculoskeletal pain (ultrasound, electrotherapy, acupuncture, etc.) made a conclusion: “There seems to be evidence from basic science research to suggest that many of the therapies could have potentially therapeutic effects. However, there appears to be limited high-quality evidence from randomised clinical trials to support the therapeutic effectiveness of these therapies”.

A group of “Quebec Task Force on Whiplash” analysed over 10,000 abstracts and articles in an attempt to identify effective treatment/s for acute whiplash injury. They did not find conclusive evidence in clinical trials that would support the efficacy of current treatment modalities for acute whiplash injury, except exercises and mobilization.

Current paradigm of treatment for acute injury comprises passive modalities: rest, icing, elevation, and compression (RICE). There is no other effective conventional therapy currently available within the first
24-48 hours after trauma. As a result, the “golden hour” for the treatment is missed, an injured tissue condition rapidly deteriorates and recovery takes a long time.


Low Energy Photonic Therapy (LEPT) is a new non-invasive multi-facet modality for pain relief and soft tissue healing acceleration that involves the irradiation of tissue with monochromatic light at intensities that do not cause thermal changes or ionisation in tissues. Low-energy photons (LEPs) are quanta of monochromatic electromagnetic waves in the visible (400-700nm) and near-infrared (700-1100nm) ranges of wavelengths. Unlike high-energy photons (ultraviolet or X-Ray), LEPs have much less (< 2ev) energy than needed to break chemical bonds or ionise biomolecules. Different monochromatic optical sources (lasers, laser diodes and light emitting diodes) can be used for LEPT depending on the particular application. Low Energy (Low Level) Laser Therapy is a subset of LEPT.

For a medicine to be effective an appropriate dose must be administered. This also applies to physical modalities but is often ignored to the detriment of patient response and the validity of clinical trials. As a result of basic and clinical research supported by the Canadian Government, Drs. Norman and Natasha Salansky discovered that:

• Specific sets of optical parameters of low energy photons are required to produce significant improvement for various soft tissue pathologies (e.g., ischemia, swelling, acute, sub-acute or chronic inflammation). These sets of optical parameters include specific wavelength and bandwidth, modulation frequency, pulse duration and duty cycle, optical fluence and fluence rate, and three-dimensional photon distribution within the tissue. These sets of optical parameters are called “therapeutic optical windows” (TOW).

• To achieve substantial acceleration of healing for particular medical condition (e.g., acute trauma, tendonitis) it is required to combine several specific sets of optical parameters (TOWs) that work in synergy to produce maximum therapeutic effect. Combined therapy is provided by the integrated clinical protocols of LEPT.

The above concepts, the knowledge of “therapeutic optical windows” and integrated clinical protocols have been implemented in the medical device LEP2000 Multi-Modality Therapeutic System (IMI Inc., Toronto) for LEPT. A portable field unit with similar efficacy was developed for the use by the injured person. The field unit is small (cigarette pack size), light (less than 0.5 pounds), simple to use (a push button to activate requested optical protocols) and is battery operated. The unit is built using surface mount technology and it has demonstrated same level of efficacy for a specific application as the office unit.

LEP2000 Multi-Modality Therapeutic System has been used in all our basic and clinical research presented in this paper.

1.3 Basic Concepts In Pain Research. Innovation And Advantages Of Drs. Salansky’s Integrated LEPT Protocol For Acute Trauma

There are multiple pathologies that contribute to pain, soft tissue pathology, and function impairment after trauma (e.g., cell ischemia). The following basic approaches to the treatment of pain could be considered:

1. To attenuate or abolish transmission and/or reception of pain signal
2. To improve soft tissue pathologies that cause pain and accelerate tissue healing
3. A combination of 1 & 2.
The vast majority of current research on new therapies for pain relief is focused around the first approach in attempts to induce inhibitory control over pain transmission or pain receptors. However, despite the explosion of new knowledge in pain processing and in molecular background for neuroplasticity, this progress has unfortunately not resulted in a radical improvement of the ability to treat pain.

We use for pain control and soft tissue healing acceleration after acute trauma special integrated protocol of LEPT (Drs. Salansky’s protocol) that is aimed to improve soft tissue pathologies as well as to induce a release of pain-relieving biomolecules (a combination of the above approaches 1 & 2, see section 4.4). This protocol of LEPT consists of several specific procedures that are aimed to improve specific soft tissue pathologies (e.g., reduce cell ischemia, swelling) involved in acute trauma. These treatment procedures used in sequence one after another and include applications of single and cluster optical probes with required sets of optical parameters to the injured area.

Clinical research suggests that proposed integrated protocol of LEPT results not only in fast pain relief after an acute trauma: this protocol also appears to accelerate both inflammatory and repair phases of soft tissue healing and a functional recovery after an acute trauma. The acceleration of soft tissue healing is believed to be a direct result of the targeted by LEPT healing mechanisms involved and synergy between them. Advantages of the proposed integrated LEPT protocol for acute trauma include:

- It is non-invasive, no side effects, safety is proven.
- The treatment procedure is short, comfortable, simple (anybody could be trained to use it) and could be applied immediately after injury with or without ice.
- Uses synergy between several “therapeutic optical windows”. Results in extensive set of therapeutic effects (pain, swelling, inflammation relief).
- Pain relief and function improvement is almost immediate.

This integrated LEPT protocol is hypothesized to produce both antinociceptive effect and, in addition, acceleration of soft tissue healing and function recovery.

1.4 Early Intervention With LEPT Is Critical. 3-Hour And 72-Hour Time Windows To Achieve Substantial Acceleration Of Recovery After Acute Trauma

Substantial therapeutic benefits of LEPT can be achieved at any stage (acute, sub-acute, or chronic) after acute trauma. Drs. Salansky’s integrated clinical protocols are adjusted accordingly to the stage of the healing process. However, clinical research indicates that early LEPT treatment is critical to achieve maximum therapeutic benefits, immediate pain relief and fastest function recovery. LEPT could be applied immediately after trauma in combination with or without ice. Clinical data suggest that there appear to be 2 time “windows” where the most extensive therapeutic benefits are achieved.

The best time window is to administer LEPT within 3 hours after trauma.

If LEPT was administered within 3 hours after trauma, in many cases of Grade 1 sprain/strain injuries immediate complete resolution of symptoms and function recovery was observed, as the injury has never happened, and swelling and inflammation did not develop. In most cases of injuries (Grade 2, ISS<9) LEPT resulted in extremely fast resolution of symptoms.

If LEPT was administered within 72-hour window after trauma (preferably within 24-hours after trauma) the following results for Grade 1 & 2 sprains/strains (ISS<9) were obtained:

- 30-60% pain relief and substantial improvement of function after a single treatment
- 50-100% pain relief and 50-100% function recovery after 2-4 LEPT treatments within 96 hours. In cases of partial tendon tear (Grade 2 sprains), substantial acceleration of symptom
resolution and function recovery was observed, however, the actual tendon healing took longer as it was tested using diagnostic ultrasound

- early LEPT intervention appears to prevent the development of chronic conditions after injuries.

2. BACKGROUND FOR THE USE OF LEPT IN REHABILITATION MEDICINE.
BASIC RESEARCH (*IN VIVO, IN VITRO*)

2.1 General Comments On LEPT Mechanisms

A large body of cell culture and animal studies suggests that low energy photons (LEPs) can affect a broad range of biological processes vital for tissue healing. Unlike allopathic medicine, low energy photons with appropriate parameters can induce simultaneously a broad range of interconnected and interrelated photo induced phenomena at different levels of biological structures: cellular, tissue, and systemic. Substantial scientific evidence has been accumulated that low energy photons with specific parameters can induce acceleration of healing and improvement of quality of repair in various body tissues:

- Skin
- Muscles
- Nerves
- Connective tissue (tendons and ligaments)
- Bones.

Comprehensive analysis of mechanisms and therapeutic effects of LEPT in various tissues will be presented in the book “LOW ENERGY PHOTONIC AND LASER THERAPY: basic science, dosimetry, clinical applications, new developments”\(^1\).

As an example, we would like to outline the photo induced healing phenomena that can be observed in nerve tissue (based primarily on the research\(^1\) by S. Rochkind and associates):

- Immediate protective effects which increase the functional activity of the injured peripheral nerve
- Maintenance of functional activity of the injured nerve
- Reduced scar tissue formation at the injured site
- Reduced degeneration in corresponding motor neurons of the spinal cord
- Stimulation of axonal growth and myelinization
- Activation of Schwan cell proliferation
- Enhancement of sprouting of nerve processes.

The effects induced by LEPs in cells could be of substantial magnitude. Below are several examples of such magnitude effects induced by LEPs in vitro:

- Two to five-fold increases in growth-phase-specific DNA synthesis in normal fibroblasts, muscle cells, osteoblasts, and mucosal epithelial cells in tissue cultures\(^14\)
- A 1.9-fold increase in cellular ATP\(^15\). Two to three-fold increase in percentage of dividing fibroblasts and keratinocytes\(^16\)
- Four-fold increase in procollagen production in human skin fibroblast cultures\(^17\). Three-fold increase in keratinocyte motility\(^18\)

\(^1\) The references can be easily found in appropriate data bases.
Five-fold increase in IL-8 and 2.3-fold increase in IL-1α and their respective mRNA expressions by human keratinocytes\(^{19}\).

Substantial therapeutic effects or favourable alterations of cellular or tissue processes could be induced by LEPs only using specific protocols (sets of optical parameters and an appropriate three-dimensional (3D) photon distribution within the target tissue). We call them “therapeutic optical windows”. In the next section we will describe more in depth LEPT phenomena specific for wound healing acceleration.

2.2 Basic Photo Induced Phenomena Vital For Wound Healing

2.2.1 LEPT Induced Phenomena Vital For Wound Healing

2.2.1.1 Effects of LEPT on keratinocytes

Keratinocyte motility and proliferation have been found to increase after helium-neon (HeNe) laser (632.8 nm) irradiation\(^{20,21}\). HeNe laser irradiation (0.5, 1, and 1.5 J/cm\(^2\)) also has been shown to enhance the release of IL-1α and IL-8 from cultured human keratinocytes\(^{22}\). Maximum secretion (4.2-fold increase in both IL-1α and IL-8) of cytokines was observed at 1.5 J/cm\(^2\). Significant inhibition of kidney epithelial cell proliferation by a HeNe laser at much higher doses (12-140 J/cm\(^2\)) has been reported\(^{23}\).

2.2.1.2 Effects of LEPT on fibroblasts and collagen production

Numerous studies have shown increased fibroblast proliferation, collagen formation after exposure to laser irradiation\(^{24,25,26,27,28,14}\), while other studies failed to find any alterations\(^{29,30}\).

2.2.1.3 Effects of LEPT on the immunocompetent cells

Bacterium phagocytosis of leukocytes was considerably increased by a pulsed ruby laser (694.3 nm, pulse duration 1 ms) \textit{in vitro} at an energy density of 0.05 J/cm\(^2\) and inhibited at energy densities of (2-4 J/cm\(^2\)).\(^{4}\) Young demonstrated that macrophages release factors that stimulate fibroblast proliferation \textit{in vitro} after exposure to LEPT at wavelengths of 660 nm, 820 nm, and 870 nm\(^{31}\). When stimulating human peripheral blood monocytes with mitogens after the irradiation with HeNe laser substantial alterations of interleukin-1α, interleukin-2, tumour necrosis factor-α, and interferon-γ levels in the supernatants of the cultures were observed\(^{32}\). Significantly increased levels of all cytokines were detected after 30 min of irradiation (18.9 J/cm\(^2\)), whereas after 60 min of irradiation (37.8 J/cm\(^2\)) cytokines levels were found significantly decreased. Yü et al found that LEPs (660nm) enhanced secretion of basic fibroblast transforming growth factor (bFGF) by cultured fibroblasts\(^{33}\) in a dose dependent manner. Substantial increase (by 180-250%) of both spontaneous and Candida-induced reactive oxygen species (ROS) release by spleen phagocytes after an exposure to HeNe laser with energy densities of 10-30 mJ/cm\(^2\) was reported by Dr. Karu’s group\(^{34}\). Neutrophil chemotaxis was substantially depressed by HeNe laser irradiation with energy densities of 1, 2, and 4 J/cm\(^2\)\(^{35}\).

2.2.2 LEPT In Vivo. Effects Of LEPT On Healing Of Animal Model Wounds

There is clear evidence that LEPT does modify cellular activity (rate of ATP, DNA, RNA and protein synthesis, reproduction rate and cell secretion) \textit{in vitro}, depending on the specific wavelength and optical parameters, but the current literature paints an indistinct picture of its \textit{in vivo} effectiveness in wound healing\(^{36,37,38,39,47,48,49,50}\). Several studies showed significant increase in tensile strength of laser-treated wounds\(^{40,41,42,27}\), enhanced leukocyte infiltration and fibroblast proliferation\(^{43}\), epidermal thickening\(^{44}\), dermal vascularity\(^{43}\), and early epithelialization\(^{33,44}\) after HeNe laser irradiation of wounds in rabbits, rats and guinea
pigs. Several authors reported acceleration of wound healing in albino rats\textsuperscript{43} (632.8 nm, 4 J/cm\textsuperscript{2}) and white mice\textsuperscript{45} (694.3 nm, 1.4 and 5 J/cm\textsuperscript{2}), and burn healing in white mice (694.3 nm, 1.1 J/cm\textsuperscript{2})\textsuperscript{45} after LEPT. Irradiation with either HeNe laser or non-coherent lamp of the same wavelength accelerated healing of full thickness wounds in rats, while Argon laser failed to do so\textsuperscript{46}. Although the above early studies in loose-skinned animals (mainly rats, mice and rabbits) could show benefit on wound healing after LEPT, there were conflicting results from other studies that failed to show any significant improvement\textsuperscript{47,48,49}. Unfortunately, the comparison between the above studies is difficult, since optical parameters used were different. Although some of them had positive results, their validity was limited since most of them were either poorly controlled or were performed in loose-skinned animal models. Loose-skinned animals, such as rats, rabbits, dogs and guinea pigs are less adequate models for human wound healing, since they heal primarily through contraction, while tight-skinned animals, like pigs or human, heal primarily through epithelialization. We are aware of only a few studies on the effect of LEPT on wound healing in pigs\textsuperscript{28,38,50}. Two of them failed to show any effect of LEPT on wound healing in pig models while a 6.5-fold increase in type I procollagen levels was observed in the skin of pigs treated with HeNe laser in the third one.

Another series of studies on the acceleration of wound healing by LEPT in Sprague-Dawley (SD) rat model of normal wound healing were accomplished by Al-Watban et al\textsuperscript{51,52,53,54}. Acceleration of wound healing was achieved using HeNe laser with energy densities of 7 to 60 J/cm\textsuperscript{2}, while doses outside this range were found to be ineffective. Maximum acceleration (by 33\% in days to complete healing and by 54\% in size reduction) was achieved at the optimal dose of \~ 25 J/cm\textsuperscript{2}. Argon laser (514 nm) was found to induce an acceleration of wound healing in SD rats with doses of 7 to 60 J/cm\textsuperscript{2}. HeNe laser was found to be more effective for wound healing than Argon laser. Doses higher than 130 J/cm\textsuperscript{2} inhibited wound healing.

Two studies reported beneficial effect of LEPT for compromised wound healing. Statistically significant difference in the rate of healing of wounds contaminated with Staphylococcus aureus\textsuperscript{55} was observed in the LEPT (904 mW, 76.4 mJ/cm\textsuperscript{2}) treated group of SD rats. Yu et al\textsuperscript{56} used genetically diabetic mice to compare the effect of bFGF, LEPT (630 nm, 5 J/cm\textsuperscript{2}), and a combination of the growth factor and LEPT. Their results indicated that all three treatments significantly enhanced wound closure, with LEPT alone or in combination with topical application of bFGF being most effective. Histological evaluation showed higher leukocyte infiltration at the initial stage of wound healing, improved wound epithelialization, granulation tissue formation, and collagen deposition in LEPT group as compared to the control.

3. BACKGROUND FOR THE USE OF LEPT IN REHABILITATION MEDICINE.
CLINICAL RESEARCH

3.1 General Comments On LEPT Dosimetry

3.1.1 Low Energy Lasers (LELs) Showed Promise As A Possible Therapeutic Modality For Acceleration Of Soft Tissue Healing And Pain Relief. Inconsistency Of Clinical Results.

A large body of \textit{in vitro} and some animal studies that had been accumulated by 1990 demonstrated a surprisingly broad spectrum of photo-induced phenomena in mammalian cells and mammals by so-called low energy lasers (LELs). Many scientists supported the idea that LELs could be used for the acceleration of soft tissue healing and pain relief. However, most attempts to confirm putative therapeutic effects of LELs in rigorous controlled clinical trials failed\textsuperscript{57,58,59,60,61}. Brockhaus and Elger did confirm analgesic effect of needle acupuncture and did not find any analgesia using laser acupuncture. There were a number of negative controlled and double blind studies on the use of LELs for pain and musculoskeletal pathologies published.
3.1.2 Is Coherence And Polarization Important For Healing Phenomena? Dosimetry Is Critical.

In 1985, Dr. Salansky embarked upon the most fundamental aspects of light-biotissue interaction in an attempt to understand the real therapeutic potential of LELs and the reasons of the clinical failures\textsuperscript{2,6,3,6,4,6,5,6,6,6,6,6}. This research was initially supported by NRC of Canada and, later on, by DoD of Canada.

One of the basic questions was whether or not the coherence and polarization (specific laser features) was essential for photobiomodulation phenomena? Dr. Salansky discovered that a laser beam quickly loses its coherency and polarization in the biological tissue, except speckles, because of scattering phenomena and forwarded a hypothesis that coherence and polarization may not be important for many biomodulating effects of LEP irradiation. Several scientists presented data supporting this hypothesis, which is generally accepted today. There were enormous ramifications of this discovery, including the possibility of creating a therapeutic device based on light emitting diodes (LEDs, not on lasers) that could safely be used by patients for self-treatment.

Unlike plant cells, mammalian cells could exhibit dramatic responses to LEPs only within very specific narrow ranges of optical parameters. However, the incident optical parameters are very different from those that are actually “seen” by the skin cells because of photon scattering, absorption, reflection, and refraction. This could substantially affect \textit{in vivo} dosimetry of LEPT (sets of optical parameters including wavelength, three-dimensional light (3D) distribution of photon fluence and fluence rate, modulation frequency, etc.). Therefore, theoretical analysis of 3D distribution and laser/LED induced temperature changes within the skin was initiated using several mathematical and computer approaches (diffusion approximation, Monte Carlo, etc.)\textsuperscript{69,70}. Based on these theoretical calculations and the data from \textit{in vitro} and \textit{in vivo} studies we created the first LEPT clinical protocols for wound and soft tissue healing acceleration for further testing in animal and clinical studies.

As a result of photon scattering and absorption, a complex 3D distribution within the tissue takes place. A classical approach to photon distribution modelling in the tissue is based on Monte-Carlo (MC) algorithm\textsuperscript{71,72}. However, this approach does not permit to obtain photon distribution for different optical source parameters in real time. A neural network (NN) approach was developed\textsuperscript{73,74} which could be used for fast multi-parametrical solutions. We combined MC modelling with the NN approach for fast 3D photon distribution observation in the tissue in real time and adjusted the clinical protocols accordingly providing high efficacy.

3.2 Effects Of LEPT On Healing Of Human Wounds

Mester et al reported the first encouraging results on the use of LEPT for wound healing. As a result of an uncontrolled clinical trial on the use of HeNe (632 nm) and Argon (488 nm) lasers for wound healing, he reported 78\% of complete healing of recalcitrant ulcers of different etiology\textsuperscript{4}. In a series of case studies and an uncontrolled clinical trial Schindl et al. demonstrated complete healing of recalcitrant ulcers of several etiologies (diabetes, arterial insufficiency, radiation damage, and autoimmune vasculitis) with the use of LEPT\textsuperscript{75,76}. Dr. Salansky’s group reported complete healing or substantial improvement of 75\% of chronic and bacteria contaminated leg ulcers in uncontrolled clinical trials\textsuperscript{7,8}.

However, numerous attempts to prove efficacy of low energy lasers for wound healing in well controlled randomised comparative clinical trials have failed\textsuperscript{77,78,79,80}. There are only a few controlled clinical studies that provide some evidence of beneficial effects of LEPT for wound healing\textsuperscript{81,82,83}. LEPT has been shown to be beneficial for improving skin circulation at patients with diabetic microangiopathy, diabetic
ulcers or gangrenes in a randomised double blind controlled study by Dr. Schindl’s group. Another, well-controlled study demonstrated that if given prophylactically LEPT could lessen the severity of mucositis experienced by patients undergoing chemotherapy.

Jointly with the University of Toronto Dr. Salansky’s group demonstrated statistically significant superior efficacy of LEPT for chronic venous skin ulcers as compared to placebo in a recent double blind, small sample study. In this study nine patients with a total of twelve venous ulcers were randomised to either a LEPT treatment group or to placebo. The patients who were randomised to placebo treatment received placebo irradiation from an identical appearing light source from the same delivery system. In addition to LEPT both groups received wound care consisting of regular saline rinse followed by dry gauze dressing. The LEPT treatment group received therapy with two wavelengths, 660 nm and 880 nm, for 10 weeks. The ulcers were evaluated in weeks 0, 3, 7 and 10. The clinical parameters used for comparative healing were: change in ulcer area compared to baseline, percentage of ulcer area that remained unhealed, rate of ulcer healing (mm²/week). LEPT was found to be significantly more effective than placebo at weeks 3, 7 and 10, when the efficacy parameters were: change in ulcer area, percentage of ulcer area that remained unhealed. At week 10 (end of the study) patients receiving LEPT had extensive healing of their wounds. Only 24% of their wound area remained unhealed at 10 weeks compared to 85% in the control (see Fig. 1).
LEPT was reported in this trial to significantly decrease pain associated with leg ulcers. In most cases of chronic skin ulcers treated with LEPT in Canada significant pain relief was reported within first 2 weeks of LEPT. In one case\(^2\), 50-year-old male had been suffering from pyoderma gangrenosum ulcer for 10 years. He experienced excruciating pain and had to take narcotic Darvocet, 1-2 tablets every 4 hours in conjunction with 3-4 Advil every 2 hours in between. After 10 LEPT sessions the patient was free of narcotic. After 4 months of LEPT 60% of the ulcer healed (see Fig. 2).

Fig. 2. LEPT Took off the Patient from Narcotic Medication

LEPT appears to be effective for chronic skin wounds of different etiology (venous insufficiency, decubitus, vasculitis, radiation) including diabetic. Below is a picture of an infected diabetic ulcer in a 72-year-old male that successfully healed after 25 sessions of LEPT\(^3\)(see Fig. 3).

Fig. 3. Infected Diabetic Ulcer in a Patient with CVD

\(^2\) Courtesy of Scarborough Hospital, General Division (Toronto)

\(^3\) Courtesy of Scarborough Hospital, General Division (Toronto)
3.3 LEPT Is Effective For The Restoration Of Median Nerve Conduction And Resolution Of Symptoms In Patients With Carpal Tunnel Syndrome (CTS)

Carpal tunnel syndrome is a debilitating painful disease with high prevalence in many industries that require a lot of manual work. For instance, a staggering seventy-five percent of army dental hygienists reported having hand problems, and 56% exhibited probable or classic symptoms of CTS in a recent study. Jointly with the University of Toronto, we accomplished a prospective, open protocol clinical trial investigating if abnormal median nerve conduction could get restored in patients with persistent CTS. This trial was a new development from our previous clinical study that resulted in a complete resolution of symptoms in 15 (71.4%) patients with CTS after a course of LEPT. Upon completion of a course of LEPT treatment, most patients experienced substantial pain relief and improvement of their sleep at night (see Fig.4). In addition, normalization of mean median nerve latency from pre-treatment mean value 4.68msec (range 4.0-6.0) to 3.99msec (range 3.5-4.3) suggests a healing effect of LEPT on nerve regeneration (see Fig.4).

Fig. 4. LEPT efficacy for pain relief, sleep restoration, and nerve function recovery in patients with CTS

![Graphs showing pain VAS, sleep disturbance, and distal latency before and after LEPT treatment.]

3.4 LEPT Induced Substantial Pain Relief After Extracorporeal Shock Wave Therapy (ESWT) Procedure

Recently we had an opportunity to test the efficacy of LEPT for “acute-on chronic” pain in 13 cases of chronic foot pain that was treated by ESWT followed by LEPT in a non-randomised comparative clinical trial. ESWT is a novel treatment that is hypothesized to convert a chronic pathology to an acute one and, in addition, to numb nerve endings. This is believed to result in pain reduction. LEPT being applied after ESWT induced substantial pain reduction (see Fig. 5) as opposed to ESWT used alone or in conjunction with the other LEPT system Bioflex (Meditech International Inc.).

![Graph showing pain VAS before and after LEPT treatment.]

4 Courtesy of ESWT Pain Clinic (Toronto)
3.5 LEPT Is Effective For Chronic Whiplash Associated Disorder. Pilot Placebo Controlled Randomised Clinical Trial

While many individuals recover from MVA injury with conventional therapies including active exercise program, others develop chronic pain related to whiplash associated disorder (chronic WAD, >3 months). Recent Cochrane Database review did not reveal any effective therapies or treatment guidelines available for chronic WAD. Active exercise program does not appear to work for the individuals with chronic whiplash. Persistent pain appears to be a stumbling block for their recovery, and, in some cases, their pain is getting worse after exercises.

In this pilot clinical trial integrated protocols of LEPT (real or placebo) were applied to patients with neck and back pain that persisted more than 3 months after car accident. In addition, all patients received conventional therapy (CT, active exercise program and chiropractic care). Treatments were administered twice a week for 6 weeks (12 treatments total). After the course of treatment substantial statistically significant improvements in neck and back pain, and neck and back disability indexes were observed in the real LEPT group (see Fig. 6 -9). There was no improvement in the placebo treated group. Range of motion improved in both groups, however, it was greater in the LEPT treated group.
Fig. 6 Comparison of mean back pain VAS prior to and after the course of treatment (LEPT vs Placebo)

Fig. 7 Comparison of mean low back disability index prior to and after the course of treatment (LEPT vs Placebo)

Fig. 8 Comparison of mean neck pain VAS prior to and after the course of treatment (LEPT vs Placebo)
3.6 LEPT Provided Fast Pain Relief Of 56.8% For Chronic Post-Traumatic Sport Injuries That Did Not Respond To Conventional Therapies

This prospective clinical trial was carried out at the Institute of Sport Medicine & Wellness Centre (Toronto). 15 patients with chronic post-traumatic pain that was not completely resolved with the use of conventional therapies including other LEPT system Bioflex (Meditech International Inc.) received 1-4 treatments (2 treatments on average) with customized protocols of LEPT for a particular patient using LEP2000 system (IMI Inc.). The mean duration of their symptoms was 14.8 months. Significant pain relief by 56.8% was produced by LEPT after 2 treatments on average (see Fig. 10). This pain relief was found to be statistically significant (p<0.001) as compared to the baseline pain score.

Fig. 9 Comparison of mean neck disability index prior to and after the course of treatment (LEPT vs Placebo)

Fig. 10 Mean VAS pain relief after 1-4 LEPT sessions
4. LEPT AS A NEW PARADIGM FOR ACUTE TRAUMA MANAGEMENT

4.1 Cell Ischemia (ATP Depletion) As A Central Pathology In Acute Trauma. Cell Deterioration And Death

A large body of research suggests that impairment of microcirculation and ATP depletion in ischemic injured tissues plays a key role in the progressive cell injury. On average, a body cell has ATP resources (10^8-10^9 molecules of ATP per cell) that can support its life for 1-2 minutes without additional ATP synthesis. Under disruption of microcirculation, cellular ATP synthesis declines and progressive cell injury occurs. Cell deterioration begins with functional changes in the cell and cell membrane. Membrane transport and membrane potential declines, Na^+ enters and K^+ leaves cells, and Na-K ATPase is activated. ATP is depleted, and mitochondria are stimulated as increased lactate produces acidosis. Cell energy and cAMP levels decrease, ATPase is depleted, Ca^{2+} regulation is compromised, and nuclear function and protein synthesis are depressed. The cell swells, and further membrane changes occur with altered hormonal effects and mitochondrial uncoupling. Finally lysosomes leak, intracellular and mitochondria disruption occurs, and the cell is destroyed.

4.2 Early Intervention With Intravenous ATP Was Proven To Be Beneficial For Ischemia/Reperfusion Injuries

Based on the understanding of ATP depletion as the leading mechanism of cell death after injury, an intravenous (IV) intervention with a compound of ATP and a vasodilator MgCl2 to prevent ischemia/reperfusion injury was developed and tested in numerous studies. Benefits of the treatment with ATP-MgCl2 have been achieved on myocardial preservation during cardiac operations, kidney preservation for transplantation, and as metabolic support for the injured and septic patients. Substantial reduction or full resolution of I/R injury was achieved with early intervention of compound ATP-MgCl2. However, in many studies it has been emphasized that the efficacy of this approach strongly depends on the timing of the IV intervention with ATP-MgCl2. The maximum benefit of the therapy was achieved with prolonged intravenous infusion of ATP-MgCl2 introduced early prior to reperfusion. In a battlefield environment it may often happen that IV intervention becomes available too late, after substantial ischemic tissue damage has occurred.

4.3 Specific Protocols Of LEPT Are Proven To Increase Cellular ATP (In Vitro, In Vivo Studies)

Many therapeutic phenomena of LEPT in live organisms are believed to be based on the activation of ATP synthesis in cell mitochondria by LEPs. It is hypothesized that LEPs being absorbed by the primary photoacceptors in live cells may induce substantial activation of mitochondrial enzymes, components of the respiratory chain. This, in turn, is leading to the activation of ATP synthesis. Additional energy resources resulting from LEPT are used for cell and soft tissue repair. If ATP synthesis is activated in blood cells in substantial quantities, it becomes available for all body tissues via systemic circulation in a matter of minutes. Beneficial phenomena for soft tissue healing that can be induced by LEPT are described below.

Irradiation of low energy (typically 0.01-10Joules/cm² of skin or cell culture) could induce substantial enhancement of ATP synthesis and cell metabolism. For example, ATP synthesis was increased by 70% in liver cell mitochondria after exposure to He-Ne laser of 5J/cm² density. Similarly, DNA and RNA synthesis was increased by 60-100% after exposure to LEPs of energy density 0.01J/cm². The result of LEPs-live cell interaction is almost immediate, although it might have a long-term healing effect even after one LEPT treatment.
In this section we would like to present some encouraging data on the activation of ATP synthesis and other positive phenomena induced by LEPs in different experimental models and some clinical trials. Authors of\textsuperscript{95} hypothesized that photo-irradiation with Argon-dye laser (red emission, wavelength 660 nm) could increase ATP in isolated rat hearts (pre- or post- storage), and, therefore, reduce ischemia and improve their functional preservation. Both pre and post-storage treatment groups showed significant improvement in recovery of aortic flow, cardiac output, and work compared to the corresponding control groups. Investigation using isolated rat cardiomyocytes found that both end-storage ATP and end-reperfusion catalase activity in the laser-treated group were significantly higher than those in the untreated cells. The authors draw the conclusion that photo-irradiation improves functional recovery of the cold-stored rat heart possibly via conservation of ATP and antioxidant enzyme activity.

Dr. Yu and co-authors\textsuperscript{96} investigated the effect of photo-irradiation with Argon-dye laser on rat survival in the experimental model of sepsis. The cecal ligation and puncture (CLP) rat model was used. 36 Sprague-Dawley rats were divided equally among four groups: control (non-operative), sham operation, CLP treated with laser irradiation, and CLP without laser irradiation. The peritoneal cavity of each animal in CLP/laser group was irradiated immediately after CLP using an Argon-dye laser at a wavelength of 630 nm and at a fluence of 5 J/cm\textsuperscript{2}. Laser irradiation (LI) significantly improved ex-vivo lymphocyte proliferation of cells from septic rats (179.7 vs. 129.5) and survival in septic rats (79\% vs. 42\%). LI significantly stimulated lymphocyte proliferation in the presence of mitogenic stimuli and enhanced lymphocyte ATP synthesis. The conclusion was drawn that LI may be useful as an adjuvant therapy for sepsis.

Positive phenomena of intravenous laser blood irradiation (IVLBI) were observed in a dog model of I/R injury.\textsuperscript{97} The effect of low-intensity intravenous laser blood irradiation (IVLBI) on morphofunctional characteristics of erythrocytes and circulation parameters has been studied experimentally on 22 adult inbred anesthesized dogs, of both sexes, during a 2-hour hemorrhagic shock, and in the first hours after resuscitation. It has been established that the use of IVLBI during 45 min of hemorrhage shock stabilizes erythrocyte membranes and improves myocardial function.

Efficacy of IVLBI with He-Ne laser was investigated as an adjunct therapy in the management of peritonitis involving 350 patients.\textsuperscript{98} The patients were randomised to two groups: Group 1: 245 patients were treated with IVLBI as an adjunct therapy to conventional therapies; Group 2: 105 patients were treated with conventional therapy only.

The conclusion was made after the completion of the study that IVLBI with He-Ne laser was effective as an adjuvant therapy in the integrative management of peritonitis. Mortality in the laser-treated group was 2.8\% as opposed to 14.2 \% in the control group. The percentage of post-surgical complications (sepsis, purulent wounds, lung and heart failures, thrombophlebitis, etc.) was reduced from 53.3\% in the control group to 21.2\% in the laser-treated group. The average stay in the hospital was reduced from 27.4 days in the control group to 18.5 days in the laser-treated group. In the experimental group of patients faster resolution of acute inflammation (leucocytosis, neutrophilia, lymphopenia), normalization of body temperature and blood indexes were noticed as compared to control group.
4.4 Drs. Salansky’s Integrated Clinical Protocol For Acute Trauma. Hypothesized Therapeutic Effects.

It is well known that acute trauma can lead to multiple underlying pathologies of the injured tissue, e.g.:

- Cell ischemia
- Physical distention of the joint capsule or fascial compartments by blood or tissue fluid transudate
- Tissue damage followed by activation of kinin and prostaglandin systems
- Massive release of pain and proinflammatory mediators
- Disturbance in acid-alkaline balance
- At later stage extravagated blood or necrotic tissue will initiate non-bacterial inflammation and secondary release of pain and inflammatory mediators.

Each of these pathologies could contribute to the compound pain and function impairment. After thorough analysis of basic and clinical studies, and theoretical modelling of LEPT phenomena, we came to the conclusion that it is not possible to improve all above pathological conditions with a single LEPT approach. Hypothetically, for each particular soft tissue pathology a specific LEPT set of optical parameters (specific “therapeutic optical window”) is required. Therefore, in order to increase efficacy of LEPT, we developed a concept of integrated clinical protocols when several LEPT procedures are applied one after another in physiologically justified sequence.

We propose to use for pain control immediately (or any time within 2 weeks) after acute trauma an integrated protocol of LEPT that includes several specific treatment procedures of LEPT applied in sequence one after another. Each of these specific LEPT procedures for acute trauma was designed to induce one of the following healing macro-mechanisms in the injured soft tissue that can result in fast cumulative pain relief and soft tissue condition improvement:

- Photo-induced release of pain-relieving molecules by local stimulation. Additional non-invasive stimulation of related acupuncture points (no-needle acupuncture) could enhance analgesic effect.
- Direct photo-activation of ATP synthesis in the injured tissue. This protocol is believed to reduce ischemic pain in the injured tissue.
- Transient photo-induced vasoconstriction in the injured area. This protocol is believed to reduce edema and, therefore, attenuate pressure pain.
- Photo-immunomodulation is believed to accelerate removal of cell debris from the injured area, reduce the duration of inflammatory stage, and reduce concentrations of pain mediators in the injured tissue: bradykinin, proinflammatory prostaglandins, etc., and, as a result, to attenuate pain related to inflammation.
- Photo-induced relief of abnormal muscle activity (in particular, muscle spasm) that is often accompanies injury. This is believed to relieve pain that may be induced by muscle spasm.

Based on the animal and clinical studies, this integrated clinical LEPT protocol appears to produce substantial fast pain and swelling relief and wound healing acceleration if applied early enough after acute trauma. It was also demonstrated in numerous case stories that cumulative pain relief is greater after application of all above specific procedures one after another as compared to an application of a single procedure only.
4.5 LEPT Is Effective For Acute Whiplash Injury And Post-Traumatic Pain In Joints

4.5.1 LEPT Is More Effective And Provides Faster Recovery After Acute Whiplash Injury Than Conventional Therapies: Controlled, Randomised, Comparative Clinical Trial

This study had an objective to verify if LEPT being applied in addition to conventional therapies could accelerate patient recovery after acute whiplash injury. 54 patients after acute whiplash injury were randomly allotted to the following groups: Group #1 (17 pts) received Chiropractic Manipulative Therapy (CMT); Group #2 (18 pts) received CMT plus Exercise Therapy (Ex); Group #3 (19 pts) received CMT plus Ex plus LEPT. Therapy was administered three times per week during 8 weeks. Extensor muscle strength (EMS) was measured prior to the course and at weeks 2, 4, 6, and 8 of therapy. Uninterrupted sleep (US) at night was evaluated prior to the study and at week 8 using sleep questioner. Statistical analysis revealed that EMS recovered faster in LEPT group in comparison with 2 other groups: statistically significant increase in EMS was observed at week 4 (see Fig.11) as opposed to week 8 in Ex group. There was no statistically significant increase in EMS in the group treated with CMT until the completion of the trial. There was higher increase in EMS (by 23%) in LEPT group in comparison with Ex group (by 15%) and CT group (by 9%) after 8 weeks of therapy. In addition, patients in LEPT group slept better as compared to other treatment groups (see Fig. 12).

4.5.2 LEPT Is More Effective Than Ultrasound And Placebo For Joint Pain Relief: Proof Of Concept In 2 Double Blind Clinical Trials

Jointly with McMaster University (Hamilton) and a teaching hospital of the University of Toronto (Scarborough Hospital, General Division) were accomplished 2 double blind studies on the efficacy of LEPT for pain relief. LEPT (both real and placebo) equipment and clinical protocols for the trials were provided by IMI Inc.

4.5.2.1 Study 1

This study was prospective, randomised, comparative, and double blind. 75 subjects (41 females and 31 males with mean age of 47.5 years, range, 18-78) suffering from sub-acute (2 weeks – 6 months duration) or chronic (more than 6 months duration) pain around joints were enrolled in this study in accordance with the inclusion and exclusion criteria: Group #1 (26 subjects) received LEPT; Group #2 (24 subjects) received
placebo LEPT; Group #3 (25 subjects) received ultrasound (US). Two treatments of selected in accordance with the random number treatment modality were administered to the patients on the first and third days of three consecutive days.

No pain, anti-inflammatory medication or steroid injections were used during the study period. Pain at rest by 10 cm vertical visual analogue scale (VAS) was taken prior to and 20 minutes after each treatment. In addition, pain pressure threshold (PPT) using dolorimter was measured at the most tender spot of the affected area prior to the first treatment. Initial measurements of PPT were included to the study to make assessment of local baseline soft tissue tenderness in different study groups and investigate if randomisation in the study was not biased in regards to this local tenderness parameter.

Neither the patients nor the physiotherapist administering the treatment were aware of which treatment modality (placebo or real LEPT) was used. Outcome measurements were taken by a different evaluator in a separate room from the treatment area. The evaluator, the biostatistician and the investigators were completely blinded in regards to the treatment modality used: ultrasound, placebo or real LEPT. 72 subjects completed the study. There were 3 patients who did not come for the second treatment: one patient dropped out from the placebo group and 2 patients dropped out from the ultrasound group.

Fig. 13 LEPT efficacy for sub-acute and chronic pain relief compared to Placebo and Ultrasound

![Graph showing pain relief comparison between LEPT, Placebo LEPT, and Ultrasound]

There were no drop-outs from the LEPT group. One-way Analysis of Variance (ANOVA) revealed no statistically significant differences in mean age, symptom duration, initial pain level rating nor pain pressure threshold among the Groups. Chi-square analysis revealed no statistically significant difference among the groups in gender, chronicity, or the relative size of each group. It was no statistically significant difference between groups in the relative numbers of each joint (ankle, knee, wrist or elbow). The lack of significant
differences in the above variables suggests that the methods of randomisation used in the study were effective and the randomisation was not biased. ANOVA revealed a statistically significant difference in the mean change in VAS-measured pain level ratings between Groups (F=7.78; df 2.71; p=0.0009). Post-hoc analysis revealed a statistically significant difference in the mean change in VAS-measured pain level ratings between real LEPT and placebo LEPT (p=0.005). There was also a statistically significant difference in the mean change in VAS-measured pain level ratings (PRLs) between the real LEPT and US groups (p=0.002). The difference in the mean change in VAS-measured PRLs between placebo LEPT and ultrasound was not statistically significant. Mean VAS-measured pain level ratings declined by 40% (from 4.26 to 2.52) after 2 LEPT treatments in real LEPT group (see Fig. 13). Whereas, these pain measurements indicated, on average, only a 14% decline (from 3.73 to 3.22) in the placebo LEPT group and only a 10% decline (from 4.48 to 4.02) in the US group.

4.5.2.2 Study 2

This study had the same design as the previous one. The difference was in the duration of symptoms. 22 patients suffering from acute (6-14 days) or sub-acute (2 weeks – 6 months duration) pain around joints were randomly allotted to the following treatment groups: Group #1 (7 pts) received LEPT; Group #2 (8 pts) received placebo LEPT; Group #3 (7 pts) received ultrasound. Statistical analysis confirmed statistically significant difference in the mean change in VAS-measured pain level ratings (from pre- to post- treatment) between real LEPT and placebo LEPT (p=0.0044). There was also a statistically significant difference in the mean change in VAS-measured pain level ratings (PRLs) between the real LEPT and US groups (p=0.019).

Mean VAS-measured pain level ratings declined by 53.5% (from 4.46 to 2.07) after 2 LEPT treatments in real LEPT group (see Fig. 14). Whereas, these pain measurements indicated, on average, a 20% decline (from 3.59 to 2.87) in the placebo LEPT group and a 31.6 decline (from 3.70 to 2.53) in the US group.

![Fig. 14 LEPT efficacy for acute and sub-acute pain relief compared to Placebo and Ultrasound](image-url)
4.6 LEPT Assisted To Wound Healing Acceleration In A Partial Thickness Pig Wound Model

In a collaborative effort with the University of Miami a placebo controlled pilot study on LEPT in partial thickness wounds in a pig model\textsuperscript{101} was accomplished. The pig model was chosen for our experiment because the skin of pigs is the closest animal model available to that of humans\textsuperscript{102}. All previous published attempts to find acceleration of wound healing in pig models by LEPT have failed.

One animal was treated with LEPT and the other one served as the control. Wounds were induced on each animal and divided in four groups. A total of 280 wounds were assessed. LEP 2000 (IMI Inc., Toronto) system for LEPT was used in this study. Beginning on Day 4 and each day thereafter Epidermal Migration Assessment was performed to five wounds of each treatment group. Epidermal Migration Assessment is a method for assessing epidermal wound healing.\textsuperscript{103,104,105,106,107} In addition, studies to measure the immune response of the experimental pig to LEPT were also performed.

As one can see in Fig. 15, the greatest rate of healing was observed in the experimental pig in the group that received LEPT prior to and after wounding. This group displayed an increase of 32\% in the rate of wound healing observed when compared with control animal. To date, the most effective treatment for this type of wounds is occlusive dressings. The effect of LEPT that we obtained for wound healing in pig is comparable to that induced by the occlusive dressings and various growth factors using this model\textsuperscript{98}. An acceleration of 27\% was observed in the rate of wound healing for both groups of wounds treated with only red low energy photons and the group treated with the combination of red and infrared low energy photons. The rate of wound healing was accelerated by 16\% in air-exposed wounds of the experimental animal, in comparison to the control animal. These results suggest that LEPT accelerates wound healing locally and may have some systematic effects. Moreover, the concept of inducing acceleration of wound healing by pre-treatment with LEPT could be very appealing. It is obvious its potential relevance to clinical applications in which patients are scheduled for elective surgery, as reduction of complications and acceleration of post surgical recovery are of great importance.

![Fig. 15 Percentage of Wounds Healed](image-url)
4.7 Systemic Immunomodulation Phenomena Induced By LEPT

We accomplished several pilot studies aimed to investigate if red LEPT (non-coherent monochromatic light of 660 nm wavelength) could induce alterations in systemic blood indexes and nonspecific immune system of animals (rabbits, rats, pigs) and healthy human volunteers. Phagocytic leukocyte oxygenation activity (PLOA) and differential blood count were chosen as initial markers of systemic body response to LEPTs exposure. Numerous studies suggest that PLOA is very sensitive to changes in the organism, its activity, or environment, in particular, to wounding, trauma, inflammation, pathological stress, air pollution, etc. The changes in PLOA in response to LEPT are almost immediate what allows utilizing this measurement as a feedback for a body response to LEPT.

PLOA has been studied using luminol-amplified chemiluminescence measurements in highly (1:400 or 1:800) diluted whole blood stimulated by opsonized zymozan. Research suggests that release of reactive oxygen species (ROS) by challenged neutrophils contribute more than 90% of whole blood chemiluminescence (WBCL) under these experimental conditions. The advantage of measuring PLOA in whole blood is that it could be measured immediately in intact cells avoiding a procedure of isolating neutrophils that could induce alterations in their PLOA. WBCL is proportional to the concentration of active phagocytes in blood and their individual activity. Normalized oxygenation activity per neutrophil has been calculated dividing WBCL value by neutrophil concentration N (WBCL/N).

For the immune studies in our above pig wound model blood was drawn from an ear vein and measurements made prior to and after each LEPT irradiation. Wounding in the control animal resulted in the moderate (18%) activation of neutrophils after wounding, with rapid return (<24 hours) to near baseline activity. This would be expected in an animal with an acute injury and no further trauma or infection. Interestingly though, the LEPT treated animal experienced a slight increase in total WBCL (by 6.2%) and a substantial activation (by 67%) of reactive oxygen species release per neutrophil (WBCL/N) after pre-treatment with LEPT. The neutrophils remained activated for several days thereafter although wounding did not increase the level of activation any further. The results of the differential count of neutrophils and lymphocytes indicated that at the control animal there was neutrophilia and lymphopenia (70% neutrophils, 24% lymphocytes), which persisted after day 4, in response to the wounding. At the same time, there was no neutrophilia or lymphopenia in the LEPT treated animal (see Fig. 16).
Our experiment suggests that local exposure of porcine skin to LEPT with the given optical parameters could induce systemic immunomodulation phenomena, in particular, an activation of individual PLOA per neutrophil. The findings support the hypothesis that LEPT induces not only wound reepithelialization but also activation of non-specific immune responses during the early stages of wound healing and faster resolution of the inflammatory stage.

4.8 LEPT For Acute Trauma. Case Reports.

4.8.1 LEPT Was Administered Within 3 Hours After Injury

4.8.1.1 Case 1. Immediate resolution of symptoms and function restoration with LEPT after punch injury

Ms. N.D., 26 y.o.
Wrist injury sustained in kick-boxing from a punch. VAS 7-8/10. ½ ROM available into both flexion and extension. Unable to weight-bear on hands (e.g., push-up). Within 3 hours, LEPT (protocol for acute trauma) was used, 4-6 minute treatment in total. Immediately post-treatment, there was no pain (0/10), full, pain-free range of motion, and no pain with weight bearing. No further treatment was used, no pain returned. Sport was returned to as normal.

5 Courtesy of Honsberger Physiotherapy Clinics (Toronto)
4.8.1.2 Case 2: Immediate relief of pain and swelling reduction following LEPT for hand injury

This 47-year-old male presented to the hospital with an acute hand crush injury within 3 hours after the injury. LEPT (acute trauma protocol) was administered to the hand during 20 minutes; no ice was applied. The patient did not change his hand position. The pictures (Fig. 17) were taken prior to and immediately after LEPT. Swelling and pain substantially reduced.

![Prior to LEPT](image1) ![Immediately after 20 min of LEPT](image2)

**Fig. 17 Fast resolution of pain and swelling after LEPT**

4.8.2 LEPT Was Administered Within 24 Hours After Injury

4.8.2.1 Case 1: Fast resolution of symptoms and function recovery after acute Grade II ankle sprain

This 18-year-old female, competitive volleyball player received first LEPT treatment 24 hours after a Grade II lateral ankle sprain. Immediately after the first LEPT session the pain reduced by 45% and weight bearing substantially improved followed by a substantial swelling reduction 3 hours later. After second LEPT session pain became minimal and stayed at this level since ever (see Fig. 18). In 72 hours she resumed training (like stationary cycling), 9 days after the injury she played in a volleyball tournament with an assistance of ankle support. A follow-up diagnostic ultrasound confirmed complete healing of the tendon. At 6-months follow-up there was no complaints on the ankle and she was able to play beach volleyball. In a testimonial Ms. C.N. stated that similar previous injuries to her ankles kept her away from sports for at least for 5 weeks.

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6 Courtesy of the Scarborough Hospital, General Division (Toronto)
4.8.2.2 Case 2. Fast resolution of symptoms after toe fracture with LEPT. No complaints for 2 years.

Testimonial
September 14, 2001
B. K., B.A.Sc., CA

To whom it may concern

I would like to bear witness to the beneficial effects that Low Energy Photon Therapy (LEPT) had for me for the trauma I experienced last year. Last fall I fractured my toe. I was in severe pain and had great difficulty walking. There was substantial bruising and swelling in the trauma area. My family doctor identified toe fracture. Next day after the injury (on Friday) I received treatment with Low Energy Photon Therapy at the Pain and Injury Clinic (Toronto) using LEP2000 device. After the first treatment I experienced a substantial immediate pain relief that lasted about 2 hours. Then pain stabilized at the lower level as compared to the pre-treatment condition. Next 2 days (it was weekend) I was treating myself with the portable home device for LEPT in accordance with the instructions given to me at the Clinic. On Monday I received second LEPT treatment at the Clinic. Again, I experienced substantial immediate pain relief. After this treatment my pain stayed at low level and I was able to conduct most of my regular activities without any substantial discomfort. In 10 days after the injury my pain was gone. Since that time I did not experience any pain or discomfort in the injured area.
4.8.2.3 Case 3: Professional cricket player was able to continue his participation in the International Cricket Cup (ICC) after Grade 2 muscle strain with LEPT treatments

Mr. L. was seen at the on-site clinic covering ICC. He presented with a one-day history of a calf strain. The pain by verbal analogue scale (VAS) was rated at 8/10. Mr. L. demonstrated a limp during gait, had marked tenderness through the right lower leg muscles. The overall assessment indicated a grade 2 muscle strain of the lower leg muscles. His initial treatment included Low Energy Photonic Therapy (LEPT), myofascial release, education in proper stretches and icing protocols, and complete rest from training and competition. The next day Mr. L. was again seen in the clinic. He reported his pain level to be 6/10. His physiotherapy included LEPT and myofascial release. He was to take one more day off, but had a probable return to play next day. Mr. L. was seen the day after playing. Even though playing a full cricket match (7-8 hours), his pain level still dropped. His gait pattern was much improved. Treatment was as above. Mr. L. was seen successfully over the next 3 days. The level of pain continued to drop and he continued to play with full participation.

4.8.2.4 Case 4: Fast resolution of symptoms with early LEPT intervention after double (nose and rib) fractures. No complaints at 20 months follow-up

This 79-year old lady sustained double fracture (nose and rib) from the fall in her bathroom. First LEPT session was administered within first 24 hours after the injury. The nose was pain-free after 2 LEPT sessions, the rib was pain-free after 4 LEPT sessions (see Fig. 19).

![Fig. 19 LEPT for the treatment of double fracture](image)

40 hours after injury, 24 hours after 1st LEPT session

12 days after injury, the nose was pain-free after 2 LEPT sessions; the ribs were pain-free after 4 LEPT sessions

7 Courtesy of Honsberger Physiotherapy Clinics, Toronto
4.8.3 LEPT Was Administered Within 96 Hours After Injury

4.8.3.1 Case 1. Fast healing of 2-3-degree burn using portable LEP2000 device for LEPT

This 56-year-old female presented with 2-d-to-3d degree domestic burn that she sustained 4 days prior to the first LEPT session. She complained excruciating pain (8 on a 0-10 verbal analogue scale) and drainage from the wound. She started treating herself with the portable LEP2000 device (IMI Inc., Toronto), twice a day. After several LEPT sessions pain substantially reduced (to 2 on a 0-10 VAS) and drainage stopped. 6 days after 1st LEPT treatment the wound was 60% epithelialized and 40% covered by a crust. 7 days after 1st LEPT sessions the crust was off. In 10 days (20 LEPT sessions) the burn completely healed (see Fig. 20). Usually healing of such burn may take 3-4 weeks.

4.83.2 Fast resolution of bruising with LEPT

This 71-old female sustained severe head injury from a fall. She has been suffering insulin-dependent diabetes type II. She presented with severe bruises around her eyes and on the face, especially on the right side. She complained a moderate pain in the injured area. LEPT was focused on the forehead and the eyelids as the areas of main concern. After 2 LEPT daily sessions, bruising was substantially reduced and pain was gone. In 6 days after 4 LEPT sessions bruises around her eyes and on the forehead disappeared (see Fig. 21. Make a note that the bruise on the non-treated chick area was not resolved. Usually resolution of such bruises in elderly patients may take over 2 weeks. Follow-up 1 year after the injury she did not have any complaints related to the injury.
4.9 Comparative Analysis Of The Rate Of Symptom Relief And Function Recovery After Acute Ankle Sprain (Grade II) Using LEPT Versus Conventional Therapies (CT)

Current passive treatments for acute ankle sprain could not be considered as satisfactory. A recent 6-18-months follow-up (after medical evaluation) survey revealed that 72.6% of patients who had suffered an acute ankle sprain reported some unresolved residual symptoms. Of these, 40.4% reported at least one moderate to severe symptom.

The following table represents comparative analysis of the rates of recovery using LEPT (early administration within 72 hours) and conventional therapies. This analysis is based on case reports on LEPT use and existing literature data on CT.
Table 1 Comparative Analysis Of The Rate Of Symptom Relief And Function Recovery After Acute Ankle Sprain (Grade II) Using LEPT Versus CT

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Low Energy Photonic Therapy</th>
<th>Conventional Therapy\textsuperscript{114,115}</th>
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</table>
| Pain      | 1. Walking VAS pain reduced by (30-60\% immediately after 1-st LEPT session as compared to baseline.  
               2. Walking VAS pain reduced further by (50-100\% immediately after 2-d LEPT session as compared to baseline.  
               3. After 2-3 daily LEPT sessions walking pain is minimal or non-existent | Patients suffer significant pain for at least 1 week after the injury. This pain gradually subsides during the second week after the injury with a residual pain that may last over 2 weeks. |
| Swelling  | 1. Swelling is visibly reduced in 1-3 hours after 1-st LEPT session. Next day swelling is much less compared to baseline.  
               2. After 2-3 daily LEPT sessions swelling is minimal and restricted mostly to the area surrounding tendon tear or sprain as assessed by a diagnostic ultrasound | Swelling and inflammation is substantial for at least one week after the injury followed by a gradual decline during second week. |
| Inflammation |                                                                                             |                                                                                                              |
| Weight bearing | 1. Weight bearing with reduced pain restored immediately after 1\textsuperscript{st} LEPT session.  
               2. Weight bearing with low pain level (0-3 out of 10 by VAS) is possible after 2 LEPT sessions.  
               3. After 2-3 daily LEPT sessions pain-free or minimal pain weight bearing is restored. | Weight bearing is accompanied by substantial pain for at least one week after the injury followed by a gradual improvement at weeks 2-3. |

4.10 Summary Of Case Reports.

If LEPT (Drs. Salansky’s protocols) was administered within 3-hour window after trauma, in most cases of Grade 1 sprain/strain immediate complete resolution of symptoms and function recovery was observed, as the injury has never happened.

In most cases of Grade 2 injuries (ISS<9) LEPT resulted in fast (within 1-3 days) significant improvement of symptoms and function recovery. Tendon healing as it was assessed by diagnostic ultrasound took longer.
In a small percentage of cases, individuals overused the injured area that was treated with LEPT as they did not have restrictions related to pain and swelling. This was followed by a short exacerbation of pain and restriction of function. After additional LEPT sessions the cases were resolved. Further research is needed to identify the range of functional activity appropriate after fast resolution of symptoms with LEPT.

In several cases of bilateral injury only the worst side was treated by LEPT. In a short-term pain was resolved bilaterally. However, at a follow-up (3-6 months) the untreated areas had pain while treated with LEPT areas continued to be pain-free. This may be suggestive that early intervention with LEPT may reduce chronicity development. Further research on prevention of chronicity development with LEPT is indicated.

5. SUMMARY

1. This paper describes basic concepts and advantages of new LEP therapy for pain and wound management after acute trauma.
   This therapy is non-invasive, easy to use and could be applied immediately after trauma. The therapy provides significant pain relief even after a few minutes of treatment and has no side effects.
   This therapy is available at institutional environment (office unit) and at the battlefield (with a portable field unit), weight less than half-pound.

2. Background data (basic research *in-vivo* and *in-vitro*, as well as clinical results) are presented to elucidate physiological mechanisms involved in the therapy and substantiate clinical applications of LEPT. The photo-induced healing mechanisms represent justification for LEP therapy.

3. The concept of “therapeutic optical windows” is presented and 3D dosimetry considerations provide a scientific basis for the technology development and explain high efficacy achieved in the clinical trials and case reports with the use of LEP2000 Therapeutic System (IMI Inc.).

4. LEP therapy (LEP2000) has been tested for treatment of different cites of traumatic injuries (neck, ankle, wrist, etc.) exhibiting consistent positive results for both short term (immediate symptom relief) and cumulative healing effects after a course of therapy.

5. Comparative data with conventional therapies are presented that suggest that LEPT using LEP2000 Therapeutic System is more effective than conventional therapies.
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Delta-Selective Glycopeptides Related to Enkephalin Produce Profound Analgesia with Reduced Side Effects in Mice

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ANALGESICS BASED ON ENDOGENOUS NEUROPEPTIDES

The use of endogenous neuropeptides such as enkephalins and endorphins as drugs has remained an elusive goal since the 1970’s. The principle reason for this is that peptides generally do not cross the blood-brain barrier, and are quickly degraded in the blood stream prior to delivery to opiate receptors in the brain. Animal research with glycosylated enkephalins and endorphins (dynorphins) indicates that potent analgesia is possible after intravenous or sub-cutaneous injection. Glycopeptides derived from delta-selective opioid agonists have 2-3X the potency of morphine, and lack many of the side effects associated with mu-agonists such as morphine. Morphine is still used on the battlefield for combat casualty care, and it is anticipated that further development of the glycopeptide analgesics will result in superior analgesics with greatly reduced side effects. Recent developments in this area are reported.

1.0 INTRODUCTION

There is no question that the American Civil War was a medical learning experience for the doctors involved in it. The American Civil War provided the setting for the first genuinely effective care of combat casualties with the introduction of field hospitals on or near the battlefield, and early treatment of casualties. (Figure 1) The pharmacopoeia of the day was not extensive by today’s standards, but among the most effective agents were ether and chloroform anaesthetics used during amputations and other procedures, and morphine, used for the treatment of pre- and post-operative pain.1 In the South, the scarcity and expense of imported drugs forced the Confederate Army to establish several medical laboratories to manufacture drugs for military use.2 Empirical testing in military hospitals helped determine the clinical value of indigenous remedies. During this war morphine, both in its pure form and in various impure preparations of opium, gained its first widespread use on the battlefield, and in hospitals far removed from the field of battle. While there have been many advancements and refinements in combat casualty care in the intervening 130 years, morphine and its congeners are still used extensively, with many of the same unwanted side effects that were noted by the physicians of the 1860’s. Chief among these unwanted side effects were respiratory depression and lowered blood pressure. It will never be known for certain, but it is very likely that opiates given to Stonewall Jackson in the course of his “diligent care” contributed to his death 8 days after the successful amputation of his left arm. It has recently been concluded that hemorrhagic shock and pneumonia, both possible sequellae of opiate administration, contributed to the death of this Confederate general, and consequently dealt a serious blow to the Confederate cause.3 The problems of opiate induced respiratory depression are followed closely by the problems associated with tolerance and physical addiction. So widespread was the problem of opiate addiction of former soldiers after the war that it was given the term “veteran’s disease.”

2.0 ENDOGENOUS OPIOID PEPTIDES

Long before the discovery of the opioid peptides, it was suspected that mammals produced an endogenous substance with morphine-like effects. Eventually, with the aid of immunocytochemistry, these substances were discovered, and eventually isolated and chemically characterized. Three major classes exist: the relatively large dynorphins and endorphins (sometimes collectively referred to as endorphins), and the much smaller enkephalins (methionine enkephalin and leucine enkephalin). All of these peptides are enzymatic hydrolysis products of much larger precursor proteins that have a wide variety of neuropeptides embedded within their sequences. The enzymatic cleavage of these precursor peptides into the neurotransmitters and neuromodulators that are secreted by neurons allows for many pathways for regulation, and is a complex issue that will not be discussed here.4

3.0 ENHANCED STABILITY AND BBB TRANSPORT OF GLYCOPEPTIDES

With the discovery of the endogenous opiate peptides in the 1970’s, and the recognition of their high selectivity and potency, it was initially anticipated that a new pharmacopoeia based on met-enkephalin, leu-enkephalin, or β-endorphin would emerge. Since these peptide opiates are degraded to pharmacologically inert amino acids, whereas morphine and similar alkaloidal pharmaceuticals produce a cascade of biologically active metabolites, it was logically (and correctly) assumed that peptide analgesics would possess a limited side effect profile. Problems associated with the physicochemical features of peptides, including their metabolic liability have been largely solved in the intervening years with the introduction of un-natural and/or D-amino acids, and by covalent modifications of the peptide backbone. Unfortunately, the pharmacodynamic behaviour of most peptides is still poor, and the blood-brain barrier (BBB) remains as a significant and largely unsolved deterrent to the effective delivery of peptide-based central analgesia. The BBB is not only a physical barrier represented by the tight junctions of the cells of the brain microcapillaries, but is also an enzymatic barrier caused by a broad spectrum of proteolytic enzymes and specific peptidases.

A significant advance was made in the transport of enkephalins was reported in 1994, when it was noted that glycosylated enkephalins penetrate the BBB to produce centrally mediated analgesia in mice after i.v. injection. A series of glycopeptides were synthesized5 with varying types of O-linked glycosides attached to
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Ser$^{6}$ of a potent δ-selective sequence first studied by Roques$^{6}$ (Figure 2). O-Linked glycosylation of the relatively lipophilic Leu-enkephalin C-terminal amide YdGFS*-CONH$_2$ led to enhanced surfactant properties$^{7}$ of the molecule, which in turn led to increased interaction with membranes and membrane mimics.$^{8}$ Although these relatively short glycosylated neuropeptides had no defined conformation in aqueous solution (e.g., they existed as random coils), in the presence of sodium dodecyl sulphate (SDS) micelles or other membrane mimics they adopted a very restricted and well-defined set of conformations, as indicated by circular dichroism (CD) and $^1$H-NMR analysis.$^{9}$

![Figure 2: Glycosylated Enkephalin Analogues. Glycosyl hexapeptides were synthesized using solid-phase Fmoc chemistry. The Fmoc serine glycosides were incorporated as the peracetates, and synthesized using methods developed in the Polt group.](image)

<table>
<thead>
<tr>
<th>YdGFLS*-CONH$_2$ Glycoside</th>
<th>Glucoside Moiety</th>
<th>δ Binding (nM)</th>
<th>μ Binding (nM)</th>
<th>MVD IC$_{50}$ (nM)</th>
<th>GPI IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (peptide control)</td>
<td>—</td>
<td>2.1</td>
<td>7.5</td>
<td>2.7</td>
<td>25</td>
</tr>
<tr>
<td>2 (glucomorphin)</td>
<td>β-D-Glc</td>
<td>2.4</td>
<td>7.6</td>
<td>1.6</td>
<td>34</td>
</tr>
<tr>
<td>3 (maltomorphin)</td>
<td>α-D-Glc-(1→4)-β-D-Glc</td>
<td>9.9</td>
<td>30.8</td>
<td>1.7</td>
<td>52.6</td>
</tr>
<tr>
<td>4 (maltotrimorphin)</td>
<td>[α-D-Glc-(1→4)]$_2$-β-D-Glc</td>
<td>3.8</td>
<td>15</td>
<td>7.7</td>
<td>71.7</td>
</tr>
<tr>
<td>5 (lactomorphin)</td>
<td>β-D-Gal-(1→4)-β-D-Glc</td>
<td>17.3</td>
<td>40</td>
<td>5.72</td>
<td>34.8</td>
</tr>
<tr>
<td>6 (biomorphin)</td>
<td>α-D-Gal-(1→6)-β-D-Glc</td>
<td>5.6</td>
<td>36.6</td>
<td>6.06</td>
<td>43.8</td>
</tr>
</tbody>
</table>

Table 1: *In Vitro* Binding Activity and Functional Assays for Glycosylated DTLES. IC$_{50}$’s for δ- and μ-opioid binding were determined using displacement of $^3$H-labeled radioligands from rat brain homogenates. Functional assays were performed using electrically stimulated mouse *vas deferens* and *guinea pig ilium.*
Delta-Selective Glycopeptides Related to Enkephalin Produce Profound Analgesia with Reduced Side Effects in Mice

Figure 3: Glycopeptide Stability and Transport. (a) Octanol-saline distribution for the unglycosylated peptide 1 and glycopeptides 2, 3, and 4. The addition of 1, 2, or 3 glucose units to the opioid peptide message significantly decreases lipophilicity. (b) The in vitro stabilities of the peptide and glycopeptides were measured in mouse brain and serum. Increased glycosylation led to significant increases in stability in both brain and serum. Brain stability increased with each additional glucose. However, in the serum, the stability of the trisaccharide was lower than that of the disaccharide. (c) Brain delivery of the peptides measured by in situ perfusion studies. Addition of glucose to the peptide significantly increased uptake. Uptake to the brain was improved further for the disaccharide, giving the maximal delivery. The trisaccharide produced no further increase in BBB penetration.

Classical pharmacological theories of BBB transport suggest that peptides are not lipophilic enough to diffuse into the brain. Glycosylation decreases lipophilicity even further. Despite this, greatly increased transport rates in rat brain have been observed for the glycosylated enkephalins (Figure 3). Previous studies with the glucoside indicated that the increased transport was due to a saturable mechanism, thus further ruling out simple diffusion. Reversible interaction of the glycopeptides with the membrane is believed to promote transport through the brain capillaries by transcytosis. Several other possible modes of transport (simple diffusion and receptor-mediated processes) have been ruled out. Maximum transport rates (and maximum biological effects) are observed when the optimum degree of glycosylation is achieved. For this peptide, the disaccharide produces both the optimal transport and stability in vivo. In general, glycosylation leads to
enhanced stability of the peptide “message” in both serum and brain. The identity of the individual sugars does, however, contribute to the overall biological effect, which is a product of both BBB transport rates and the stability of the peptide in serum, as well as metabolism and excretion by the liver and kidneys.

4.0 ANALGESIC EFFECTS OF GLYCOSYLATED ENKEPHALINS

The extent of antinociception was shown to be comparable to, or even superior to the effects of morphine in mice after i.c.v. and i.v. administration using the warm water tail flick assay. The representative glycopeptides all produced full agonist effects in these assays with the potencies exceeding that of morphine.
on a µmol/kg basis in some cases. (Figure 4) Additional analgesic assays involving visceral, chemical and inflammatory pain states were also used to gauge the effectiveness of 2 and 5 after i.v. and s.c. injection.

Figure 5: Non-analgesic effects of opioids on mice. Both mice have received equi-analgesic (A90) doses of drug. (a) and (b) Glycopeptide-based analgesia did not induce Straub tail. (c) Morphine-induced analgesia induced large increases in locomotor activity, stereotypic circling, compared to equi-analgesic doses of glycopeptide 2 (d).
Two well-known effects of morphine in rodents are increases in locomotor activity\textsuperscript{15,16} with stereotypic patterns of movement,\textsuperscript{7} and increases in muscular rigidity, including Straub tail.\textsuperscript{18} Unlike morphine and other \(\mu\)-selective opioids, at equivalent s.c. \(A_{90}\) antinociceptive doses, or even supramaximal doses, the glycopeptide analgesics produced minimal increases in locomotor activity, and did not produce Straub tail (Figure 5). These results were confirmed in two different strains of out-bred mice.

**5.0 MECHANISM OF TRANSPORT**

Evidence obtained from \textit{in vivo}\textsuperscript{19,20} as well as \textit{in vitro} experiments\textsuperscript{9} with the glycopeptides are consistent with an endocytotic mechanism of transport. Receptor mediated transport and diffusive mechanisms have been ruled out, and further work strongly suggests that adsorption to the endothelial membrane of the brain capillaries is required for BBB transport. While the drug must adsorb strongly to the membrane in order to undergo endocytosis or transcytosis, this must also be a reversible adsorption, otherwise the drug will bind tightly to the first membrane it sees, resulting in poorer transport. This concept is demonstrated clearly with the amphipathic \(\alpha\)-helices, \textsuperscript{14, 15, and 16}. (Table 3)

Our work began with glycosylated enkephalins that were designed to have potent \(\delta\)-agonist activity, but still have appreciable \(\mu\)-agonist activity. While it is possible to produce some analgesic effects through the \(\delta\)-receptor alone, previous work has shown that \(\mu\)-agonists are much more effective in this regard. It was hoped that mixed \(\delta/\mu\)-agonists would show reduced side effects, relative to \(\mu\)-selective agonists, \textit{e.g.} morphine. Other researchers have proposed \(\mu\)-agonist/\(\delta\)-antagonists as drug candidates for analgesia with reduced side-effects.\textsuperscript{21} An important aspect that is not fully understood is the role that “address” segments play in determining receptor selectivity.

Helices are the most commonly occurring secondary structural elements in globular proteins, accounting for one-third of all the residues.\textsuperscript{22} Linus Pauling first proposed the \(\alpha\)-helix as an important motif of secondary structure in proteins in 1948,\textsuperscript{23} interestingly, without any experimental evidence.\textsuperscript{24,25} Segrest first theorized the \textit{amphipathic} (a.k.a. amphiphilic) helix to be an important structural motif of integral membrane proteins in 1974.\textsuperscript{26} It is estimated that over 50% of all \(\alpha\)-helices in nature are amphipathic.\textsuperscript{27} These proteins are unique in that they possess hydrophobic and hydrophilic parts either by primary structure (highly hydrophilic N-terminus and hydrophobic C-terminus) or by secondary structure, with polar residues pointing one to face and the non-polar residues on the opposite face. This allows them to “float” in a cell membrane, exposing the hydrophilic side to the aqueous exterior of the cell and the hydrophobic side to the lipophilic membrane.\textsuperscript{28,29} This peptide-membrane interaction is believed to be important for two reasons. First, the amphipathic nature of the helix can help guide a drug or hormone to its specific receptor by narrowing the receptor search from a 3-dimensional search to one in 2-dimensions. Surface-assisted “reduction-of-dimensionality” calculations, performed by Polya in 1921, were examined by Max Delbrück in which he quantitatively demonstrated the viability of this theory.\textsuperscript{30} Assuming that no other forces are at work (\textit{e.g.} convection), and that the membrane is fluid, the probability of a substrate finding its corresponding receptor is much better in 2-dimensions (\textit{e.g.} a cell surface) than in 3 (\textit{e.g.} in solution)— almost 100% when the search is reduced to 2-dimensions.

Second, membrane insertion may allow the portion that interacts with the receptor (pharmacophore or “message”) to be fixed in a specific geometry. By restricting mobility in the membrane near the binding site, the amphipathic \(\alpha\)-helix can dramatically alter the peptide-receptor interaction.\textsuperscript{31} In addition, membrane insertion can also induce a specific conformation in the ligand, different from its solution conformation. It seems clear that the bioactive conformation of a peptide is the membrane-bound conformation, and that membrane insertion is actually the first step in receptor activation.
Delta-Selective Glycopeptides Related to Enkephalin Produce Profound Analgesia with Reduced Side Effects in Mice

The endogenous neuropeptide β-endorphin is a 31-residue naturally occurring opioid peptide. The first 5 residues of β-endorphin are identical to Met-Enkephalin. It has been shown that the α-helical structure of C-terminal region of β-endorphin play a role in the receptor binding and opiate activities, and resistance to proteolysis. Kaiser proposed that β-endorphin consists of the [Met]-enkephalin peptide sequence at the N-terminus, a hydrophilic linker region from residues 6—12, and an amphiphilic helical region between the residues Pro13 and Gly30, which were assumed to be “helix breakers.” This hypothesis has been supported by the conformational analysis of a number of β-endorphin mimics with artificial C-terminal helical regions with amphipathic character. All of the analogues were α-helical by CD measurements, as the monomer or oligomers, and showed strong opioid agonism in vitro when compared to natural β-endorphin. These studies clearly suggest that amphipathicity of the entire peptide is more important than the identity of specific amino acids present in the helical C-terminus. This has been further supported by the work by Kyle, who synthesized several potent peptide analogues containing the α-helix-promoting residues α-aminoisobutyric acid (Aib) and N-methyl alanine (MeAla) near the C-terminal region of nociceptin, the natural ligand for the recently identified opioid receptor-like 1 receptor (ORL-1). According to Schwyzer, the N-terminal “message” is steered toward certain receptors and away from others by the C-terminal “address” segment, which interacts with the membrane to orient the message with respect to the receptor.

Dynorphin A (1-17) is an endogenous opioid heptadecapeptide which binds preferentially to the κ opioid receptor. Dynorphin consists of a N-terminal message identical to Leu-enkephalin, and an address sequence that imparts selectivity for κ receptors. Dynorphin A is believed to adopt an extended and/or random coil structure as determined by various spectroscopic measurements. In the presence of DPC micelles Dynorphin A is believed to contain a less ordered N-terminus, a well defined α-helix segment spanning between Phe4 and Pro10 or Lys11 and a β-turn from Trp14 to Gln17. Based on NMR results, the authors concluded that both the α-helix and the C-terminal β-turn may be a consequence of dynorphin’s interaction with the micelle, and may be important structural features of the full-length peptide when bound to the cell membrane in vivo. The α-helix could have multiple roles in positioning the amphipathic helix for interaction with the receptor, as amphipathic helices have many roles at interface.

<table>
<thead>
<tr>
<th>Helix Glucoside</th>
<th>Glycopeptide Sequence</th>
<th>Retention Time (RP-HPLC)</th>
<th>% Helicity (CD)</th>
<th>i.c.v. Analgesia IC50 (picoMol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>YtGFLGELAS*KWFNALE</td>
<td>8.85 min</td>
<td>69%</td>
<td>insoluble</td>
</tr>
<tr>
<td>8</td>
<td>YtGFLGELAS<em>KWFNALES</em></td>
<td>7.95 “</td>
<td>55%</td>
<td>270</td>
</tr>
<tr>
<td>9</td>
<td>YtGFLGELAS<em>KWFNALES</em>F</td>
<td>9.91 “</td>
<td>53%</td>
<td>insoluble</td>
</tr>
<tr>
<td>10</td>
<td>YtGFLGELAS<em>KWFNALES</em>FW</td>
<td>12.48 “</td>
<td>68%</td>
<td>insoluble</td>
</tr>
<tr>
<td>11</td>
<td>YtGFLGLLKS<em>FAES</em>WS*NF</td>
<td>6.69 “</td>
<td>34%</td>
<td>~ 30</td>
</tr>
<tr>
<td>12</td>
<td>YtGFLGKS<em>FAELWS</em>NFLS*</td>
<td>5.35 “</td>
<td>14%</td>
<td>~30</td>
</tr>
<tr>
<td>13</td>
<td>YtGFLGLLKS<em>FWES</em>WS*NF</td>
<td>8.25 “</td>
<td>37%</td>
<td>~30</td>
</tr>
</tbody>
</table>

Table 2: Glycosylated Endorphin Analogues.
Delta-Selective Glycopeptides Related to Enkephalin Produce Profound Analgesia with Reduced Side Effects in Mice

<table>
<thead>
<tr>
<th>Helix Glucoside</th>
<th>Glycopeptide Sequence (3rd Generation)</th>
<th>Retention Time (RP-HPLC)</th>
<th>Per Cent Helicity (CD)</th>
<th>MVD IC50 (nM)</th>
<th>GPI IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>YtGFL((p))NLBEKALKS*L-CONH₂</td>
<td>31.57</td>
<td>21</td>
<td>34.5</td>
<td>63.1</td>
</tr>
<tr>
<td>15</td>
<td>YtGFL((\beta A))NLBEKALKS*L-CONH₂</td>
<td>33.50</td>
<td>26</td>
<td>23.0</td>
<td>354</td>
</tr>
<tr>
<td>16</td>
<td>YtGFL((GG))NLBEKALKS*L-CONH₂</td>
<td>30.30</td>
<td>14</td>
<td>18.8</td>
<td>196</td>
</tr>
<tr>
<td>—</td>
<td>Morphone</td>
<td>—</td>
<td>—</td>
<td>258</td>
<td>54.7</td>
</tr>
</tbody>
</table>

Table 3: Glycosylated Endorphin Analogues.

Figure 6: (a) Plot of Retention Time vs Degree of Helicity. (b) Mouse BBB Transport Data.

The first- and second-generation endorphins were also based on the \(\delta\)-selective YdGFL~ opioid message. Formed by simple truncation, the first generation helices, 7—10, were designed to probe the minimum length for helix formation. Essentially, we overshot the target, and all of these compounds were extremely helical, but they were not water soluble enough to work with, with the exception of helix 8. This compound possessed appreciable antinociceptive activity, however. All of these compounds were quite soluble in the presence of SDS micelles. Since these compounds are so stable in their helical form, they probably form aggregates, and fall out of solution in the absence of the detergent. The second generation helices, 11—13, were designed to be less lipophilic, and consequently were more water soluble, and showed much less helicity in the presence of micelles.

The third-generation helical endorphin-based glycopeptides, 14—16, used the same \(\delta\)-selective peptide DTLET first studied by Roques, and showed much superior properties, both in the chemistry lab and in the mouse. Using \textit{in situ} methods in the mouse, not rat studies as before, Egleton was able to measure BBB transport rates independently of analgesia, and Bilsky has been able to demonstrate the analgesic effects of these larger glycopeptides using \textit{i.c.v.} tail flick results in the mouse. Initial studies with these glycohexadecapeptides indicated that BBB transport rates were determined by the amphipathic nature of the glycopeptides, rather than the lipophilicity of the compound, per se, and that they actually show BBB transport rates that are similar to, or better than the shorter enkephalin analogues.
These endorphin analogues have the same N-terminal YdGFL~ opioid message contained in the enkephalin analogues 1—6, and the same C-terminal amide address sequence ~NLBEKALKS*L-CONH$_2$, where B is the helix-stabilizing $\alpha$-aminoisobutyric acid (Aib) residue, and S* is the serine glucoside residue. The “linker region,” which is intended to “break” the helix, and prevent propagation of the helical address into the opioid message, is different in the three glycopeptides: 14 => proline, 15 => $\beta$-alanine, and 16 => glycyglycine.

Figure 7: Lipid Bound Helix. One structure of glycopeptide 14 in the presence of micelles, as determined by NOE-constrained molecular dynamics calculations. The message segment is labelled in yellow, and the helix indicated with the overlaid ribbon. The structure on the right has the hydrophobic (blue) and hydrophilic (red) surfaces labelled. The structures were rendered with the MOE® software package.

Figure 8: Lipid Bound Helix-Bend. One structure of glycopeptide 17 in the presence of micelles, as determined by NOE-constrained molecular dynamics calculations. The message segment is labelled in yellow, and the helix indicated with the overlaid ribbon. The structure on the right has the hydrophobic (blue) and hydrophilic (red) surfaces labelled. The structures were rendered with the MOE® software package.

While the data presented in Figure 6 is interesting, and perhaps even compelling, it is also clear that one cannot only use the degree of helicity to predict amphipathicity. NMR evidence, in conjunction with Monte Carlo calculations (NOE constraints not discussed here) shows that the glycopeptides bind to micelles, and
adopt a very restricted set of conformations. For the helices 14, 15, 16, and the disaccharide 17 (not pictured in Table 3, but is the top-most data point in Figure 6a) we see two membrane bound conformational ensembles, one that is very helical, (e.g. Figure 7) and one that has a helix-bend motif (e.g. Figure 8), but is none-the-less very amphipathic. The peptide sequence for 17 is the same as the sequence for 14, but the compounds differ in that 14 is glycosylated with the β-D-gluco side, and 17 is glycosylated with the disaccharide β-lactose. These two compound both show the same conformations in their micelle-bound ensembles based on NMR, and similar helicities based on CD, but slightly different population densities.

While it there is still much to be learned about the details of both the transport and binding processes of the amphipathic glycopeptides, an important principle has emerged concerning transport. It seems clear that one must have a glycopeptide that essentially has two states: 1) A state defined by one or more membrane-bound conformations that permit or promote endocytosis. 2) A state defined by a water-soluble, or random coil state that permits “membrane hopping.” The key to efficient transport is to balance these two states so that the compound is neither retained in the membrane, or held in solution so that it cannot undergo adsorptive endocytosis. It may also be true that aggregation of glycopeptides on a membrane surface may actually initiate and promote endocytosis.

6.0 CONCLUSIONS

Based on the results obtained so far, it would seem that further pre-clinical studies are warranted to test the viability of the glycosyl enkephalin analogues (e.g. compounds 2, 5 or 6) as a replacement for morphine on the battlefield. Anecdotal studies in mice suggest that these compounds possess an extremely low level of toxicity, even at super-analgesic doses. The notion that one could administer a large sub-cutaneous dose of a non-toxic glycopeptide that would have prolonged analgesic effects without respiratory depression or the risk of overdose is particularly appealing. Further research needs to be completed in order to quantify the effects of the glycosylated δ-agonists on respiration and blood pressure, particularly in hypovolemic animals to gauge the propensity of these compounds to induce hemorrhagic shock. Complete absorption, metabolism and excretion studies (ADME) need to be completed, and oral bioavailability needs to be explored. The fact that the glycosylation strategy seems to be effective with the much larger endorphin analogues (e.g. compounds 14 and 15) suggest that this approach may have general applicability to BBB transport of non-analgesic (or even non-opioid) neuropeptides, which could lead to novel treatments for anxiety, stress-related disorders and depression.

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Antimicrobial Bone Graft Substitutes

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ABSTRACT

Open fractures account for approximately 20% of all combat-related injuries in soldiers. Because of the severity of wounding mechanisms and high rates of wound infection, open fractures from combat pose greater problems to surgeons and result in higher morbidity than similar injuries suffered by civilians. Standard care for open fractures requires multiple procedures, including irrigation, débridement, stabilization, and antibiotic therapy. A commonly used method to provide local antibiotics through elution and to fill dead space is the application of polymethylmethacrylate (PMMA) combined with a broad-spectrum antibiotic, rolled into beads, and placed in the bone defect. Although effective for treating infection, these cement beads are not bioabsorbable and will eventually retard bone growth if not removed during a second surgical procedure. Yet a third procedure for bone autografting is often required for definitive treatment. Current standard care with multiple procedures and delayed definitive treatment results in high patient morbidity and increased cost of treatment. To evaluate potential alternatives to current standard care we have developed a large animal open fracture model in goats. Thus far we have tested different osteoconductive or osteoinductive bone graft substitutes impregnated with antibiotics to determine the ability of each product to reduce infection rates. All products have been as effective as standard care treatment in preventing infection in this contaminated bone defect model. Current research is evaluating the use of similar, but commercially available products that have been approved, or are pending approval, by the United States Food and Drug Administration. The use of antimicrobial bone graft substitutes may reduce morbidity, the number of surgical interventions, and associated medical costs for military personnel and civilians alike.

1.0 INTRODUCTION

Open fractures, defined as broken bone in communication with the environment, account for approximately 20% of all injuries sustained on the battlefield [1-3]. These fractures, which are frequently the result of high-energy impact, are difficult to manage because of segmental defects, neurovascular damage, or inadequate soft tissue coverage. In addition, associated bacterial contamination presents an enormous surgical challenge and considerable patient morbidity. Despite meticulous treatment, open fractures have high rates of delayed union and non-union, causing complications such as chronic osteomyelitis that can threaten the viability of the limb and even the life of the patient [4]. Bone defects typically require treatment with a bone graft to augment

1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
fracture healing. However, a bone graft cannot be placed in a wound immediately following injury, due to wound contamination and the high risk of infection. The wound bed must first be thoroughly cleaned, and the patient placed on systemic antibiotics. In some instances, to treat severely contaminated wounds, a high concentration of antibiotics is mixed with polymethylmethacrylate (PMMA) bone cement to form small beads that are temporarily placed into the contaminated fracture site for periods as long as six weeks. The beads preserve soft-tissue tension and decrease the amount of dead space [5] while slowly eluting high levels of antibiotic into the surrounding environment; this level of medication would be toxic if delivered systemically [6]. After the wound is thoroughly cleaned, the beads are removed and, if necessary, definitive bone grafting is performed.

Despite clinical studies demonstrating the efficacy of this local antibiotic delivery technique, there are several disadvantages. Most importantly, PMMA is not biologic, necessitating a second surgical procedure for removal. Furthermore, since the beads are not osteoinductive, (i.e., cannot induce bone growth), definitive bone grafting and mature fracture healing cannot begin until the beads are removed. In addition, when autologous bone graft is used, harvest may result in significant morbidity due to blood loss, operative time, postoperative pain, and difficulty with ambulation [7]. The goal of our research is to find an all-in-one substitute for the current long and drawn out process of staged repair to reduce morbidity, shorten recovery time, and reduce cost. The ideal bone graft product should be osteoconductive, to provide a scaffold for bone growth, as well as osteoinductive. It should also be antimicrobial and available in unlimited supply.

We have tested two bone graft substitutes in animal studies—tobramycin-impregnated calcium sulfate pellets and tobramycin-impregnated calcium sulfate pellets combined with demineralized bone matrix (DBM)—that meet some of these criteria. Biodegradable antibiotic delivery systems have been used in Europe for many years with excellent clinical results [8, 9]. However, biodegradable products containing antibiotic delivery systems have not yet been approved for clinical use in the United States. In the United States only biodegradable products without antibiotics, such as calcium sulfate and calcium phosphate, have been approved by the FDA.

Calcium sulfate, which has been used since 1892 as a bone defect filler and is known to be osteoconductive [10], can be impregnated with an antibiotic. Demineralized bone matrix (DBM) is also commercially available, is osteoinductive, and has been shown to promote bone healing. Turner, Urban and Gitelis, using a canine model, showed that the combination of calcium sulfate pellets and DBM was more effective as a bone graft substitute than either component alone [11]. Additionally, they demonstrated that the combination was as effective as autogenous bone graft at six weeks following treatment. We speculated that the combination of tobramycin-impregnated sulfate pellets and DBM could be used as a local antibiotic delivery system that would be osteoinductive as well as osteoconductive.

We have teamed with industry and academia to use existing and emerging products and information to identify a better treatment for open fractures. In order to accomplish this goal, a large animal contaminated bone defect model has been developed to evaluate the effectiveness of various treatments in an ongoing series of experiments. We describe two completed studies that evaluated experimental treatments for reducing infection rates of open fractures. These treatments have the potential to reduce the number of surgeries, patient morbidity rates, total cost of the treatment, and to speed the soldier’s return to duty.
2.0 METHODS AND MATERIALS

2.1 Experimental Design

We developed a large animal model \textit{(vide infra)} to mimic a contaminated open fracture. Study I used this model to evaluate the effectiveness of OSTEOSET® (Wright Medical Technology, Arlington, TN), an antibiotic-impregnated calcium sulfate bone replacement material. Animals were divided into four groups: negative control, positive control, calcium sulfate, and experimental. The negative control group received no treatment. The positive control group received current standard care of PMMA beads impregnated with 4% tobramycin by product weight. The beads were prepared and rolled by hand in standard clinical fashion and placed in the metaphyseal defect. The calcium sulfate group received 15 pellets of the commercially available product OSTEOSET® (Wright Medical Technology, Arlington, TN). The experimental group received 15 pellets of OSTEOSET®, which are calcium sulfate and 10% tobramycin by product weight. Study II evaluated the effectiveness of an antibiotic-impregnated calcium sulfate bone replacement material when combined with DBM; the combination of calcium sulfate and DBM has been shown to be effective in stimulating bone growth. Animals were again divided into four groups: negative control, positive control, DBM, and experimental. The negative and positive control groups received the same treatment as Study I. The DBM group received 2.5 ml of DBM (ALLOMATRIX™ injectable putty; Wright Medical, Arlington, Tennessee). The experimental group received 15 pellets of OSTEOSET T® and 2.5 ml of DBM.

2.2 Surgical Technique

Adult male Spanish goats were used for our studies. A 2.5-cm longitudinal skin incision was made over the medial proximal metaphyseal region of the tibia centered at a point approximately 2 cm medial and 2 cm distal to the tibial tubercle. After elevating the periosteum with a periosteal elevator, a unicortical, 12-mm circular defect was produced with a coring reamer. Next, the bony defect was inoculated with an aliquot of bacteria (30 µl of solution with $10^6$ CFU/ml of \textit{Staphylococcus aureus}). The bacterial strain used was American Type Culture Collection (ATCC) 29213, which was further modified by our institution to be resistant to streptomycin. Thirty µl of a $10^6$ CFU/ml solution of bacteria has been shown to be sufficient to cause infection in greater than 70% of non-treated animals without producing overwhelming sepsis [12].

2.3 Wound Grading System

The animals were followed daily for 21 days for clinical signs of infection. After the dressing was removed on postoperative day 4, three independent examiners graded each wound daily. The goat’s treatment group was masked to the graders for the duration of the study. We used a clinical grading system established in a previous caprine study [12]. The condition of the wound was graded by the following criteria: a score of 0 was assigned for no signs of contamination; 1 for inflammation, swelling, or serous drainage without frank purulence; 2 for frank purulence at the wound site, or purulent discharge upon aspiration or incision and drainage. A score for each wound was calculated by adding the score from each of the three observers each day for 21 days. The clinical determination of infection was defined by a score of at least 5 on two consecutive days for a given wound. Hence, a wound had to exhibit two consecutive days of purulent drainage, as identified by at least two of the three examiners, to be considered infected.

2.4 Necropsy and Microbiologic Analysis

On postoperative day 21, the animals were euthanized and the treated hindlimb was disarticulated at the hip and radiographs were obtained. The bony defect was transected at its mid portion with a Gigli saw. Culture
swabs were obtained from the proximal and distal intramedullary canals. A number 5 surgical curette (0.5 g tissue) was used to harvest marrow and trabecular tissue from the canal.

The tissue and swab samples were sent for standard qualitative quantitative and microbiological analysis. Isolates were identified by routine microbiological procedures. Each *S. aureus* isolate was tested for streptomycin resistance to determine whether it was the same strain as the initial inoculation.

2.5 Outcome Measures

The outcome measure used to define the presence of deep wound infection was the recovery of the streptomycin resistant *S. aureus* strain ATCC 29213 from intramedullary cultures at 21 days. The threshold for infection was set at $10^4$ CFU/g of marrow [12]. Cultures with bacteria present but less than $10^3$ CFU/g marrow were considered contaminated. If the quantitative analysis identified between $10^3$ and $10^4$ CFU/g marrow of bacteria at final tissue culture, the clinical score was used to determine infection (i.e., the wound had to be considered infected by our clinical scoring criteria to receive definitive identification).

3.0 RESULTS

3.1 Study I

The primary determination of infection in this study and Study II was the recovery of the inoculated bacteria from the intramedullary cavity. *S. aureus*-R was found in the deep wounds in eleven of the twelve goats in the negative control group ($6.9 \times 10^7 \pm 4.0 \times 10^7$ CFU/g) and in all twelve wounds in the calcium sulfate group ($2.2 \times 10^8 \pm 1.1 \times 10^7$ CFU/g). No *S. aureus*-R was recovered in any of the bony defects of the goats in the positive control and experimental groups. The amount of bacteria recovered from the intramedullary cavity differed between the tobramycin-treated group and the group that was not treated ($p<0.002$). The carrier group clinical infection rate was higher than the positive control and treatment groups ($p<0.02$). In addition, gross pathologic and radiographic evaluation confirmed a difference between the groups that were and were not treated with tobramycin. There was purulent replacement of marrow and trabecular bone in the negative control and calcium sulfate groups, whereas the positive control and treatment groups appeared to have normal marrow and trabecular bone. The radiographic evaluation of the negative control and calcium sulfate groups further suggested infection by revealing evidence of periosteal new bone formation that produced involucra in all of the infected bony defects. The bony defects in the two groups that were treated with antibiotic appeared normal.

3.2 Study II

*S. aureus*-R was found in the bony defects in six of seven goats in the negative control group ($2.2 \times 10^8 \pm 1.2 \times 10^8$ CFU/g) and in seven out of eight goats in the DBM group ($1.3 \times 10^8 \pm 8.1 \times 10^7$ CFU/g). No *S. aureus*-R was recovered in any of the deep wounds of the goats in the positive control and experimental groups. The amount of bacteria recovered from the deep wounds differed between the groups that were treated with tobramycin and those that were not treated ($p<0.02$). The DBM group had a significantly higher rate of clinical infections than did the experimental, positive control, and negative control groups ($p=0.0058$, $0.01$, and $0.0187$, respectively).

The gross pathologic and radiographic evaluation results were similar to Study I. The radiographs revealed pronounced periosteal reaction in the groups that were not tobramycin treated. The radiographs from the
positive control and experimental groups qualitatively displayed less periosteal reaction than the other groups. Gross pathological analysis revealed purulent replacement of marrow and trabecular bone in the negative control and DBM groups. The tobramycin treated groups appeared to have normal marrow and trabecular bone (fig. 1).

Figure 1: Gross anatomy samples from negative control group (left) and experimental group (right). The negative control group, which received no treatment showed purulent replacement of bone marrow and trabecular bone. The experimental group in which defect was treated with antibiotic-impregnated calcium sulfate bone replacement material combined with demineralized bone matrix (DBM) showed normal bone marrow and trabecular bone.

4.0 DISCUSSION

These studies demonstrate that an antibiotic-impregnated bone graft substitute can be used effectively in a contaminated fracture model. We feel that this is a valid model to evaluate a product’s ability to prevent an infection in an open fracture. In our tibial defect model, similar to open fractures in humans, bacteria have access to the intramedullary space. Bacteria are able to colonize this wound if it is left untreated and the wound becomes infected. However, in this model, because wounds become infected without causing overwhelming sepsis, the goats are not subjected to excessive stress and do not have to be euthanized prior to
the completion of the study. In addition, the unicortical defect does not require stabilization, and the goats are able to ambulate within hours of surgery.

Standard care often dictates a high level dose of antibiotic-impregnated PMMA that would be toxic if delivered systemically. PMMA beads “hold the space” by preventing the formation of fibrous tissue while the local dose of antibiotic is eluted. This procedure is generally the first stage of a procedure requiring a second operation that then leaves a bone void. An autograft or allograft is routinely placed in the void to stimulate bone growth. Calcium sulfate pellets with tobramycin as used in our study act similarly to PMMA beads and fill the dead space and prevent the formation of fibrous tissue while eluting a local dose of antibiotic. Calcium sulfate, however, is resorbable, which could prevent the need for a second operation to remove the substance that is not biologic. Unfortunately, calcium sulfate is only osteoconductive, and in a canine model it takes 24 weeks for calcium sulfate pellets to stimulate as much bone as autogenous bone graft [11]. When osteoconductive calcium sulfate pellets and osteoinductive DBM are combined the resulting product can stimulate as much bone growth as autograft in just 6 weeks in the same model [11]. Theoretically, by adding tobramycin, the combination should be antimicrobial as well, but this combination had yet to be challenged in a contaminated fracture model.

Our preliminary studies provide the groundwork for future studies. In the contaminated fracture model used in the second study, tobramycin-impregnated calcium sulfate pellets and DBM combination was not significantly different from tobramycin-impregnated cement beads in preventing establishment of *S. aureus* infection. However, use of the antibacterial calcium sulfate pellet and DBM combination could decrease the morbidity of open fractures by eliminating the need for surgical bead removal and by reducing the need of an autograft. Combining this calcium sulfate product with DBM could potentially accelerate bone formation. Reducing multiple procedures to a single-stage treatment will also reduce the overall risk to the patient.

### 5.0 SUMMARY

The current standard of care for battlefield open fractures is not acceptable, as evidenced by the high rates of infection and nonunion. The treatments that we have evaluated were as effective as antibiotic impregnated cement beads and they have the potential to reduce the number of surgeries, morbidity, and return to duty time because they are resorbable and promote bone growth. Currently, we are testing commercially available products that have been approved, or are pending approval, by the United States Food and Drug Administration. These products will reduce the number of procedures that the surgeon must perform to treat the fracture and are closer to finding a definitive treatment. Future studies will focus on the effects of products on bone healing and will evaluate the effectiveness of tobramycin in preventing infection when the wound is contaminated with gram negative bacteria (*Pseudomonas aeruginosa*).
6.0 REFERENCES


Bi-Layer Wound Dressing System for Combat Casualty Care

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SUMMARY

Burn injuries remain a significant cause of morbidity and mortality during modern military conflicts and peacekeeping operations. Considering that commercially available dressings are not designed to meet the challenges of treating combat burn wounds, DRDC Toronto has designed a novel, absorbent and medicated bi-layer wound dressing to address key requirements for treating external war wounds. In the present report, we assessed our dressing’s bactericidal efficacy, wound healing properties, and skin-cooling characteristics using various pre-clinical models. Biopsies taken from full-thickness, pig wounds infected with Ps. aeruginosa and Staph. epidermidis showed a 2- to 5-log reduction in the bacterial load of antiseptic-treated wounds compared to those of control wounds. Though increasing the frequency of dressing changes led to a greater reduction in the wound bacterial load, the contamination levels of all antiseptic-treated wounds remained below 10⁵ CFU/g of wound. Our results also show that 97% of partial-thickness, non-contaminated porcine wounds treated with the DRDC dressing healed within 7 days. In contrast, 92% of the wounds treated with commercial dressings healed within 9 days. Finally, the application of a moist DRDC dressing (to simulate a condition of exudate absorption; DRDCmoist) on a scald burn covering 25% of the dorsal area in rats reduced skin temperature (Tskin) by 1.7°C for 5 min, Tskin in DRDCmoist being comparable to that of control burned rats (BURN) after 25 min. While there were no significant differences between the body temperature (Tip) in BURN and DRDCmoist throughout the 90-min experiment, application of a commercial hydrogel dressing markedly decreased Tip after 90 min (3.03±0.55 °C). These data show that the DRDC dressing is effective in: a) delivering medications, such as an antimicrobial agent, to the wound bed; b) promoting faster healing of the treated wound; and c) providing a transient, but beneficial cooling effect to the skin contact-site, without the adverse effect of inducing whole-body hypothermia.

1.0 INTRODUCTION

Burn injuries have been considered an ubiquitous and potentially life-threatening insult, commonly associated with modern front-line military operations. The risks of burn injury during combat are well recognized, and are unlikely to diminish because of the rapid proliferation of new and powerful thermobaric weapons that target the inherent vulnerability of the soldier to sustain burns [1]. The incidence of accidental burn injuries has also been increasing in recent peacekeeping and training operations due to the mishandling of flammable materials, propellants, chemical agents, munitions, and hot liquids [2, 3]. Military deployment will also expose personnel to accidental burn injuries unrelated to combat (e.g., road traffic accidents, smoking and the use of unguarded flames for cooking, lighting and heating liquids). Clearly, burn injuries can potentially have not only detrimental pathological effects on the soldier but also a tremendous impact on both military and civilian facilities.

Management of wounds sustained during military operations offers challenges that are different from treating comparable wounds in a civilian setting. First, since delayed evacuation of casualties is not uncommon, immediate wound care may need to be self-administered or facilitated by untrained personnel under extremely challenging and probably hostile environment [4]. Second, the front-line wound care management strategy must also take into consideration that wounds are prone to contaminations and infections in the battlefield environment by soil, organic material, and debris. Data from the Somalia conflict as well as the Falkland Island campaign have shown that approximately 15% to 20% of combat wounds will result in infection [5, 6]. The morbidity and mortality associated with a given wound size may become markedly enhanced if the wound becomes contaminated. Furthermore, it appears that standard field dressings applied on mild to moderate wounds will often slide distally once the casualty resumes normal activities, thereby failing to protect the wounds from further tissue damage and bacterial infection [7]. Reports from medics involved in Operations Enduring Freedom and Iraqi Freedom have also confirmed the emergence of multi-resistant bacteria that do not respond to recommended systemic antibiotic therapies, suggesting the need for a topical antimicrobial treatment to counteract the rapid formation of bacterial biofilms at the wound site. Lastly, another consideration applying specifically to the treatment of burns remain the reduction of the progression of the severity of tissue injury by providing short-term, immediate cooling of the burned skin to prevent further tissue damage [8].

Commercially available dressings are not designed to meet simultaneously the challenges of treating combat burn wounds. DRDC Toronto has therefore designed a novel, absorbent and medicated bi-layer wound dressing to address key requirements for treating external war wounds. The DRDC dressing is composed of a hydrogel wound-contacting layer surimposed by a proprietary polyurethane foam layer. In the present report, we assessed our dressing’s bactericidal efficacy, wound healing properties, and skin-cooling characteristics using various pre-clinical models.

2.0 METHODS

2.1 Preparation of wound dressings

The DRDC dressing is composed of a thin (38 mil) hydrogel wound-contacting layer surimposed by a proprietary soft, hydrophilic polyurethane foam layer (10 mm thick; Figure 1). An outer semi-permeable polyurethane membrane can also be added to: allow the controlled release of moisture vapour; provide an effective barrier to water or wound exudate; and, prevent the passage of bacteria through the back of the dressing. All non-medicated dressings were prepared by Avitar Inc. (Canton, MA) under aseptic conditions, and packaged individually in aluminum foil bags (VWR Canlab, Mississauga, ON). Assessment of the sterility of each batch of dressings was performed at DRDC using standard microbiological procedures prior to the application of the samples on wounds.

All medicated DRDC dressings were prepared by immersing for 30 min the material in an aqueous solution containing the antimicrobial agent under study. The drug-loaded dressings were then squeezed under a sterile custom-built benchtop rolling press for 60 s, to retain a standardized amount of the drug solution. Preliminary experiments have shown that the drug is loaded in both layers of the DRDC layers, with the foam layer acting as a drug reservoir (unpublished data).

The protocols of the experimental studies described herein were approved by the institutional animal committee, and conducted in accordance with the guidelines of the Canadian Council on Animal Care.
Figure 1. Schematic representation of the DRDC bi-layer wound dressing, consisting of a medicated polyurethane foam over a thin tissue-contacting hydrogel layer to reduce adhesion to the wound. Colored circles represent two types of drug loaded in the pores of the foam layer. For the sake of clarity, no drug was represented in the hydrogel layer.

2.2 Study I: Assessment of in vivo Bactericidal Efficacy

Three male Yorkshire pigs (20-25 kg) were allowed to adapt to the environmental conditions (20-25°C, 12 h light/dark cycle) for at least 7 days before undergoing surgery. Domestic pigs were selected because of the morphological and functional similarities of pigskin with the human skin, and the ability of the pig models to predict wound healing in humans [9]. Animals were housed individually, and had free access to pig chow and water at all times during the study period.

2.2.1 Bacterial Challenge

Clinical isolates of Ps. aeruginosa, Staph. epidermis, and Fusobacterium sp. were used to infect the wounds. The bacterial strains were grown at 37°C in nutrient broth for 18 h in a shaking water bath to obtain a log-phase growth culture. The suspensions were washed 3 times in sterile phosphate-buffered saline (PBS), re-suspended in sterile PBS, and diluted to approximately 10^7 colony forming units (CFU) per mL. Serial dilutions were plated on Pseudomonas Isolation agar (for Ps. aeruginosa), Staphylococcus Medium 110 (for Staph. epidermis) or Tryptic Soy agar (for Fusobacterium) to assess bacterial concentrations in the inoculum. On the experimental day, the three bacterial cultures were mixed together in a ratio approximating 1:1:0.5 (Ps. aeruginosa: Staphylococcus: Fusobacterium; 10^7 CFU in 50 mL).

2.2.2 Experimental Procedures

On the experimental day, each pig was pre-anaesthetized with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.) followed by gas inhalation (oxygen: 1-2% isoflurane). The dorsal and lateral thorax were clipped, and the skin prepared for wounding by washing with an antibiotic-free soap. Columns of wounds on the dorsum were labelled (using an indelible marker) as A through D, and rows
marked as 1 through 4. Eighteen full-thickness (down to the deep fascia) wounds were created using a 2-cm diameter tissue trephine. Wounds were made 4 cm apart, with columns B & C set 2 cm on each side of the pig’s spine. Sterile gauze compresses (Nu Gauze, Johnson & Johnson, New Brunswick, NJ) were applied on the wounds, soaked with a saline/epinephrine solution (1:100 v/v), and allowed to remain in situ until complete haemostasis had occurred.

The wounds were loosely packed with 2.5 cm x 2.5 cm sterile gauzes, and inoculated with 3 mL of the bacterial suspension. The wounds were then covered for 20 min with an occlusive sterile film (Saran Wrap®, SC Johnson, Racine, WI) to prevent drying. At the end of the infection procedure, the gauzes were removed, and the wounds were each dressed with a 2-cm DRDC bi-layer dressing containing either 1 mL of PBS (Control), or were loaded in aqueous solutions of 0.5 % chlorexhidi ne digluconate or 1 % chloramphenicol, as described previously. Each experimental dressing was then covered with a piece of sterile parafilm. Layers of adhesive PVC tape (Elastoplast™, Smith & Nephew, Lachine, QC) were then applied to hold down firmly the experimental dressings. The entire trunk of the pig was then wrapped with a layer of elastic self-adhesive bandage (Elastoplast™, Smith & Nephew, Lachine, QC). A dose of narcotic (i.m. buprenorphine, 0.1 mg/kg body weight) was administered and a Duragesic® patch (Janssen Pharmaceutica, Titusville, NJ) delivering 50 µg/h fentanyl for 72 hours was then applied prior to returning the animal to its pen.

Dressings were replaced daily or every other day during the 4-d study (n=6 per experimental group per dressing change). A 4-mm biopsy was taken at the centre of 6 wounds in each experimental group at each dressing change. Tissues were placed into pre-weighed tubes, homogenized in cold PBS, and plated serially on SM110 and PIA to determine the bacterial counts. Animals were humanely euthanized at the end of the 4-d study period using T-61 (i.v.; Hoechst-Roussel Canada Ltd, Montreal, QC) following sedation (ketamine, 15 mg/kg body weight; acepromazine, 0.5 mg/kg body weight, i.m.).

2.3 Study II: Assessment of Wound Healing Properties

2.3.1 Surgical Procedures

On the experimental day, 5 male Yorkshire pigs (20-25 kg) were anaesthetized, and their skin prepared for wounding as previously described. Seventy partial-thickness (0.4 mm deep) 1-cm² wounds (five columns of seven wounds on each side of the pig’s spine) were marked using an indelible pen on the back of the animal with a custom-built Lexan® template. The wounds were then individually created, 1 cm apart, at the marked location using a dermatome (Tyler Research Corp., Edmonton, AB). Sterile gauze compresses were applied on the wounds, soaked with a saline/epinephrine solution (1:100 v/v), and allowed to remain in situ until complete haemostasis had occurred. Groups of 5 adjacent wounds were then randomly covered with strips (2.5 cm x 15 cm) of sterile, non-medicated DRDC tri-layer dressing or Allevyn® (Smith & Nephew, Lachine, QC) (n=125 wounds per experimental group). Layers of adhesive PVC tape were then applied to the back of the pigs to hold down firmly the experimental dressings. The trunk of the pig was finally wrapped with a layer of elastic self-adhesive bandage. No experimental or securing dressings were applied to the last row of wounds on each pig (air-exposed control; n=100 wounds). Analgesia was administered as previously described.

2.3.2 Experimental Procedures

Since wound healing in this model rarely occurs within the first 4 days after wounding [10], the following procedures were performed daily from day 5 to day 9 only. Briefly, pigs were anaesthetized and all
dressings were removed. Rows of wounds were harvested in one strip using a 2 cm x 2 cm blade set at 0.8 mm depth, according to a pre-determined harvesting protocol (maximum 15 wounds per pig per day). The wound strips were placed in a sodium bromide solution for 24 hours to provide an assessment of the extent of wound healing. A new experimental dressing was then applied to all wounds that had not been harvested, and the animal was returned to its pen.

Following sodium bromide incubation, dermal and epidermal layers were separated, and the presence of holes in the epidermal sheet was noted. Epidermal sheets harvested intact and not demonstrating any defects were scored as being completely healed; any defects was scored as not being healed. Animals were humanely euthanized at the end of the 9-d study period as previously described.

2.4 Study III: Assessment of Skin Cooling Properties

Forty male, Sprague-Dawley rats (250-275 g) were allowed to adapt to the environmental conditions (22°C, 12 h light/dark cycle) for at least 7 days before undergoing burn injury. Animals were housed in groups of 5 until the injury, and individually thereafter. They had free access to food and water at all times during the study period.

2.4.1 Burn Injury Protocol

Our rodent model of scald injury was adapted from Walker et al. [11] and Martineau et al. [12]. Briefly, rats were anaesthetized (2.0% halothane:N₂O:O₂), and received analgesics (buprenorphine, 50 ug/kg body weight; s.c.). Their back was then clipped, depilated, and cleansed using standard procedures. A 25 percent total body surface area full-thickness scald of the dorsum was produced under anaesthesia by placing the animal in a custom-built Teflon® template, and dipping the clipped, exposed dorsum in water at 90°C for 10 s. Sham-burned rats (SHAM) were immersed in warm (37°C) water for the same time interval. The rats were then rapidly removed from the restraining device, and their skin pat-dried for 5 s with a sterile gauze pad. All animals received a subcutaneous injection of sterile saline.

2.4.2 Experimental Procedures

Immediately following completion of the burn injury procedures, one of 4 small thermistors threaded into a sterile stainless steel tether was inserted 2 cm into the abdominal cavity through a small incision (5 mm), 1 cm below the edge of the burn wound. The thermistor was maintained in place with a nylon non-absorbable suture (Johnson & Johnson, New Brunswick, NJ) threaded through a loop made in the thermistor. The incision was closed in 2 layers with non-absorbable interrupted sutures. The remaining 3 thermistors were sutured 2.5 cm apart on the dorsal burned skin, 1 cm lateral to the spine. The first probe was positioned approximately 2 cm distal to the nape of the neck. The second and third probes were randomly located on alternate sides of the spine on each animal.

The entire burn wound in 3 experimental groups (n=8 per group) was then covered with a hydrogel dressing (2nd Skin®, Spenco Medical (UK) Inc., Wako, TX), a standard, tri-layer DRDC dressing (5 mm thick; 5 cm x 10 cm; DRDCdry), or one hydrated to 50% of its maximum absorption capacity with warm water (DRDCmois). The latter group simulated a condition of high exudation from the wound. The experimental dressings were applied within 20 min following the burn injury; this time interval was selected based on the estimated delay in attending to a burn wound sustained in the battlefield. A single layer of a 6-cm wide self-adherent non-woven wrap (Coban™, 3M, London, ON) was then used to further secure in place the.
experimental dressings (i.e., 2nd Skin; DRDC\textsubscript{dry}, and DRDC\textsubscript{moist}), and to cover the untreated burn (BURN) or sham burn (SHAM). The animals were returned to their cage, the tether being positioned well out of reach of the animals. Temperature data were then acquired for 90 min using a small data logger secured to the cage lid. All rats were euthanized by cervical dislocation (under general anaesthesia) once the data acquisition was completed.

2.5 Statistical Analysis

Statistical analyses were completed using Statistica (Version 6.1, Statsoft, Inc.). In all studies, a two-way analysis of variance (ANOVA) for repeated measures with two within-subject variables (frequency of dressing change or time elapsed since application of dressing; type of wound dressing) was used to determine statistical significance in: bacterial counts in the wounds (Study I); wound healing (Study II); and, intra-abdominal and skin temperature (Study III).

For all analyses, p-values were calculated using the Greenhouse-Geisser epsilon correction for repeated measures. When statistical significance was determined for main or interaction effects, a Neumann-Keuls post-hoc analysis was completed to locate significant differences. Significance was deemed to exist when p<0.05.

3.0 RESULTS

3.1 Study I: Assessment of in vivo Bactericidal Efficacy

All animals survived for the duration of the experiment. The DRDC dressings were easily removed from the wounds, no residual layer of gel being left behind. The effect of daily wound dressing changes on \textit{Ps. aeruginosa} and \textit{Staph. epidermidis} growth in infected, full-thickness porcine wounds are shown in Figures 2 and 3. \textit{Ps. aeruginosa} and \textit{Staph. epidermidis} bacterial counts in the control wounds increased to $1.10 \times 10^6$ CFU/g and $1.03 \times 10^8$ CFU/g within 48 h, respectively, these levels plateauing for the next 48 h (Figures 2 and 3). In contrast, biopsies taken from the wounds showed a 2- to 5-log reduction in the bacterial load of antiseptic-treated wounds compared to those of control wounds. \textit{Ps. aeruginosa} counts in the wounds treated with chloramphenicol-loaded dressings were 2-log lower for the first 24 h than when applying the chlorhexidine-loaded dressings (Figure 2). However, there was no statistical difference in the \textit{Ps. aeruginosa} counts in the wounds on day 4, following daily changes of either antiseptic-loaded dressings. Interestingly, chlorhexidine showed a 75% greater bactericidal efficacy against \textit{Staph. epidermidis} than chloramphenicol did throughout the study (Figure 3). Nevertheless, daily application of the antiseptic-loaded DRDC dressings maintained the wound bacterial load below $10^4$ CFU/g for both bacteria throughout the 4-d study.

The effect of altering the frequency of dressing changes on \textit{Ps. aeruginosa} and \textit{Staph. epidermidis} growths on day 4 in infected, full-thickness porcine wounds is shown in Figures 4 and 5. Increasing the frequency of dressing changes led to a significantly greater reduction (p<0.05) in the number of viable bacteria in the wounds. However, this bactericidal effect was significantly greater for \textit{Staph. epidermidis} than for \textit{Ps. aeruginosa}. It is noteworthy that the contamination levels of all antimicrobial-treated wounds remained below $10^5$ CFU/g of wound, irrespective of both the drug loaded in the wound dressing and frequency of wound dressing change.
Bi-Layer Wound Dressing System for Combat Casualty Care

Figure 2. Effect of daily wound dressing changes on \textit{Ps. aeruginosa} growth in porcine full-thickness wounds contaminated with an inoculum containing $10^7$ CFU \textit{Ps. aeruginosa}, \textit{Staph. epidermidis}, and \textit{Fusobacterium} sp. (1:1:0.5). Wounds were treated with a drug-free DRDC dressing or one containing either 0.5% chlorhexidine or 1% chloramphenicol. Data are expressed as means ± standard error of the mean (SEM; n=6). * Significantly different from control (p<0.05) † Significantly different from chloramphenicol (p<0.05). ‡ Significantly different from previous time interval (p<0.05).

Figure 3. Effect of daily wound dressing changes on \textit{Staph. epidermidis} growth in porcine full-thickness wounds contaminated with an inoculum containing $10^7$ CFU \textit{Ps. aeruginosa}, \textit{Staph. epidermidis}, and \textit{Fusobacterium} sp. (1:1:0.5). Wounds were treated with a drug-free DRDC dressing or one containing either 0.5% chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control (p<0.05) † Significantly different from chloramphenicol (p<0.05) ‡ Significantly different from previous time interval (p<0.05).
Figure 4. Effect of altering the frequency of dressing changes on *Ps. aeruginosa* growth at day 4 in porcine full-thickness wounds contaminated with *Ps. aeruginosa*, *Staph. epidermidis*, and *Fusobacterium* sp. Wounds were treated with a DRDC dressing containing no drug, 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control (p<0.05)
‡ Significantly different from daily dressing change (p<0.05).

Figure 5. Effect of altering the frequency of dressing changes on *Staph. epidermidis* growth at day 4 in porcine full-thickness wounds contaminated with *Ps. aeruginosa*, *Staph. epidermidis*, and *Fusobacterium* sp. Wounds were treated with a DRDC dressing containing no drug, 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control (p<0.05)
† Significantly different from chloramphenicol (p<0.05) ‡ Significantly different from daily dressing change (p<0.05).
3.2 Study II: Assessment of Wound Healing Properties

All animals survived for the duration of the experiment. Since the experimental dressings remained securely fastened, all wounds were therefore included in the calculations. Figure 6 depicts the number of non-contaminated, partial-thickness wounds healed over a 9-d period when applying daily either a non-medicated DRDC dressing or Allevyn®. It is noteworthy that the wounds healed poorly when left air-exposed for the duration of the study, with only 25% of them healed after 9 days. In contrast, 36% of the wounds covered with the DRDC dressing had healed after 5 days, this healing rate being 4.5 times greater (p<0.05) than that observed for the Allevyn®-covered wounds. While the wounds covered with Allevyn® thereafter healed faster for the next two days, the number of wounds healed remained significantly higher (p<0.05) in the DRDC dressing group for 7 days (Figure 6). No further increase in the number of partial-thickness wounds healed was observed in either experimental group for the remainder of the study. However, our results show that while 97% of the partial-thickness wounds treated with the DRDC dressings healed within 7 days, a comparable value was only reached after 9 days when covering the wounds with Allevyn®. While all DRDC dressings were not or minimally adherent to the wounds, 20% of the Allevyn® dressings were embedded in the wound bed at day 5.

![Figure 6](image-url). Kinetics of wound healing in a porcine model of partial-thickness, non-contaminated wounds when applying daily either a non-medicated DRDC dressing or Allevyn® (n=25 per time point per group). Control wounds were left air-exposed (n=20 per time point). Data are expressed as means ± SEM. * Significantly different from air-exposed (p<0.05) † Significantly different from Allevyn® (p<0.05) ‡ Significantly different from previous time interval (p<0.05).

3.3 Study III: Assessment of Skin Cooling Properties

All animals survived for the duration of the experiment. There were no significant differences in the skin temperature (T_{skin}) between the 3 dorsal sites recorded for any of the rats; the recorded T_{skin} of all sites were therefore used for comparing the different experimental treatments. Figures 7 and 8 depict the changes...
in $T_{\text{skin}}$ and intra-abdominal temperature ($T_{\text{ip}}$) over a 90-min application of hydrogel dressings (2nd Skin®) or DRDC dressings (dry or moist) on burn wounds covering 25% of the total body surface area in rats. Burn injury increased $T_{\text{skin}}$ by 0.50±0.20 °C within 5 min (p<0.05), $T_{\text{skin}}$ slowly increasing by 0.78±0.25 °C above the pre-burn injury value for the remainder of the study. $T_{\text{ip}}$ in BURN decreased by 1.36±0.27 °C within 20 min of the burn injury, $T_{\text{ip}}$ plateauing for the remainder of the study (Figure 8). This reduction in $T_{\text{ip}}$ while observing a raise in $T_{\text{skin}}$ can likely be explained by a reduction in the level of activity of the rat due to the burn injury.

![Figure 7. Changes in skin temperature ($T_{\text{skin}}$) when applying hydrogel dressings (2nd Skin) or DRDC dressings (either dry or moist) for 90 min on burn wounds covering 25% of the total body surface area in rats. The animals in the SHAM and BURN groups were dressed with one layer of the secondary dressing only. Data are expressed as means ± SEM (n=24). Filled symbols indicate a difference from BURN (p<0.05). Dashed lines indicate significantly different from 2nd Skin (p<0.05). DRDC (moist) was different from SHAM and DRDC (dry) at all time intervals (p<0.05). There were no significant differences between DRDC (dry) and SHAM throughout the experiment.](image)

Application of a dry DRDC dressing (DRDC$_{\text{dry}}$) to burn wounds for 10 min further increased $T_{\text{skin}}$ by 0.60 °C compared to that of BURN (p<0.05), this increase in $T_{\text{skin}}$ doubling by 90 min. Furthermore, DRDC$_{\text{dry}}$ prevented the burn-induced reduction in $T_{\text{ip}}$ throughout the study (Figure 8). Interestingly, there was no statistical difference in $T_{\text{ip}}$ or $T_{\text{skin}}$ between SHAM and DRDC$_{\text{dry}}$ throughout the study period, suggesting that the polyurethane foam layer did not act as an insulator. In contrast, the application of a moist DRDC dressing (to simulate a condition of exudate absorption) reduced $T_{\text{skin}}$ by 1.7°C for 5 min (p<0.05) compared to that of BURN. A greater reduction in $T_{\text{skin}}$ was observed following application of the hydrogel dressing compared to that of DRDC$_{\text{moist}}$ (p<0.05). While $T_{\text{skin}}$ in DRDC$_{\text{moist}}$ and BURN were comparable within 25 min, $T_{\text{skin}}$ remained 1.5 °C lower following application of the hydrogel dressing throughout the 90-min experiment compared to that of BURN. While there were no significant differences between $T_{\text{ip}}$ in BURN and DRDC$_{\text{moist}}$ throughout the experiment, application of a hydrogel dressing markedly decreased $T_{\text{ip}}$ after 90 min (3.03±0.55 °C).
4.0 DISCUSSION

Several new types of wound dressings have been commercialised in recent years, as it is accepted that designing an ideal wound dressing that will simultaneously meet all of the clinical requirements for treating the various types of wounds is impossible. Our data show that we have designed a layered wound dressing that will meet the unique challenges of treating combat wounds. Indeed, the combination of the foam and hydrogel layers: facilitates the reduction of bacterial load of wounds through incorporation of therapeutic agents; protects the wounds from additional trauma and contamination; absorbs wound exudates while maintaining a moist environment; is minimally adherent; promotes wound healing; and, provides surface cooling without inducing significant whole-body hypothermia. These properties make our dressing an excellent wound care management for the treatment of wounds sustained during military operations, especially burn wounds.

The types of bacteria used in the present studies represent common wound pathogens. While *Ps. aeruginosa* remains the most common and dangerous pathogen on burn injuries, the presence of *Staph. epidermidis* strains has also increased in many types of wounds, due to their prevalence on human skin, their ability to adhere to biomaterials, and a greater resistance to most antibiotics [13]. Bacteria can colonize unprotected burn wounds within 12-24 h, with microbial levels reaching 10⁸ microbes per g tissue within 48 h [14]. Furthermore, conventional dressings are susceptible to strikethrough of wound fluid, thereby providing a route for the bacteria into the wounds [15]. Although suppression of skin flora with an appropriate topical antiseptic agent kills bacteria, the suppressed microflora can rapidly grow back, and invade the compromised...
wound site. Furthermore, therapeutic agents applied to the wounds will undergo inactivation by body fluids and other organic debris. It is therefore not surprising that the early application of medicated wound dressings that continuously deliver antiseptic to the wounds remains the most effective way for preventing invasive wound infection.

Selection of the ideal antiseptic for treating contaminated wounds remains a controversial decision. Chlorhexidine and chloramphenicol were loaded in our dressings due to their broad-spectrum antimicrobial activity, and their ability to maintain a local residual antibacterial activity for hours without being neutralized by blood and wound exudates [16, 17]. However, late onset hypersensitivity to chlorhexidine has been observed following its application on both intact skin and wounds [18]. Furthermore, topical application of chlorhexidine has been shown to delay wound healing at concentrations above 0.1% [19], partly due to its cytotoxicity to newly formed keratinocytes [20], and its immunosuppressive effects on exposed macrophages [21]. Interestingly, irrigation of corneal abrasions with 1% chlorhexidine did not impair the rate of re-epithelization compared to that of control wounds [22]. Although we used a 0.5% chlorhexidine solution to load the drug into our dressings, the amount that was effectively incorporated during these procedures was likely much lower. Indeed, Loke et al. [23] have shown that the amount of chlorhexidine gluconate incorporated in their carboxymethyl-chitin hydrogel material was 3 to 8 times less than the loading concentration used. The magnitude of this relationship is likely to be material-specific, as we have previously shown a two-fold reduction in the amount of ciprofloxacin loaded into a proprietary hydrogel compared to the loading solution [24]. The amount of chlorhexidine incorporated into our dressings was sufficient to maintain the bioburden below the 10^5 CFU/g for the duration of the experiment, a bacterial threshold that has been shown to allow wound healing to proceed uneventfully, despite the wound bacterial colonization [25]. Thus, it appears that bacteria remain more detrimental to wounds and wound healing than the perceived injurious effects of antiseptic agents, which may explain that chlorhexidine is included in many commercial wound care products such as Bactigras™ (Smith & Nephew), Serotulle™ (Leo Laboratories, Ltd.) and BioPatch™ (Johnson & Johnson). Nevertheless, we are currently undergoing studies to investigate the usefulness of other antiseptic agents with lesser side effects on wound healing.

Our finding of comparable bactericidal efficacies of chlorhexidine-loaded wound dressings against both *Ps. aeruginosa* than *Staph. epidermidis* is in agreement with that of Kearney et al. [26] showing that chlorhexidine retained its activity against these bacteria for 13 and 21 days, respectively. While our data show a sustained bactericidal effect *in vivo* for up to 4 days, irrespective of the frequency of dressing change, we have observed that the *in vitro* drug delivery is that of a “burst” release, the bactericidal efficacy of the chlorhexidine-loaded dressings lasting up to 10 days (unpublished data). However, it is likely that the drug release kinetics from our dressing will be a function of the hydration status of the material. Furthermore, bacterial biofilms can be formed as early as 40 min following contamination of biological surfaces, thereafter becoming harder to eradicate [27]. While this data suggest the need to apply antiseptic-loaded wound dressings to the wounds soon after injury, such wound care strategy is likely impractical in the battlefield due to the hostile environment where the injuries occur. However, Gisby and Bryant [28] have reported that mupirocin cream was effective in reducing the bacterial load of foreign-body induced skin wound infections even when the treatment was delayed by up to 30 h. Similarly, we have shown the effectiveness of liposomal ciprofloxacin-loaded hydrogel dressings in significantly reducing the bacterial load in superficial muscles in a rat model of established wound infection [24]. Although it appears an important consideration that the dressing kills bacteria in the wound very rapidly, it is perhaps more important that the antimicrobial activity be maintained for the wear time of the dressing.

While we evaluated the bactericidal efficacy of our antiseptic-loaded dressings in a porcine model of full-thickness wounds, we elected to determine its wound healing properties on partial-thickness skin defects.
Indeed, the mechanical pressure exerted from the insertion of either of the experimental dressings to completely fill the wounds would have resulted in lesser rates of re-epitheliazation than those observed in the present study [29]. Although the mechanisms for healing partial- and full-thickness wounds differ, the conclusions regarding the effects of the different experimental dressings should remain unchanged. There is also an excellent degree of concordance between human and pig studies investigating the effect of various dressings on the healing of partial-thickness wounds [9]. In agreement with the literature [30-32], the dressing-covered healed faster than those that remained air-exposed. However, the rates of wound healing were quite variable at any given time interval, likely because some of the wounds may have become infected, thereby showing a much slower rate of healing compared to marginally contaminated wounds. As expected, the presence of a hydrogel layer in our dressing contributed to its minimal adherence to the wound bed [33], a desirable property that likely promoted the initially faster rate of wound healing of our dressing compared to that observed when applying Allevyn® by reducing the trauma to the new epithelial layer. Furthermore, dressing adherence to wound bed may lead to pain during its removal. While the control polyurethane foam dressing possesses a non-adherent wound-contacting membrane, it has been shown to induce scab formation on low-exudating wounds such as partial-thickness defects, which will then adhere to the dressing [34]. The latter observation, taken together with our finding of comparable maximum absorption capacities for both dressings (unpublished data), would suggest the greater polyvalence of our dressing.

Immediate cooling of both experimental and clinical burn injuries with cold water has been repeatedly shown to be the most effective way to: reduce skin temperature; reduce the severity of tissue damage; decrease initial local oedema; and, improve wound healing [8, 35-37]. For obvious reasons, this wound care strategy remains impractical in the battlefield. Our finding of a transient decrease in skin temperature following the application of the moist DRDC dressing to rat burns is in agreement with previous studies reporting the cooling properties of hydrogel dressings [36, 38]. However, the magnitude of the change in skin temperature varied greatly depending on the type of hydrogel dressing applied, ranging from approximately 1.5°C to 4°C. This discrepancy is likely related to differences in the water content (and thus evaporative cooling capacity) of the various gel formulations. Furthermore, Coats et al. [38] have shown that increasing the air movement over a hydrogel dressing could reduce the skin temperature by up to 10°C. Interestingly, a large afterdrop in skin temperature after removal of hydrogel dressings was attributed to exposure and subsequent evaporation of volatile substances and water in the residual layer of gel that was left on the skin after removal of the dressings [38]. While excessive cooling of a scald has been shown to be detrimental to wound healing [39], our data show that even a relatively small (1°C) reduction in skin temperature following application of a hydrogel on the large burn can lead to a significant hypothermia. These negative effects of hydrogels on wound healing and body cooling may contribute to their controversial use on burns.

It is unclear from our data whether the small, short-term reduction in skin temperature that we observed upon application of the moist DRDC dressing would be of clinical relevance. Interestingly, application of a moist prototype DRDC dressing prepared in our laboratories to the forearm of healthy volunteers resulted in a 3°C reduction in skin temperature for up to 90 min (unpublished data). This data, taken together with the fact that there was no effect on skin temperature in the present experiment when applying a dry DRDC dressing on the burn wounds, might suggest that the extent of the decrease in skin temperature is related to the moisture content of the foam layer of the DRDC dressings. It is interesting to speculate that the use of the DRDC dressing on low-exudating wounds (e.g., partial-thickness defects) or on surfaces that require maintenance of their moisture level (e.g., spilled guts) would not reduce their temperature. Nevertheless, care must be taken in interpreting the results from the present experiment in relation to human thermal injury.
5.0 CONCLUSION

These data show that the DRDC dressing is effective in a) delivering medications, such as an antimicrobial agent, to the wound bed; b) promoting faster healing of the treated wound; and c) providing a transient, cooling effect to the skin contact-site, without the adverse effect of inducing whole-body hypothermia like conventional hydrogel wound dressings.

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6.0 REFERENCES

Bi-Layer Wound Dressing System for Combat Casualty Care

Mechanical Ventilation in Hypobaric Atmosphere – Aeromedical Transport of Critically Ill Patients

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INTRODUCTION

Mechanical ventilation is used in the most of the aeroevacuations of critically ill patients. Patients and mechanical ventilators suffer from variations in the environmental Pressure, Partial Pressure of Oxygen, Humidity, Luminosity, Accelerations and Vibrations. We must describe briefly the history of Mechanical Ventilation and aeromedical transport:

Vesalius was the first author on describe one method of ventilation with positive pressure; 400 years later was applied for first time to a patient. Robert Hook in 1667 applied continuous flow ventilation to a dog. Woillez in 1876 made the first mechanical ventilator with negative pressure over the thorax, but the first “iron lung” was built in 1928 by Drinker and Shaw and after modified by Kroghs and Emerson. In 1955 the poliomyelitis epidemic was the main factor for the great success of the mechanical ventilation, with the device of the “Emerson Company” (Boston, Massachusetts) applying Mechanical Ventilation with positive pressure for the respiratory treatment of the patients affected by poliomyelitis. It could be the beginning of Mechanical Ventilation and possibly the Critical Care also.

The first aeromedical evacuation described was done in Paris in 1870 with aerostats, but the source never has been trusted. During the First World War the French Army carried out some aeroevacuations and also the US Army Air Service during the twenties. The first Military Flight Ambulance Unit was organized by Major Epanlard in the French campaign of the Riff. The German Army was pioneering on long distance aeroevacuations in the Spanish civil war, using JU-52 aircrafts rising altitudes of 18000 feet. During the
Second World War some aeromedical units were very famous like the 38th Medical Air Squadron from the US Army. Korea and Vietnam were the big success for the Rotatory Wing Aeroevacuations.

The Doctrine show us that the Rotatory Wing must be used for aeroevacuations shorter than 300 kilometres, and the Fixed Wing aircrafts for the bigger ones. One critical difference is that the planes usually can pressurize their cabins and the helicopters not. The planes can rise higher altitudes, for this reason the inner pressure has to be under control and also the temperature.

The factors that can damage the patients and also the devices in aeromedical transport are:
- Pressure.
- Temperature.
- Accelerations.
- Vibrations.
- Humidity.
- Luminosity.

Our study was focused on the effects of the changes on the environmental pressure over the system: Oxygen Tank + Mechanical Ventilator + Patient.

The Advanced Life Support (Cardiac or Trauma) have been improved for the use of aircrafts (fixed or rotatory), the response-time has been decreased and the success of the EMT systems is bigger also. But the Technology has its own price, and new devices inside different scenarios implies new factors to control. In the aeromedical transport the pressure changes in the environment has to be one variable to study, specially when the mechanical ventilation is applied. Primary or secondary aeroevacuations can need mechanical ventilation on flight. Secondary aeroevacuations of patients with Adult Distress Respiratory Syndrome are happening every day with one bigger frequency. The decrease that the Partial Pressure of oxygen suffers with the decrease of the pressure in one environment, is another factor to study about the mechanical ventilation applied to aeromedical evacuation.

In 1969 Robert Kirby et al, from the USAF studied the use of the Mechanical Ventilator Bird Mark VIII on altitude from 8000 to 34.000 feet. This mechanical ventilator cycled under airway pressure. Nowadays the Mechanical Ventilators cycle by the Tidal Volume or specifically by flow and time.

The Boyle-Mariotte law show us that the volume of one gas with constant temperature, is inversely proportional to the pressure that this gas receive. The decrease of pressure inside the cabin of the aircrafts, involves the increase in the volume of the gases inside. The effects of this phenomenon over the medical gases on mechanical ventilation produces changes in the mechanical devices and also can produce physiopathological consequences to the patients that suffer aeromedical evacuations with mechanical ventilation.

The basic parameters of mechanical ventilation are: Respiratory frequency (FR), Tidal volume (Vt), Minute Volume (Vm), Inspiration Fraction of Oxygen (FiO2), Positive Expiratory End Pressure (PEEP) and all of them changes if the environmental pressure does. Resistance of the Airway and the difference Compliances (Dynamic, Static and Specific) play a main role in the control of the mechanical ventilation during aeromedical transport.

The increase of the alveolar pressure can produce lung injuries. The Ventilation Induced Lung Injury (VILI) is a great risk on mechanical ventilation applied during Aeromedical Evacuations. The Barotrauma, the Volutrauma and their consequence the Biotrauma, can be produced for the effects of the
changes of the pressure in the environment during aeromedical evacuations of patients receiving mechanical ventilation.

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*Table 1: Relationship among environmental pressure, temperature and altitude.*
Mechanical Ventilation in Hypobaric Atmosphere – Aeromedical Transport of Critically Ill Patients

**Table 2:** Relationship between the outer altitude of the aircraft with the inner altitude (equivalent pressure), and the differential pressure between them on flight.

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* Study objective:

We tested the changes that decreased environmental pressure produced in a mechanical ventilator and also in the individuals that were connected to this device receiving mechanical ventilation, with the main task being to evaluate the changes in Tidal Volume. Our Hypothesis was that one system with oxygen tank + mechanical ventilator (flow time cycled in Assisted/Controlled modality) + patient, can be seriously damage for the increase in the gases volumes (specially the Tidal Volume), due to the decrease in the pressure of the environment, and this phenomenon happens inside the cabin of the aircrafts during the aeromedical transport of critically ill patients with mechanical ventilation.

**Material and Methods:**

We applied Invasive Mechanical Ventilation (endotracheal tube) in Assisted/Controlled modality with a transport mechanical ventilator (Dräger Oxylog 2000), with a Fraction Inspiration (FiO2) of Oxygen of 100%, Respiratory Frequency of 12-16 breaths per minute and Positive End Expiratory Pressure of 5-6 centimetres of water to 10 beagle dogs with weights from 8-17 kilograms under intravenous sedation. The animals with the mechanical ventilator and the oxygen tank were introduced into the Hypobaric Chamber for Physiological Studies of the Spanish Unit of Aviation and Space Medicine of the Air Force. The pressure conditions of a profile of High Altitude Flight (from 2000 to 35000 feet) were applied inside the chamber.

The dogs were ventilated for 45 minutes before the experimental flight and along the whole test. Parameters from the mechanical ventilator were measured and also vital signs (monitoring) from the animals. The controls of the Tidal Volume were measured at 14 altitudes in the climbing phase and 6 in the descent phase. All the measures were taken one minute after to arise the altitudes and to stabilize the pressures. The Tidal Volume was measured three times every altitude (equivalent atmospheric pressure).
Results:

The Tidal Volume was always bigger with the decrease in the environmental pressure and even in the
descent phase, we found bigger Tidal Volumes that in the same altitudes in the climbing phase. The
increase of the Tidal Volume with the decreasing atmospheric pressure was checked statistically with a
bilateral significance of P< 0,01 applying one Pearson Test. But the increases on the Tidal Volumes were
not as big as expected by the Boyle-Mariotte law.

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* Table 3: Results of the different parameters measured at the beginning of the flight (first altitude) of the
10 dogs. These data were measured in twenty different altitudes.

Conclusions:

The Tidal Volume increases or decreases with changes in the environmental pressure when the mechanical
ventilation is used. This is very important in the transport of critically ill patients. These changes can
produce damages to patients that are under mechanical ventilation during aeromedical evacuations.

Correction rules can be calculated for this phenomenon using the Compliance, the Saturation of Oxygen,
the different Airway Pressures, the Expiratory Tension of CO2, the continuous Monitoring of the
Mechanical Ventilation applied (specially curves and loops), the Oxygen Partial Pressure and finally the
continuous Blood Arterial Gas Analysis. This is a new investigation area in which the monitoring of
different parameters (specially the Compliances) plays the main role to control the Mechanical Ventilation
during Aeromedical Transport.
Mechanical ventilation on medical transport (and especially on aeromedical) is a different concept of mechanical ventilation than the one applied on Emergency Medicine. The devices (mechanical ventilators) have to fit to these different scenarios, or different mechanical ventilators should be used for every one.

The perfect Mechanical Ventilator should change by itself (its parameters and modality of mechanical ventilation), with the analysis of the atmospheric changes of the scenario and the repercussions to patients, for avoiding the VILI.

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Closing the Loop on Critical Care Life Support for Military *En Route* Care Environments*

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**SUMMARY**

The Future Force (FF) war-fighting concept includes maintenance of medical functionality as close to the action as possible for 72 hrs without re-supply and possibly without air evacuation assets. The most challenging casualty for the Forward Surgical Team (FST) is the critically injured soldier requiring immediate life-saving surgery and transport while on life support equipment. The FF battlefield envisions long evacuation distances, exceeding the 2 hr. flight capabilities of the current UH60 Blackhawk used for MEDEVAC. This situation requires greater holding capability at the FST and enhanced en route care capabilities in both ground and air evacuation vehicles with 10 to 12 hour transport times. The Automated Critical Care Life Support (ACCLS) capability under development within the US Army's Medical Research and Materiel Command, will provide automation of life support functions, through the development of computer-driven closed loop control of ventilation, fluid, drug and oxygen administration. This closed loop approach to life support will not only optimize the patient’s life support but will result in significant conservation of IV fluid and oxygen resuscitation resources.

**1.0 INTRODUCTION**

Clearing the battlefield quickly and efficiently while providing the patient the best possible care is a priority mission of the US tri-service, military medical community. At the same time, reduction in the size of the medical footprint and enhancement of the mobility of our MTFs is also a high priority. Expedient movement of the critical care patient population would contribute significantly to the attainment of these goals [20]. Since World War II, 90% of all combat battle deaths occurred within the first hour in Echelon I before reaching the first level of medical care. This statistic has remained unchanged thru the Viet Nam War. Hemorrhage and CNS trauma were the leading factors that contributed to early death of these casualties.

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* Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Although a small fraction of the total casualty population, about 10% requiring immediate surgery these patients require a disproportionately large number of man-hours and logistical support in the field. [16]

Current US military evacuation procedures require that a patient be held in a field MTF until ready to return to duty or until stable enough to be evacuated [6]. For the critical patient, this can be several days or more before he can withstand the added stresses of ground or air evacuation with the attendant high ambient noise and vibration, and in the case of rotor-wing air evacuation and the MV22, low atmospheric pressure conditions. Although there is little reason to believe that the high acoustic noise and vibration are, in themselves, medical hazards, they would, and do, significantly degrade the attendant care-giver's diagnostic and therapeutic capabilities en route. Heart and lung sounds are difficult if not impossible to detect above road or aircraft noise and even simple palpation of peripheral pulses can be very challenging if not impossible in the hypotensive patient, in high vibration environments. In addition, the training of the air and ground medics attending these patients does not include management of the critically injured on life support equipment. These conditions create a significant hazard for the patient since a medical crisis may escape detection en route. On the other hand, delaying the evacuation for the time required to get the patient off his life support equipment is not desirable either as this compromises the mobility of the Forward Surgical Team which is charged to stay within a few miles of the forward edge of the battlefield. It is for these reasons that a portable, lightweight, self-contained system that provides capability for continuous monitoring of casualty status, including assessment of early markers of physiological deterioration, automated alarming, and closed-loop, automatic control of life support equipment and interventions would be of value to medical personnel after casualty stabilization and during evacuation.

1.1 Military Rationale for Closed Loop Life Support

The key to successful management of the critically ill patient is vigilance. Monitoring the patient with sufficient frequency to detect life-threatening events early enough to implement lifesaving therapy is challenging enough in the controlled civilian hospital environment. However, this challenge is magnified enormously when care of the critically ill patient on life support equipment is taken into the battlefield setting, where personnel and supply constraints are much greater and the vigilance factor is further compromised by environmental challenges such as high noise, and in the Medevac setting, high vibration. The goal of any critical care unit, through both staffing efforts and the design of effective alarm systems within the monitoring equipment, is to minimize the time between detection of life-threatening events and the implementation of a corrective or therapeutic action. Computerized monitoring combined with digital control capabilities built into life support equipment offers an opportunity to automatically accomplish this goal and offers the possibility of significantly enhancing the responsiveness of the life support system.

The Army Transformation's FF Force (FF) war-fighting concept, includes maintenance of medical functionality as close to the action as possible for 72 hrs without re-supply and possibly without air evacuation assets. The most challenging casualty for the Forward Surgical Team (FST) is the critically injured soldier requiring immediate life-saving surgery and life support equipment. The FF battlefield envisions long evacuation distances, exceeding the 2 hr., flight capabilities of the current UH60 Blackhawk used for MEDEVAC. This situation requires greater holding capability at the FST and enhanced en route care capabilities in both ground and air evacuation vehicles with 10 to 12 hour transport times. The ACCLS capability proposed here would provide automation of life support functions, providing computer-driven closed loop control of ventilation, fluid, drug and oxygen administration in the first iteration. This system would optimize the patient's treatment, while minimizing resource utilization (i.e. oxygen and resuscitation fluid). The ACCLS platform will also be a significant critical care enabler for the small FST staff and for the 91W staffing of air and ground ambulances. Currently, ambulance medical attendants are not trained critical
care specialists, a situation not anticipated to change in the FF. Therefore, automation of the life support systems is a critical capability that will allow expedient movement of casualties out of the FST and will level the quality of care throughout the echelons of medical care of the critically injured. The system will also provide data logging and telecommunication capability to facilitate record keeping and to enable real time communication of patient data to the receiving hospital for assistance with monitoring and decision assistance from a remote location. The ACCLS will provide increased and improved holding capability at the FST as well as extended critical care capability within the ground ambulance platform by providing automated life support for the critically injured awaiting and during evacuation.

1.2 Requirements for the ACCLS System

1.2.1 Hardware

The physical requirements of the ACCLS system anticipated to meet the needs of the FF are as follows: A portable, self-contained, lightweight (less than 80 lb), protected environment for one casualty, capable of providing sustained, critical care monitoring and automated life support for combat casualties for up to 24 hours on the FF Force (FF) Battlefield. System capabilities will include: 1) Integral suction and ventilatory capabilities 2) Automated external defibrillation system 3) Integrated parenteral infusion system 4) On-demand oxygen production capability with compatibility with external oxygen delivery systems 5) Physiological monitoring (non-invasive & invasive) including blood chemistries and BP, ECG, HR, Core temp, oxygen saturation 6) Protection from environment, biological & chemical agents 7) Data logging and transmission capacity 8) Compatibility with military vehicle and European power sources and 9) Automated life support functionality directed at conserving resources and manpower. External dimensions will be constrained to be no greater than the cube of the existing NATO litter to maintain compatibility with existing patient movement vehicles (air and ground) and other litter support devices (litter stands, wheeled gurneys, etc). The total weight of the system with a 220 lb patient will not exceed that which is manageable with a 4 person, 5th percentile litter team (about 300 lbs). Stand-alone battery operation with all life support functions operational shall be required for a minimum of 2 hours to accommodate the transfer time between the MTF and ambulance. The system must also comply with the environmental requirements of MIL STD 810E and 462D and must comply with all airworthiness requirements of the Army rotor wing and Air Force fixed wing environments.

1.2.2 Software

The software development process follows Cleanroom Software Engineering process. Cleanroom Software Engineering is a rigorous formal methods process for generation of software requiring high reliability and is a process that fulfills the requirements of the FDA for medical device development and code traceability. This process places a heavy emphasis on the prospective generation of requirements for software before any coding begins. Once the requirements are thought to be complete, the stimulus response table is constructed linking specific conditions or stimuli to the desired response. An enumeration process follows this, which is a mathematical procedure for determining canonical and illegal states. This eliminates redundancies and identifies impossible software states. From the results of the enumeration process, state boxes are generated, then clear boxes and finally the code is written. A testing protocol is then constructed based upon a usage model and bench level testing performed. This is followed by laboratory evaluation of controller performance in animal models of the pathophysiologic states expected in the course of evacuating the patient from the front to the rear.
2.0 CLOSED LOOP DEVELOPMENT APPROACH

The basic components of a closed loop control system include 1) a sensor for the parameter to be controlled, 2) a set point target for the controlled parameter, 3) a comparator, which generates an error signal based on the difference between the set point target and the present value, 4) a transfer function, which converts the error signal into a command instruction for the effector device and 5) the effector device which implements the appropriate corrective action. This control loop emulates the components and their relationships within the physiological control systems found in the human body, which are largely negative feedback loops.

![Diagram of closed loop control elements]

Figure 1 shows the relationship of these basic closed loop components and illustrates the principle of automating the delivery rate of oxygen to the patient by driving the oxygen flow rate with the deviation of the measured arterial oxygen saturation from the desired set point.

2.1 Control of Intravenous Fluid Administration

Delivery of intravenous fluid for resuscitation in the field has been gravity-driven for more than 70 years. However, gravity-driven flow has several significant drawbacks in the field, including: 1) increased risk of exposure to enemy fire under battle conditions due to the need to hold the IV bag in the air, 2) a requirement for a fifth man to carry the IV bag in a 4-man litter carry 3) poor control of infusion rate and resuscitation. In addition, during air evacuation, previous commercial intravenous pump designs are difficult to keep running due to the small air bubbles that are produced as a result of off-gassing at altitude. These small bubbles cause the bubble detection feature to error and shut down the pump flow. Improved control of the rate and volume
of resuscitation fluid administered is required to allow implementation of new resuscitation strategies aimed at optimising tissue perfusion while minimizing fluid resource utilization. These problems could be overcome with an active pumping mechanism. Recognition of these field issues, led us to design and patent a new pumping mechanism, which will eliminate the aforementioned problems. This work uses the new commercial implementation of this patent that has the added feature of an analog control interface.

2.1.1 Control Software [Computer Assisted Resuscitation Algorithm (CARA)]

2.1.1.1 Set Point

The general strategy in the initial development of closed loop fluid resuscitation is not to discover a new resuscitation end point goal, but to simply emulate what is currently being practiced in the pre-hospital and emergency room settings today by infusing fluid to a target blood pressure end point. The classical clinical definition of shock includes systolic pressure of less than 90 mm Hg with tachycardia. Therefore, the CARA algorithm has been constructed with a blood pressure end point that would not qualify as shock by this definition. The CARA uses the mean arterial blood pressure setting as a target instead of systolic pressure since the mean pressure is less susceptible to problems encountered with improperly damped arterial pressure lines, which therefore mitigates a risk identified in the software hazard analysis. Although the mean arterial blood pressure default target is 70 mm Hg in CARA, this can be over-ridden by the care provider. The rationale for choosing this endpoint is based on the fact that autoregulation of blood flow is manifest, in most all organ systems, above a mean arterial blood pressure of 60 mm Hg. Furthermore, within the concept of operation for the use of the Automated Critical Care Life Support System, this device would serve as an asset of the Forward Surgical Team, where only resuscitative surgery would be done. In this context control of the bleeding may be tenuous and the risk of reinitiating bleeding with overly aggressive fluid resuscitation is significant. Thus, limiting fluid resuscitation efforts to sub normal blood pressure targets to minimize re-bleeding problems while providing adequate organ perfusion is the physiological goal around which CARA was designed. There is currently no other practical way to implement this strategy without automated control of fluid infusion rates since battlefield caregivers are not able to monitor the patient and titrate fluid continuously.

2.1.1.2 Comparator and Error Signal

The controller to be used is a Proportional, Integral, Derivative (PID) controller, which by definition has three components included in generation of the error signal; the proportional component, which is based on the absolute difference between the target and the current value, an integral component, which reflects the cumulative difference from the target pressure and a derivative component, which relates to the rate of change [25]. Currently it is not known whether all three of these components are required for effective control of the mean arterial blood pressure. Therefore, the coefficients on each component will be tracked throughout the resuscitation efforts to evaluate their relative contributions to the error signal. If a component contributes insignificantly regardless of the physiologic state of the animals for the type of resuscitation fluid, this component will be dropped in order to simplify the algorithm.

Clearly, the update frequency of the mean arterial blood pressure signal will significantly impact the effectiveness of the controller. Initial experiments have shown excellent control of blood pressure with five second updates of the mean arterial blood pressure, however, there are few non-invasive blood pressure sensors that can provide an update this frequently. Therefore, a major goal of this work will be to quantify the effect of blood pressure sampling frequency on the performance of the PID controller. PID controller performance will be evaluated by measuring 1) the time to achieve 50 percent and 90 percent of the target
blood pressure levels and 2) the 20 minute cumulative deviation (mmHg-min) from the target blood pressure once 90 percent of the target has been reached. In order to explore the effect of blood pressure sampling frequency on the blood pressure control, the sampling frequency of the arterial pressure line will be altered in 20 second increments starting at 5 seconds up to two minutes. These metrics of PID performance will also be quantified with each method of blood pressure measurement. The results of this series of experiments will quantify the effect on controller performance with different sampling frequencies for the blood pressure measurement. This data will be important in this selection of the blood pressure measurement modality as these data will allow for the potential of dynamically changing the sampling frequency to accommodate the clinical situation. For example, during circumstances where the desire for aggressive resuscitation is desired or circumstances where the blood pressure is falling in spite of the efforts of the controller, the sampling frequency can be increased to the level required to re-establish good blood pressure control. It is possible that oscillometric blood pressure cuffs may be sufficient in certain circumstances where the pressure is relatively stable, however a more rapidly responding sensor will be required to accommodate unstable conditions.

An adaptive quality may be conferred upon this algorithm if the responsiveness of the cardiovascular system to volume infusion is tracked as well. Therefore the pressure change associated with the volume infused will be tracked by the algorithm and will be expected to change with the state of the compensatory capability of the animal through the dynamic process of shock. It is expected that in the decompensatory phase of shock, it will be necessary to infuse more volume to attain the same pressure goal as the responsiveness of the peripheral vasomotor system deteriorates. We have observed that this low peripheral resistance state also occurs in normovolemic animals with 2.5 percent isofluorane anaesthesia in this model and therefore could serve as a surrogate for the decompensatory phase of hemorrhagic shock which is also characterized by a lack of vasomotor responsiveness to fluid and to catecholamine therapy. It is a goal of these experiments to record the pressure-volume history accumulated during operation of the controller during resuscitation to determine whether it will be possible to confer an adaptive change in the PID controller that would accommodate these pathophysiologic changes in the cardiovascular system. This information might also trigger an informational alert to the user to indicate that there has been a change in the physiologic state of the vascular system that might cue either the onset of vascular decompensation, correlate with a pharmacological effect of a drug infusion (e.g. isofluorane-induced vasodilation), or indicate volume loss from the system, such as onset of bleeding. The protocol described below addresses this issue by proposing to undermine the controller's efforts by removing blood at controlled rates to determine whether it might be feasible to detect the onset of occult bleeding.

2.1.1.3 Transfer Function

The transfer function will convert the error signal from the PID in mm Hg to a driving voltage for the pump that translates to a flow rate in ml per minute.

2.1.2 Sensor and Effector Hardware

2.1.2.1 Infusion Pump

Since there were no commercially available fluid infusion pumps which could deliver flow rates high enough for resuscitation of hemorrhagic shock, we (WRAIR) designed and patented a new, light weight, and highly efficient pumping mechanism which would meet the needs for fluid resuscitation in echelon I. The pump dimensions are 2.4”W x 3.8”L x 1.2”H and weighs 238 grams. This pump is FDA approved and is in production (Infusion Dynamics, Inc., Plymouth Meeting, PA) and is composed of three basic parts 1) a reusable control unit, 2) a sterile disposable cartridge and 3) a single-use battery, lasting 6 hrs at 100 ml/min.
It uses a sterile disposable pumping cartridge with standard Luer fittings and a built-in air eliminator. The air eliminator prevents the pump from shutting down due to off gassing at reduced atmospheric pressures, such as that encountered during air evacuation. The pump can operate as a stand-alone device with the flow rate set using a knob on the side of the pump or it can be controlled by a signal from an external device. This latter feature allows implementation of servo-controlled fluid resuscitation based on blood pressure or any other selected resuscitation end-point. The lightweight nature of the pump allows it to be attached to the patient’s arm with a Velcro strap.

![Image of intravenous infusion pump with 500 ml bag of Hespan](image)

**Fig. 2: Picture of intravenous infusion pump with 500 ml bag of Hespan**

The one-way valve arrangement and non-occlusive pumping action allows fluid to flow freely in the forward direction only. The IV lines can be primed or purged of air by simply squeezing the IV bag. This free flow feature also provides for fail-safe operation since fluids can be delivered by elevating or pressurizing the IV bag while the pump is off but still connected. The pump infuses crystalloids at rates comparable to an IV bag raised 7 feet above the patient and colloids at rates comparable to a bag raised 15 feet (6 L/hr.). Compared to inflatable pressure infusers, this pump offers typically shorter set-up times, an air elimination filter and greater control over flow rates. This latter feature is important to enabling the hypotensive resuscitation strategy to be implemented in the field as a temporizing resuscitation strategy which will minimize the potential for re-bleeding during resuscitation efforts aimed at elevating the arterial blood pressure. The electrical impedance of the infusion fluid past the air eliminator is continuously monitored and will shut down if a bubble is detected or a fluid below physiologic ionic strength is pumped. The latter prevents accidental infusion of distilled water or other fluids of unacceptable ionic strength. Alarms are also triggered with conditions of low battery and IV tube occlusion. A new cartridge allows for infusion of whole blood as well.

In the experimental animal studies, the flow delivered to the animal is quantified by the decrease in the weight of the infusion bags resting on a digital scale. Obviously, in the final of implementation of CARA, there must be a way to relatively accurately quantify the volume infused in order for the tracking of the pressure-volume history to be useful in conferring an adaptive component to the controller or to be able to issue an advisory on the change in the physiologic response to the volume as suggested above. Currently there is no flow monitor built into the infusion pump but clearly this would be a hardware improvement that would be desirable for the
reasons cited above. However, the back emf is measured which gives an indication of the motor speed that is used to validate that the motor performance is appropriate for the driving voltage instruction issued by the controller. This gives only a semi-quantitative estimation of the actual flow since other factors such as the inlet and outlet pressures can affect the absolute flow rate. Studies are ongoing to determine whether the back EMF might be accurate enough for predicting the flow with varying inlet and outlet pressures. If not, a recommendation to add a flow monitor to the pump will be made to the manufacturer for this application.

2.1.2.2 Blood Pressure Sensor

Obviously a major component of importance is the sensor responsible for measuring the mean arterial blood pressure. The sensor must be reliable, be able to provide continuous blood pressure measurements and preferably be non-invasive. The greatest reliability and accuracy is obtained from an arterial line but its invasive nature makes it undesirable for the patient on the move in the battlefield setting. However, the most ubiquitous non-invasive alternative, which is the oscillometric blood pressure cuff, cannot be used for long periods of time at sampling frequencies > twice per minute and is susceptible to motion artefact and error in high vibration environments.

Clearly, the quality of the blood pressure measurements will significantly affect the performance of the controller, however, it is not clear what the lowest blood pressure measurement frequency can be and still get good blood pressure control. Therefore, the current studies will systematically study the effect of blood pressure measurement frequency on blood pressure control using an intra-arterial blood pressure line sampled at different frequencies. This information is required for selection of the best non-invasive blood pressure measurement modality. Currently, there are three non-invasive monitoring techniques that will be evaluated against the arterial line standard for their ability to provide pressure readings of sufficient quality and frequency to meet the requirements of optimal blood pressure control. A standard oscillometric blood pressure cuff will also be included for a basis of comparison. If new options become available, they will also be evaluated.

2.1.3 Controller Performance

Figure 3 illustrates the type of experiment that is used to evaluate the performance of the closed loop controller, CARA. In this experiment, the experimental protocol was designed to emulate a severe arterial bleed in 70 kg swine resulting in a drop in the mean arterial blood pressure (MABP) to 40 mm Hg over a 15 min period. The pressure was then held there for 15 min. This resulted in the loss of 1.45 L of blood or 30% of the pig's blood volume. Resuscitation was then initiated by turning on the autocontrol software which ran on an external computer which was receiving mean arterial blood pressure readings every 5 s, calculating the deviation from the target and then applying the transfer function to alter the driving voltage every 5 sec. The volume infused was followed by monitoring the output of a digital scale on which the resuscitation fluid was placed. In this experiment, the MABP set point was 70 mm Hg and the resuscitation fluid was 0.9% saline. Metrics of the controller performance are shown in the table above the graph showing the times and volumes required to attain 25, 50, 90 and 100% of the MABP target. Although 90% of the target value was attained with 756 ml of saline within 6.3 min with the pump operating continuously at 120 ml/min, an additional 1.5 L was required to maintain the MABP at this level for the next 30 min. Comparison of the dotted line drawn over the resuscitation period shows excellent control of the blood pressure over the 40 min following the start of resuscitation. This figure also shows the effect of a transient occlusion of the pump outflow tract at 48 min that initiated the occlusion alarm. When the occlusion was released, the autocontrol resumed automatically and restored the blood pressure once again as it had fallen as a result of the 2 min occlusion.
2.2 Control of Ventilation

Seventeen per cent (17%) of patients seen by the Marine Forward Resuscitative Surgery Units during the first 30 days of Operation Iraqi Freedom required en route care and 20% were categorized as unstable. The mean time to evacuation was 8 hours and patients who were intubated and/or required ventilation accounted for 25% of the 48 indications for en route care. Since Army Medevac is staffed by only by flight medics who are not trained in ventilator management, it is important to automate ventilator operation so that minimal training, or even no training, is required for them to safely transport the critically injured patient. Currently, neither the Marine Corps FRSS or the Army FST have enough medical personnel who are familiar with ventilator operation that they can afford to lose them on a Medevac mission.

The modern mechanical ventilator has been in existence for nearly 100 years. The earliest efforts focused on ventilating the patient by applying a negative pressure in a closed space in which the patient was contained from the neck down. This iron lung concept evolved in the mid 20th-century to ventilation using intermittent positive pressure via an integrated airway. It was not long after the first automatic mechanical ventilator was developed that investigators began exploring ways to automatically control ventilator in response to patient needs. Frumin developed the first working closed loop controller based on the automatic maintenance of end-tidal CO₂ levels [5]. This system simply changed the tidal volume in small steps until the desired change in
CO₂ level was achieved; requiring an increase in the tidal volume to decrease the end tidal CO₂ levels. [12], implemented a closed loop control system based on arterial PO₂ and demonstrated maintenance of the arterial oxygen tension within 1 mm Hg of the target value even when CO₂ production rates were increased by nearly 50 percent. Chapman [2] described similar precise control of end-tidal CO₂ levels, achieving the set point goals within 60 seconds of the set point change and maintaining the end-tidal CO₂ level to within 1 percent of the set point. However, controllers that use end-tidal CO₂ levels presume that this measurement is a reasonable estimate of arterial PCO₂. This is the case as long as there are not ventilation-perfusion mismatches that create large alveolar to arterial gradients such as those observed during embolic events or in atelectasis. In these cases, the end-tidal CO₂ partial pressure decreases and the controller's response is to decrease the ventilation in an attempt to increase the end tidal CO₂ levels. This is an inappropriate response. Therefore, unless ventilation perfusion mismatches can be ruled out end-tidal CO₂ controllers are not safe.

In addition, successful control of ventilation has been achieved using intra-arterial blood pH monitors as well [4]. The principal of this control system is to adjust the arterial PCO₂ to maintain a pH of 7.4 according to the Henderson-Hasselbach equation; pH=6.1 + log[HCO₃]/(0.0301 * PCO₂). This controller would be expected to work well under conditions of metabolic acidosis and controlled ventilation, where the respiratory muscles are paralyzed and unable to respond to the chemoreceptor-mediated drive. The commercial availability of intra-arterial PCO₂ and pH monitors make controllers that use these endpoints feasible today.

Closed-loop ventilation has been a topic of active investigation for over 50 years and ventilator companies in Europe and the US have positioned their hardware for closed loop control for some time. However, at present, closed-loop ventilator systems have only been licensed for sale in Europe.

2.2.1 Software Controller

The overall approach to maintaining arterial oxygen saturation will be to first optimize oxygenation by means of ventilator adjustments (i.e. tidal volume, frequency, I:E ratio, PEEP) and then to only deliver oxygen when increases in alveolar ventilation do not resolve the desaturation problem. Evaluation of the performance of the ventilator and oxygen flow controller will first be tested independently and then in combination during specific ventilatory challenges designed to emulate anticipated clinical conditions. Once these controllers are operating satisfactorily in an independent manner, they will be evaluated together. The final phase will evaluate the combined CARA and CAVA algorithms operating simultaneously.

2.2.1.1 Set Point

In view of the problems noted above with regard to controllers that are based on end-tidal CO₂, and their inappropriate response to ventilation and perfusion mismatch conditions, a review of the literature shows that a relatively new controller developed by Laubscher offers significant advantages [7,8]. The closed loop controller that will be evaluated for inclusion into the ACCLS system in this proposal is called Adaptive Volume Ventilation which uses breath to breath analysis of the pressure volume relationship to determine the optimal tidal volume and respiratory rate required to minimize the work of breathing while achieving a minute ventilation goal which is set by the user. The advantage of this type of controller is that it is based on a physiological principal discovered by [14] who demonstrated that mammals normally breathe at tidal volume and respiratory rates that minimize their work of breathing. This is been shown to work particularly well for weaning ventilator-dependent patients in a shorter amount of time [10]. This technique is also suitable for use in both spontaneously breathing as well as paralyzed patients. The only input required from the user is an approximation of the desired gross alveolar ventilation set point, which is simply the product of the respiratory rate and tidal volume minus the anatomical dead space volume. To avoid having to set this value,
the test breath procedure described by Laubscher et al. will be used [7]. This test procedure uses a standardized breath pattern based on pressure controlled synchronized intermittent mandatory ventilation (PCSIMV) and requires synchronized measurement of airway flow, airway pressure and instantaneous expired CO₂ concentration. These breaths are analyzed in terms of tidal volume, series dead space and respiratory time constant. With knowledge of the respiratory rate the gross alveolar ventilation can be calculated based on these test breaths to initiate the ventilation start up procedure. Comparator and Error Signal

The work of breathing (WOB) calculation will be performed twice per minute and compared with the prior WOB and changes tracked.

2.2.1.2 Transfer Function

The tidal volume and frequency required to minimize the work of breathing in accordance with the work of Otis [14], will be calculated for the target gross alveolar ventilation set point as described by Laubscher [7] and the optimized settings sent to the ventilator twice per min.

2.2.2 Sensor And Effector Hardware

2.2.2.1 Sensors

Calculation of the WOB required a measurement of lung compliance which is derived from instantaneous measurement of the airway pressure and flow. Assessment of SPO₂ and end tidal CO₂ is required periodically to assure that gas exchange is adequate at the chosen gross alveolar minute ventilation. To determine whether this level of alveolar ventilation is sufficient requires periodic blood gas analysis. Within the context of the ACCLS system, it is proposed that this data be input by the user who may periodically analyze blood samples using a handheld point of care blood analysis system or the commercial intra-arterial PCO₂, pH and PO₂ fiber-optic probes may be used for continuous update of the arterial PCO₂ values. If this number is available, the gross alveolar ventilation can be automatically adjusted to compensate for any ventilation perfusion mismatch conditions that create a large alveolar to arterial gradient. This can only be done however, if the arterial CO₂ tension is known so that the gross alveolar ventilation can be corrected for the alveolar dead space ventilation. With this level of automated control the ventilator control panel could be reduced to a single knob for setting the alveolar ventilation in liters per minute, which can be simply done on the basis of a lookup table that shows minute ventilation as a function of body weight.

2.2.2.2 Ventilator

This effort will seek to adapt the preferred patient movement item (PMI) transport ventilator chosen by the Armed Services to be used in the context of en route care. Recently Impact Instrumentation, Inc. has modified its 754 series ventilator for digital control, enabling it to be microprocessor-controlled. The first phase of this effort will be to evaluate the effectiveness of the adaptive volume ventilation controller when implemented on this ventilator. These studies will be conducted in both spontaneously breathing and paralyzed and anesthetized 70 kg swine.

2.3 Control of Oxygen Administration

The US Army Combat Support Hospital planning factors for oxygen, call for 9 tons of oxygen per day (including the weight of the cylinders) to support the oxygen needs of a 256-bed hospital. This massive
requirement is logistically difficult to manage and oxygen tanks are a significant safety hazard. As a result, the Army Medical Department has initiated an effort to eliminate oxygen tanks from the battlefield and to replace them with oxygen generation systems. Both the US Army and the Air Force have significant investments in this area. However, the systems under development have been oriented toward meeting the needs based on utilization within civilian fixed facility hospitals where oxygen is considered inexpensive and readily available. As a result, efforts to conserve oxygen in the civilian medical setting have not been considered important.

Oxygen conservation systems are in common use within the diving community but have not found their way into the medical community, probably because oxygen conservation has not been a primary concern. Although the closed loop system with CO₂ absorbent is the best suited to achieving the greatest degree of oxygen conservation, its complexity is one negative aspect. Other alternatives that can accomplish conservation of intermediate value are also possible. Although not optimal, there are passive partial re-breathing approaches that have recently been commercialized that would allow a 50% decrease in flow rate to achieve the desired FIO₂ (i.e. Hyox partial re-breathing mask). In addition, pulse conservation methods are also available which deliver oxygen only during inspiration. This technique saves two-thirds of what would normally be delivered in a continuous flow, non-re-breathing circuit with an I:E ratio of 1:2. This feature is commonly found in home care oxygen delivery systems that use nasal cannulas. Each of these techniques, combined with continuous monitoring of the arterial oxygen saturation from pulse oximeter readings will allow the system to consume as little oxygen as possible while meeting the oxygenation requirements of the patient.

Closed-loop controllers have been reported recently to be very effective in maintaining the arterial oxygen saturation within 3% of the target SpO₂ in mechanically ventilated patients [19] and to be as effective or more effective than a full time nurse in maintaining the SpO₂ of low birth weight infants within a normoxic range of 88-96% [3]. Similar studies have not yet been done in trauma patients.

2.3.1 Software Controller for Oxygen Administration

Advanced Trauma Life Support (ATLS) guidelines recommend 10 to 15 L/min supplemental oxygen flow for all trauma patients. Furthermore, the ACCLS platform will need to carry intrinsic oxygen delivery capability in order to accommodate patients with either pulmonary compromise or respiratory control problems caused by head injury and to accommodate treatment at altitude. However, in the context of the ACCLS development effort, it is an objective consistent with the goals of the Combat Developer, to provide oxygen with on-demand oxygen generation systems in order to eliminate the use of oxygen cylinders on the battlefield. However, with the current state of oxygen generation systems, units that generate 10 to 15 L/min weigh nearly 100 lbs. To meet the weight goals of the ACCLS platform, it will be necessary to minimize the size of the oxygen generation system required to maintain the arterial hemoglobin oxygen saturation goals. Current MRMC investments have produced a pressure swing oxygen generation system that will deliver 3 L/min and weighs between 8 and 10 lbs. Although this is a significant improvement over pervious systems, this weight is still too much as it represents a 10 to 15% of the total weight of the system. Clearly, it will require a significant oxygen conservation effort to enable the size of the generation system to be reduced even further. Consideration of the factors leading to the high recommended oxygen flow rates suggest that the recommendations for these flow rates pre-dated the widespread availability of pulse oximeters and were aimed at covering 100% of the minute ventilation requirements of adults of any size. In addition, these high rates also help to overcome the air admixture problem that non-re-breathing oxygen masks have.
2.3.1.1 Set Point

In the context of the ACCLS development, it is useful to consider how low the oxygen flow rate can be and still meet the patient requirements throughout the whole range of clinical conditions encountered in the battlefield and during air evacuation where FIO\textsubscript{2} is reduced in proportion to the altitude/atmospheric pressure. The theoretical limit for oxygen flow that which is equal to the oxygen consumption of the patient. At rest, the oxygen consumption of a typical 70 kg man is 200 to 250 ml/min. With sedation or anesthesia, this value is decreased by 25 to 30%. This rate is at least 50 fold lower than the recommended ATLS flow rates. If the oxygen generation systems were linearly scaleable, the theoretical weight of an oxygen generation system capable of 0.3 L/min would be 1 to 2 lbs. To accomplish this theoretical limit would require a sealed rebreathing circuit with CO\textsubscript{2} absorbent and oxygen titrated into the circuit to maintain a constant FIO\textsubscript{2}. Any FIO\textsubscript{2} could be achieved by briefly flushing the inspiratory side of the breathing circuit with higher flow rates. This would be necessary in patients with large alveolar to arterial (A-a) gradients due to ventilation-perfusion mismatch or edema. Once the desired FIO\textsubscript{2} is attained, the steady state flow required to replace the consumed oxygen will be re-established and will be equal to the rate of oxygen consumption in a closed system. Thus, recording the flow rate required to replace the oxygen consumed would provide a continuous monitor of patient oxygen consumption, which is an excellent end point for resuscitation. The set point for oxygen saturation will be 90% as assessed by arterial blood gas measurement or pulse oximetry.

2.3.1.2 Comparator and Error Signal

The deviation from the arterial oxygen saturation set point will be calculated every 30 sec and the oxygen flow will be altered to accomplish the flow change using a PID controller constructed in an analogous manner to the fluid infusion algorithm described above. In both cases, the active control is unidirectional, that is, the administration rate of oxygen can be increased or decreased but cannot be removed from the system to compensate for overshooting the target. The controller can only turn off the flow and then allow passive return of the controlled variable toward the set point. Physiological factors outside of the controller's influence will determine this rate of return toward the target in these "overshoot" situations.

2.3.1.3 Transfer Function

The error signal will be converted to an instruction to the oxygen blender contained within the ventilator, to digitally alter the FIO\textsubscript{2} setting in accordance with the PID derived error signal.

3.0 CONCLUSIONS

Automation of the life support systems is a critical capability that will allow expedient movement of casualties out of the FST and will level the quality of care throughout the echelons of medical care of the critically injured. The ACCLS capability proposed here would provide automation of life support functions, providing computer-driven closed loop control of ventilation, fluid, drug and oxygen administration in the first iteration. This system would optimize the patient's treatment, while minimizing resource utilization (i.e. oxygen and resuscitation fluid). The ACCLS platform will also be a significant critical care enabler for the small FST staff and for the 91W staffing the air and ground ambulances. The closed loop Automated Critical Care Life Support System will provide improved holding capability at the FST as well as extended critical care capability within ground and air ambulance platforms by providing automated life support for the critically injured during the evacuation and holding process.
4.0 REFERENCES


Predictive Calculation of the Arterial Gasometric Variables during the Transfer of Respiratory Patients by Air

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ABSTRACT

The latest recommendations published for the air transport of patients with respiratory pathology are those of the British Thoracic Society. These guidelines describe the conditions required to allow their transport in commercial pressurized air lines. They present a management protocol for the patients based on the basal pulse oximetry at sea level, indicating those patients who will require supplementary oxygen, but without listing the specific contraindications to flying with the exception of current closed pneumothorax or active tuberculosis. A number of formulae exist that attempt to predict in-flight hypoxaemia but which, at the end of the day, have the same applicability as the protocol of the British Thoracic Society, concluding with the recommendation of whether or not to administer supplementary oxygen at two liters per minute, without individualizing the dose. In this article, our aim is to present a change in the current focus on the problem.

We propose an analysis of the clinical situation of the patient, performing arterial gasometry at ground level and calculating the alveolar-arterial oxygen gradient. Using these Data in the formula that we propose, we individualize the management of the patient during air travel, optimizing the air transport of cases of respiratory pathology.


This will avoid delay in the transfer of patients with acute respiratory pathology by ensuring a correct oxygen delivery and facilitating the early detection of complications. This is a group of particular importance in evacuations in the military environment, in both pressurized and non-pressurized transport.

In cases of chronic respiratory pathology, hyperoxia and the consequent retention of carbon dioxide, an ever-present risk in this population group, will be avoided. Their individualized management will make air transfer possible for a large group of patients for whom there currently exist general guidelines for supplementary oxygen delivery without quantifying this in an individualized manner and which can, therefore, lead to difficulties in the control of undesirable effects.

1. INTRODUCTION

In 2002, approximately 1000 million people used air travel throughout the world. It is estimated that this figure will increase in the short term.

Twenty-five years ago it was calculated that 5% of all passengers using commercial air travel had some form of pathology. Given the current prevalence of the various pathologies, the better quality of outpatient control of chronic pathologies and the wider access to air travel, it would not be unreasonable to assume that the above datum by Iglesias et al. is still valid or has now increased.

The prevalence of chronic respiratory pathology in the Western world is currently estimated between 0.8 and 10%3. The incidence of acute respiratory failure is estimated between 109 and 137 cases/100,000 persons/year4,5.

According to the latest recommendations of the British Thoracic Society for patients with respiratory pathology traveling by air, a hypoxaemia test in a hypobaric chamber is recommended for all passengers with pulse oximetry values of 92-95% at sea level and any of the following risk factors: hypercapnia, FEV1 < 50% of the calculated value, lung cancer, restrictive parenchymatous pulmonary disease, restrictive alterations of the chest wall, restrictive lung pathology due to muscle disease, mechanical ventilation, cardiac or cerebrovascular disease. The result of this test will recommend whether or not supplementary oxygen should be administered during a flight in a generic manner at 2 l/min. The use of supplementary oxygen is indicated in all passengers with pulse oximeter readings of less than 92% without the need to perform the test in the hypobaric chamber. In those patients who require supplementary oxygen at sea level, an increase in the flow rate of oxygen during the flight at cruising level is indicated.

2. RESPIRATORY PHYSIOPATHOLOGY APPLIED TO AIR TRANSPORT

2.1. Respiratory physiology

The basic purpose of the respiratory apparatus is to ensure an adequate availability of oxygen, transported by the blood. The body adjusts the respiratory rate and volume (Tidal vol.) appropriately, depending on the mixture of gases being breathed.

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The final effector organ of gas exchange is the complex formed of the alveolus (exterior body surface), alveolar epithelium-alveolar basement membrane-interstitium-vascular endothelium (semipermeable membrane that separates the exterior surface of the body from the gas transporter), and the blood (transporting oxygen and other gases).

To define the functional status of our patient, we must take a number of factors into account, particularly in view of the possibility of changes in the barometric pressure (BP):

a. Gas solubility: this characteristic is intrinsic to each gas and varies with temperature and pressure. Under physiological conditions, the alveolar temperature will be the core temperature of the patient and the changes in pressure will depend on changes in the barometric pressure, affecting the partial pressures of each gas in the mixture.

b. Gas diffusion across the respiratory functional unit (alveolus-capillary): this will depend on the characteristics of the gas, the transmembrane gradient and the characteristics of the semipermeable membrane (the respiratory functional unit) that separates the mixture of gases from the blood.

A parameter exists, the alveolar-arterial oxygen gradient (Aa grad O₂), which expresses the gradient between the alveolar oxygen pressure (measure of the gas in the air being breathed) and the oxygen pressure in arterial blood when the blood interacts with this gas mixture across the alveolar membrane-interstitium-vascular endothelium. The result reflects the total of the millions of alveolar-arterial pulmonary effector functional units of gas exchange.

In the absence of clinical changes in the pulmonary functional situation of the patient, the Aa grad O₂ remains constant, independent of the inspiratory fraction of oxygen and, therefore, of atmospheric variations in the inspired air or the supplementary supply of oxygen. It is thus an optimal reference value for studying the status of the global respiratory functional unit and for the management of the patient during air transport.

2.2. Basal situation

The respiratory situation of any patient/passenger must be known prior to exposure to a hypobaric environment.

Respiratory insufficiency is usually defined as that clinical situation in which the partial pressure of oxygen in arterial blood (PaO₂) is less than 60 mmHg.

Basically, the patients with respiratory insufficiency are divided into two groups: carbon dioxide retainers and non-retainers. This separation is due to the different behavior that these patients present when faced with an increase in the partial pressure of oxygen in inspired air that corrects the hypoxia due to the respiratory failure.

To determine the in-flight management of all these cases, we introduce a parameter that reflects the functional status of each patient’s alveoli-capillary barrier, the final effector organ of gas exchange. This parameter is the Aa grad O₂, as described above.

The Aa grad O₂ is defined as the difference between the alveolar oxygen pressure and the arterial oxygen pressure. The alveolar oxygen pressure is determined by the composition of the mixture of atmospheric gases at 100% water saturation. The arterial oxygen pressure is measured by arterial gasometry.
The composition of the mixtures of the different respiratory gases is shown in table 1

### Table 1: Composition of the mixtures of respiratory gases.

<table>
<thead>
<tr>
<th>Mixture of inspiratory gases (atmospheric)</th>
<th>Mixture of inspiratory gases (alveolus)</th>
<th>Mixture of expiratory gases</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>0,21 · (BP − PpH₂O)</td>
<td>0,21 · (BP − 0,061 BP)</td>
<td>BP varies according to altitude. PpH₂O varies according to the saturation of the atmospheric air</td>
</tr>
<tr>
<td>H₂O</td>
<td>α · SatH₂O · BP</td>
<td>0,061 · BP *</td>
<td>α at 37°C = 6,1%</td>
</tr>
<tr>
<td>CO₂</td>
<td>0,03%</td>
<td>0,03%</td>
<td>+/- Pa CO₂</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>PpN₂ = BP − PpO₂ − PpH₂O − PpCO₂ − Pp others</td>
<td>PpN₂ = BP − PpO₂ − PpH₂O − PpCO₂ − Pp others</td>
<td>The partial pressure of nitrogen is defined by the displacement that it undergoes in the presence of other gases.</td>
</tr>
<tr>
<td>Others: argon, etc.</td>
<td>Maximum 1% (including CO₂)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α: maximum water vapor carrying capacity of air at ambient temperature.

BP: barometric pressure (BP = P₀ · e⁻ⁿ·Mgh/RT). Pp: partial pressure (according to Dalton’s law, the pressure of a mixture of gases is equal to the sum of the partial pressures of the gases that make up the mixture).

* In the alveolus, the water vapor pressure of the alveolar gas is 100%, forming 6.1% of the total mixture of gases at 37°C.

### 2.3. Hypobaric environment

Air travel presents certain characteristics common to other forms of transport, such as movement, acceleration and deceleration, both antero-posterior and vertical, and the visual and auditory stimuli. None of these variables significantly effects respiratory function if they remain within the limits of commercial aviation or tactical military transport.

Having described the behavior of the mixture of gases in a medium with a constant BP, we must analyze the changes that occur in the data presented in table 1 on varying the BP according to altitude in a hypobaric situation such as air transport.

In table 2, the changes in the partial pressures of the gases according to altitude may be seen. It should be noted that the cabin pressure in commercial aircraft presents significant variability⁶. This is not a determining factor in the military flights, but must be known for the planning of health transport, as we are attempting to demonstrate in this article.

---

When arterial hypoxaemia develops in a traveler in a hypobaric environment, a series of compensatory mechanisms are brought into effect. Initially there is an increase in the respiratory volume by making use of the reserve inspiratory volume and increasing the respiratory rate. This can be resolved by the supply of supplementary oxygen, stabilizing the PaO₂.

The provision of supplementary oxygen will prevent respiratory fatigue by avoiding hyperventilation and bathypnea. However, these measures carry two risks:

- **a.** Under-evaluation of an exacerbation of the patient’s respiratory disorder; the excessive supply of oxygen may mask acute changes, impeding early detection and thus leading to the late diagnosis of complications.

- **b.** A second risk, even more frequent, is the excessive supply of oxygen to the chronic patient, blocking the hypoxaemic ventilatory stimulus with the consequent hypoventilation and carbon dioxide retention.

We shall attempt to avoid these risks by calculating precisely the oxygen requirements in a hypobaric environment according to the respiratory pathology (acute or chronic) presented by the patient.

### 3. **CALCULATION OF THE IN-FLIGHT INSPIRATORY OXYGEN FRACTION REQUIREMENT**

#### 3.1 **Formula proposed**

The proposal made here is to determine the appropriate in-flight FiO₂ using the formula presented below. The formula is derived from the re-ordered analysis of the variables that determine the patient's basal Aa grad O₂, which will be applied as a constant in the calculation of the in-flight FiO₂. This FiO₂ will be re-calculated in the event of any change in the in-flight BP (which is a known parameter) in order to achieve the target PaO₂ for the patient. The derivation of the formula is presented in table 3.
Predictive Calculation of the Arterial Gasometric Variables during the Transfer of Respiratory Patients by Air

Table 3. Development of the formula for in-flight oxygen prescription.

\[
\begin{align*}
\text{Aa grad } O_2 &= \text{FiO}_2 (\text{BP} - \text{PH}_2\text{O}) - (\text{PaCO}_2 / 0.8) - \text{PaO}_2. \\
\text{We obtain:} & \\
& \bullet \text{Patient’s Aa grad } O_2 \text{ (basal, stable pathology)} \\
& \bullet \text{PaCO}_2 \text{ (basal gasometry)} \\
\text{We calculate:} & \\
& \bullet \text{Target PaO}_2 \text{ for the patient} \\
\text{Basal Aa grad } O_2 &= \text{FiO}_2 (\text{In-flight BP} - \text{PH}_2\text{O}) - (\text{Basal } \text{PaCO}_2 / 0.8) - \text{Target PaO}_2 \\
\text{FiO}_2 &= \frac{\text{Basal Aa grad } O_2 + \text{Target PaO}_2 + (\text{Basal } \text{PaCO}_2 / 0.8)}{\text{In-flight BP} - 0.061 \cdot \text{In-flight BP}}
\end{align*}
\]

The first step is to perform an arterial gasometry on the patient, calculating the Aa grad O2 from a known BP and inspiratory fraction of oxygen.

From this moment on, 3 fundamental data are used: a known Aa grad O2, a known arterial CO2 pressure (from the gasometry performed), and the patient's clinical profile (acute or chronic respiratory pathology).

The following step is to establish the in-flight inspiratory fraction of oxygen. For this purpose it is necessary to know the planned BP at cruising altitude in the flight to be undertaken. Secondly, the desired PaO2 (target PaO2), which differs according to whether the patient has acute or chronic pathology, must be taken into account.

Finally, the proposed formula is applied to obtain a rapid calculation of the inspiratory fraction of oxygen.

3.2. Acute respiratory pathology

In patients with acute respiratory pathology, the aim is to avoid hypoxaemia and maintain respiratory stability in the patient by avoiding both the use of the reserve inspiratory volume and tachypnoea, thus ensuring patient comfort.

A fundamental aspect is the choice of the target PaO2. If the target PaO2 is too close to the lower limit, e.g. PaO2 = 60 mmHg, a small variation in the planned cabin pressure may cause the patient to hyperventilate, increasing respiratory work. If the target PaO2 is too high, e.g. PaO2 = 85 mmHg, the excessive supply of oxygen may lead to possible changes in the patient’s respiratory situation going undetected and not becoming evident until the compensatory mechanisms are overburdened.

A reasonable target PaO2, with a good safety margin, would be a PaO2 of 65-70 mmHg.
3.3 Chronic respiratory pathology

The objectives in patients with chronic respiratory pathology are two fold. The first is to avoid clinical hypoxaemia and avoid the recruitment of compensatory mechanisms that are chronically exhausted or almost exhausted. The second objective is to avoid hyperoxemia that would inhibit the hypoxic reflex that maintains the respiratory stimulus in these patients.

The target partial pressure of oxygen must be 60 mmHg or, in some patients on chronic treatment with domiciliary oxygen, even lower, e.g. $\text{PaO}_2 = 55 \text{ mmHg}$.

An example of the in-flight oxygen prescription to manage both patient profiles is given in the following table (table 4).

<table>
<thead>
<tr>
<th>Cabin pressure</th>
<th>Basal Aa grad O$_2$</th>
<th>Target PaO$_2$</th>
<th>Indicated FI$_O_2$</th>
<th>Target PaO$_2$</th>
<th>Indicated FI$_O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>760 mmHg</td>
<td>30</td>
<td>40</td>
<td>65</td>
<td>0.21</td>
<td>50</td>
</tr>
<tr>
<td>0 meters</td>
<td>60</td>
<td>40</td>
<td>65</td>
<td>0.24</td>
<td>50</td>
</tr>
<tr>
<td>0 feet</td>
<td>90</td>
<td>40</td>
<td>65</td>
<td>0.29</td>
<td>50</td>
</tr>
<tr>
<td>681.15 mmHg</td>
<td>30</td>
<td>40</td>
<td>65</td>
<td>0.23</td>
<td>50</td>
</tr>
<tr>
<td>914 meters</td>
<td>60</td>
<td>40</td>
<td>65</td>
<td>0.27</td>
<td>50</td>
</tr>
<tr>
<td>3000 feet</td>
<td>90</td>
<td>40</td>
<td>65</td>
<td>0.32</td>
<td>50</td>
</tr>
<tr>
<td>609.09 mmHg</td>
<td>30</td>
<td>40</td>
<td>65</td>
<td>0.25</td>
<td>50</td>
</tr>
<tr>
<td>1828 meters</td>
<td>60</td>
<td>40</td>
<td>65</td>
<td>0.31</td>
<td>50</td>
</tr>
<tr>
<td>6000 feet</td>
<td>90</td>
<td>40</td>
<td>65</td>
<td>0.36</td>
<td>50</td>
</tr>
<tr>
<td>543.33 mmHg</td>
<td>30</td>
<td>40</td>
<td>65</td>
<td>0.28</td>
<td>50</td>
</tr>
<tr>
<td>2743 meters</td>
<td>60</td>
<td>40</td>
<td>65</td>
<td>0.34</td>
<td>50</td>
</tr>
<tr>
<td>9000 feet</td>
<td>90</td>
<td>40</td>
<td>65</td>
<td>0.40</td>
<td>50</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

We believe that the proposed method for the calculation of the inspiratory fraction of oxygen in patients with respiratory pathology will facilitate the medical management of these patients during air travel. First, it allows us to establish in-flight therapeutic safety objectives in patients with altered respiratory function. Secondly, the onset of desaturation during the flight will give us an early warning of the onset of new respiratory events on top of the patient's basal pathology, enabling rapid action to be taken. Finally, it enables the safe transport of patients in extreme situations, e.g. patients with advanced pathology or the performing of health transport in non-pressurized flights.

During transfers within the military environment, the use of non-pressurized air transport gives rise to a variability in the BP that can only be partially controlled. The need to maintain a certain flying altitude or on missions undertaken in territory at high altitude means that the BP may be a determining factor of the patient's clinical situation. In this situation, the application of the formula using the minimum BP according to the planned flying altitude will allow us to avoid desaturation.

Secondly, high altitude, pressurized, long haul transport (transcontinental) in the repatriation of patients must be undertaken with the greatest possible safety. This most frequently involves patients with acute respiratory pathology. Some of them, due to the time course of their pathology in the area of operations, may behave as chronic patients. Long haul transfers must be correctly planned and the application of the
formula will enable the appropriate use of equipment, the correct prescription of oxygen, the prevention of complications (in patients with a chronic profile) and the early detection of respiratory complications (desaturation in patients with an acute profile). Given the complexity of these repatriation operations, we believe that facilitating the determination of respiratory requirements will simplify one of the fundamental variables in the management of these patients.
Development and Preliminary Findings of a Combat Trauma Registry for the U.S. Navy-Marine Corps

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ABSTRACT

The U.S. military services, drawing upon the experiences of civilian trauma systems in monitoring trauma care delivery, have begun to implement their own registries emphasizing injury incidence and severity in a combat environment. The current paper describes the development of the U.S. Navy-Marine Corps Combat Trauma Registry (CTR) and presents preliminary studies and analyses of combat injury patterns and casualty management within the medical chain of evacuation during Operation Iraqi Freedom (OIF). The Navy-Marine Corps CTR is configured as a data warehouse comprised of data sets that describe the events that occur to individual casualties from the point of injury, through the medical chain of evacuation, and on to long-term rehabilitative outcomes. Data was collected from Navy-Marine Corps level 1B, 2 and 3 Medical Treatment Facilities (MTFs) during OIF-1. Data from the official combat period (19 Mar – 14 Apr 2003) were analyzed to show the number, type, and location of Navy-Marine Corps MTFs operational on each day of the operation. Maps diagramming these data show the gradually expanding MTF theater laydown beginning with two Navy-Marine Corps Level 1B, 2 and 3 facilities on day 1 of the operation to an eventually 14 at the conclusion of the official combat period on day 27. In addition, results are presented that indicate 37.5% of all Navy-Marine casualties evacuated were due to battle injuries, 28.0% were due to non-battle, injuries, 26.7% to disease, 4.4% were unknown, 3.4% were due to mental disorders.

1.0 DEFINITION of ACRONYMS

CTR - Combat Trauma Registry
BAS - Battalion Aid Station (Navy-Marine Corps Level 1B facility)
DIS - Disease
EMF - Expeditionary Medical Facility (Fleet Hospital - Navy Level 3 facility)
FRSS - Forward Resuscitative Surgery System (Navy-Marine Corps Level 1B facility)
MTF - Medical Treatment Facility
MVA - Motor Vehicle Accident
NBI - Non Battle Injury
NOS - Not Otherwise Specified
OIF-1 - Operation Iraqi Freedom 1 (Marine Corps Jan-Sep 03)
OIF-2 - Operation Iraqi Freedom 2 (Marine Corps Feb 04 –present)
OEF - Operation Enduring Freedom (Afghanistan)
RPG - Rocket Propelled Grenade
STP - Shock Trauma Platoon (Navy-Marine Corps Level 1B facility)
WIA - Wound in Action

2.0 INTRODUCTION

Traditionally, studies assessing trauma care efficacy in the U.S. Navy-Marine Corps operational setting have relied on hospital deaths as the primary indicator of effectiveness. No large-scale, comprehensive Navy-Marine Corps specific repository existed for records of combat trauma incidents that described the events associated with injury, such as mechanism, use of personal protective equipment, casualty demographic data, injury profile, levels of care where treatment occurred, treatment protocols administered, or ultimate disposition. The U.S. military services, drawing upon the experiences of civilian trauma systems in monitoring trauma care delivery, have begun to implement their own registries emphasizing injury incidence and severity in a combat environment. The current paper describes the development of the U.S. Navy-Marine Corps Combat Trauma Registry (CTR) and presents preliminary studies and analyses of combat injury patterns and casualty management within the medical chain of evacuation during Operation Iraqi Freedom (OIF-1).

3.0 APPROACH

The Navy-Marine Corps CTR is a collection of data sets, configured within the design of a data warehouse. This Navy-Marine Corps CTR data warehouse, represents a collection of integrated, yet heterogeneous sources of data organized to perform queries and analyses. Each set of data within the warehouse has a single, unifying characteristic. That single characteristic is that each data set represents some part of the continuum of care and events surrounding that care administered to casualties as they move through the medical chain of evacuation.

Examination of the events surrounding the administration of care for combat casualties, especially in the forward areas, reveals a complex interaction of activities that must be viewed as a whole if the true nature of what actually is occurring to combat casualties in the medical chain of evacuation is to be revealed. Therefore, the Navy-Marine Corps CTR program has developed and implemented a data collection plan that brings together a number of diverse sets of data collected from the point of injury through the course of convalescent care in Navy hospitals in the continental U.S.
The current state of the Navy-Marine Corps CTR data warehouse concept consists of six primary sources of data. These sources of data, when taken together, are designed to provide a comprehensive view of the nature of events and the course of care administered to Navy-Marine Corps casualties form the point of injury through to rehabilitative convalescence. A simplified representation of these six sources of data is presented in Figure 1. It can be seen in Figure 1 that the first data set is the Navy-Marine Corps medical theater laydown. This data set identifies each Medical Treatment Facility (MTF) in theater during an operational deployment, the function of each MTF, and the location of each MTF on each successive day of the operation. This data set is important because data from other data sets in the warehouse are used to identify the specific patients seen at each of the MTFs in the laydown for each day that each MTF was operational and receiving patients. These data, among other uses, permit the calculation of the specific patient workload for each MTF, on each day of the operation. Knowledge of the specific patient workload of each MTF permits the estimation of the ideal mix of providers and equipment needed to optimally configure each MTF.

Figure 1: Navy-Marine Corps CTR Data Warehouse

The next component of the Navy-Marine Corps CTR data warehouse concept (Fig. 1) is the most difficult set of data for any service in any nation to assemble. However, this data set, in the opinion of the authors, is the most important element in any CTR. It is the data, primarily clinical in nature, that describes what occurred to the casualty within the theater medical chain of evacuation at and near the point of injury. For the Navy-
Development and Preliminary Findings of a Combat Trauma Registry for the U.S. Navy-Marine Corps

Marine Corps, these are data sets found in the forward areas at level 1-3 MTFs. Within these sets of data are the events surrounding the injury such as mechanism, environmental conditions at the time of injury, personal protective equipment worn (or not worn), injury profiles, patient status in terms of signs and symptoms, and the course of care administered to the casualty at each MTF in the theater of operation. For the Navy-Marine Corps, these data are obtained from a number of sources and include the first responder (self, buddy, or corpsman), battalion aid stations (BASs), shock trauma platoons (STPs), forward resuscitative surgery systems (FRSSs), surgical companies, casualty receiving and treatment ships, fleet hospitals, and hospital ships. Because of the highly chaotic, and often extreme operational tempo experienced within these facilities, capture of clinical data generally is relegated a low priority. Even when the data are captured, results obtained on the Navy-Marine Corps CTR program have shown that the clinical record is lost somewhere within the medical chain of evacuation and is virtually never reunited with the patient. Therefore, the Navy-Marine Corps CTR program has concluded that capture of these data cannot be conducted retrospectively as originally attempted. A proactive, multifaceted approach to capturing and retaining these data at each MTF in theater is required to collect data near the point of injury in sufficient quantity to be useful. A discussion of the Navy-Marine Corps plan for capturing these data will be presented later in this paper.

The third element of the Navy-Marine CTR data warehouse (Fig. 1) is the data sets derived from level 4 MTFs. In the current deployments of OIF and Operation Enduring Freedom (OEF), most Navy-Marine Corps casualties are processed through the level 4 U.S. hospital at Landstuhl Regional Medical Center, Germany. Data describing the course of care, and associated complications of care are collected at this facility directly from the patient medical record. Because this is generally the first stable, secure, fixed facility in the medical chain of evacuation, complete patient clinical records are available for review. A Navy-Marine Corps CTR registrar is assigned to capture level 4 clinical data and forward it to the Naval Health Research Center in San Diego for inclusion in the Navy-Marine Corps CTR.

The fourth component in the Navy-Marine Corps CTR data warehouse (Fig. 1), is the data sets that describe the course of care and resultant complications of care experienced by casualties once they have arrived at continental U.S. Naval hospitals. Generally, upon arrival to the continental U.S., Navy-Marine Corps casualties are processed through National Naval Medical Center Bethesda (NNMC), Maryland. From NNMC, casualties are transferred to the MTF most capable of providing care appropriate to their condition, closest to their homes or units of origin. Two primary data sets are currently being brought into the Navy-Marine Corps CTR data warehouse. The first of these data sets is the Composite Health Care System II (CHCS II), a U.S. Department of Defense patient management system. Because CHCS II will only provide a partial clinical picture of care performed once the casualties reach the U.S., a second data set, named Canopy, will also be brought into the data warehouse. Canopy is a U.S. Bureau of Medicine and Surgery developed case management system currently operational in all continental U.S. Navy MTFs. Data derived from these two systems will permit the clinical tracking of all Navy-Marine Casualties once they arrive in the U.S.

The fifth component of the Navy-Marine Corps CTR data warehouse (Fig. 1) is the data sets obtained from the U.S. Veteran Affairs administration. This component, currently in the planning stage, will be added to provide disability ratings once casualties have completed their primary recuperative phases. Data from these sources will be used to relate the course of clinical care received early on in the medical chain with long-term rehabilitative outcomes.

The sixth and final component of the data warehouse is the data sets that describe the clinical characteristics from the combat casualty population that were killed in action or died of wounds following entry into the medical chain of evacuation (Fig. 1). This component, still in planning stages, will be used to examined lethality issues within the Navy-Marine Corps, personal protective measures, and for use in models and simulations that forecast mortality estimates for medical planners.
Development and Preliminary Findings of a Combat Trauma Registry for the U.S. Navy-Marine Corps

Data from the first two components represents clinical information derived from the forward MTFs. These are the data of most interest to analysts as it is at these MTFs that the developers of the Navy-Marine Corps CTR program expect to effect the greatest benefit. Knowledge of what care was administered at these forward MTFs will be examined in the context of longer term outcome issues such as disability ratings, rehabilitative outcomes, and long-term quality of life issues. To accomplish this objective, the first-order priority for the Navy-Marine Corps CTR has been the capture and analysis of data derived from the forward MTFs. Data for populating the first two components of the CTR began during Navy-Marine Corps operations in OIF.

4.0 Results

Preliminary results from the examination of data from the first two components of the CTR will be presented for OIF. Data supporting these results were derived retrospectively from a number of sources including patient clinical records when available, MTF logbooks, Marine Corps Personnel Casualty Reports (PCRs), and ad hoc reporting conducted by MTF clinicians at the individual MTFs. The latter data source, ad hoc reporting at theater MTFs, is a result of clinicians perceiving a need to capture detailed clinical information for latter analysis and making attempts to do so at their MTFs.

The Navy-Marine Corps has participated in two primary deployments in support of OIF. The first, OIF-1, is roughly considered to have occurred from Jan - Sep 2003. The official combat period of OIF is 19 Mar 2003 – 14 Apr 2003. The following results will pertain to the official combat period. The second major Navy-Marine Corps deployment, named OIF-2, began in Feb 2004 and is anticipated to continue for a period of one year. Results for this period are not reported in the current paper.

4.1 OIF-1 Theater Medical Treatment Facility Laydown

The first component of the data warehouse calls for development of data sets that describe the specific Navy-Marine Corps MTFs that were operational on each successive day of the operation. These data have been assembled from various data sources including situation reports, medical battalion records, medical facility records, and personal accounts. MTFs for Navy-Marine Corps levels 1B-3 are reported. The level 1B-3 MTFs from OIF-1 presented in the current results include STPs 7-10, FRSSs 1-6, Surgical Companies Alpha, Bravo, and Charlie, Expeditionary Medical Facilities (EMF) Pensacola (Fleet Hospital 3), and EMF-Bremerton (Fleet Hospital 8), Rota, Spain.

Figures are presented which describe the medical theater laydown on days when the configuration of the laydown markedly changed. Figure 2 shows the initial medical laydown on day 1 of the official combat period. It can be seen from figure 2 that at the start of the combat period, the major Navy-Marine.

---

1 Battalion Aid Stations (BASs) are also considered level 1B facilities. These facilities directly support the ground element and are therefore highly mobile. Because of their highly mobile operational characteristic, day by day identification of their positions and patient streams are not currently available for reporting in the theater laydown. Shock Trauma Platoons 1-5 also directly supported the ground element under Combat Service Support Group–11. Data identifying their day by day positions were also unavailable at the time of publication.
Corps MTFs operational were EMF-Bremerton (Fleet Hospital 8, Rota, Spain) Alpha Surgical Company in Kuwait and STP-10 near the border at Breach Point West.

By day 17 of the official combat period, additional Navy-Marine Corps MTFs had become operational. Figure 3 shows that in addition to EMF-Bremerton and Alpha Surgical Company (still in Kuwait), EMF-Pensacola, Bravo and Charlie Surgical Companies, FRSSs 1, 4, and 6, and STPs 7, and 8 were now functioning and receiving patients.

Figure 2: Navy-Marine Corps Medical Theater Laydown on Day 1 of the official combat period.
On the final day of the official combat period (day 27) the full compliment of Navy-Marine Corps MTFs were operational and operating in the positions designated in Figure 4. This compliment of MTFs included two EMFs, three surgical companies, six FRSSs, and the four STPs operating with Health Services Battalion.
4.2 Patient Profiles from Levels 1 and 2 Medical Treatment Facilities

Due to the nature of the events surrounding care in Navy-Marine Corps level 1 and 2 MTFs, gathering data on patient profiles and treatment patterns is allusive. This has historically been the case as little or no data on the course of care in STPs, FRSSs, or Surgical Companies currently exists to any significant degree. The Navy-Marine Corps CTR program has been charged with beginning the process of capturing and preserving data related to the patient stream at these MTFs. It is these kinds of data, found at the level 1-2 MTFs that comprise the second component of the Navy-Marine CTR data warehouse.

To date, the Navy-Marine Corps has collected records on 1,406 patients seen during the official combat period at one or more of these MTFs. While these data may be more comprehensive and complete than has historically been possible, they are often partial records. In addition to many partial records, the true number of patients actually seen at these MTFs during the official combat period may never be truly known. This means that not only is the denominator not known at this time, but the nominator data may also never truly be a known commodity. While this unfortunately is the nature of intellectual inquiry in this area of combat casualty care, the shear volume of data currently captured so overwhelms that available from past operations that they should not be ignored. Rather, the inferences drawn from them should be limited to those areas where the data are strongest and tempered with the expectation that a portion of the picture may not as yet be fully developed.
Given these interpretational parameters, the data on the 1,406 patients seen in level 1-2 MTFs were examined to determine their composition. This population includes evacuations from Level 1B/2 MTFs and personnel treated at a Level 1B or 2 MTF and returned to duty during the official combat period. Presumably, many of the returned to duty personnel represent sick call visits. Unfortunately, it is believed that the true number of sick call visits during this period is underrepresented due to less thorough record keeping at the MTFs for the sick call population. Given this caveat, Table 1 shows the patient categories seen at Navy-Marine Corps Level 1B/2 MTFs. Table 1 shows that a large impact on Navy-Marine Corps MTFs was attributable to sickcall related encounters for disease conditions. This is remarkable as historically, sick call encounters are generally at their lowest frequency during periods of high operational tempo.

Table 1
Navy-Marine Corps Level 1B/2 Category Types for all Patients (Evacuated and Returned to Duty)

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>538</td>
<td>38.3%</td>
</tr>
<tr>
<td>Wounded-in-action</td>
<td>330</td>
<td>23.5%</td>
</tr>
<tr>
<td>Non-battle injury</td>
<td>297</td>
<td>21.1%</td>
</tr>
<tr>
<td>Injury (unspecified)</td>
<td>171</td>
<td>12.2%</td>
</tr>
<tr>
<td>Psych</td>
<td>46</td>
<td>3.3%</td>
</tr>
<tr>
<td>Unknown/Not Recorded</td>
<td>24</td>
<td>1.7%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1406</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Next, this population of 1,406 was examined to assess the primary ICD-9 diagnostic categories. The combination of WIA and NBI in this population is reflected in the high frequency of injuries and accidents. Minimal data is often captured in the return to duty sick call population which is reflected in the high incidence of uncoded encounters and for encounters with no information on the condition for which the patient presented. Table 2 also shows that dental related visits, followed by conditions of the digestive tract were also relatively common at Navy-Marine Corps Level 1B/2 MTFs.
Table 2
Navy-Marine Corps Level 1B/2 Primary ICD-9 Diagnosis for all Patients
(Evacuated and Returned to Duty)

<table>
<thead>
<tr>
<th>Navy-Marine Corps OIF-1</th>
<th>21 Mar - 15 May 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary ICD-9 Category</td>
<td>n</td>
</tr>
<tr>
<td>Injuries and Accidents</td>
<td>554</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>118</td>
</tr>
<tr>
<td>Not Coded</td>
<td>94</td>
</tr>
<tr>
<td>No Information</td>
<td>93</td>
</tr>
<tr>
<td>Symptoms, Ill-Defined</td>
<td>91</td>
</tr>
<tr>
<td>Dental</td>
<td>83</td>
</tr>
<tr>
<td>Digestive</td>
<td>64</td>
</tr>
<tr>
<td>Infectious and Parasitic</td>
<td>54</td>
</tr>
<tr>
<td>Mental Disorders</td>
<td>46</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>41</td>
</tr>
<tr>
<td>Skin, Subcutaneous Tissue</td>
<td>34</td>
</tr>
<tr>
<td>Respiratory</td>
<td>33</td>
</tr>
<tr>
<td>Nervous System, Sense Organs</td>
<td>32</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>28</td>
</tr>
<tr>
<td>Endocrine, Nutritional, Metabolic</td>
<td>14</td>
</tr>
<tr>
<td>Circulatory</td>
<td>13</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>7</td>
</tr>
<tr>
<td>Pregnancy, Puerperium</td>
<td>4</td>
</tr>
<tr>
<td>Congenital</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1406</td>
</tr>
</tbody>
</table>

Additional analyses were conducted on a subset of the 1,406 patients. This subset of 840 patients represents the combined population of patients evacuated from the STPs, FRSSs, and surgical companies Alpha, Bravo, and Charlie during the combat period. This subset was selected because it is a highly comprehensive data set consisting of records representing virtually the full complement of casualties actually evacuated from these MTFs. This was confirmed by matching these patients with records from level 3 and 4 MTFs. Patient profiles and casualty category types for all patients evacuated from the STPs, FRSSs, and surgical companies, during the official combat period are reflected.

Table 3 shows the categories of patients evacuated from combined Level 1B and 2 Navy-Marine Corps facilities during the official combat period. It can be seen that the frequency of Wounded in Action (WIA) patients is consistent with the operational tempo of the reporting period. These data are compared with U.S. Army category types for the same approximate reporting period.
Development and Preliminary Findings of a Combat Trauma Registry for the U.S. Navy-Marine Corps

Table 3
Navy-Marine Corps Level 1B/2 and U.S. Army Level 2 Evacuations by Patient Category Types

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marines</td>
<td>Marines</td>
</tr>
<tr>
<td>WIA</td>
<td>315</td>
<td>37.5%</td>
</tr>
<tr>
<td>NBI</td>
<td>235</td>
<td>28.0%</td>
</tr>
<tr>
<td>DIS</td>
<td>224</td>
<td>26.7%</td>
</tr>
<tr>
<td>Unknown</td>
<td>37</td>
<td>4.4%</td>
</tr>
<tr>
<td>PSY</td>
<td>29</td>
<td>3.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>840</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Next, using this same population of Navy-Marine Corps Level 1 and 2 evacuations, an examination of the mechanism of injury, primary IDC-9 category, and primary site of injury was conducted on the WIA group. Table 4 shows the mechanism of injury for each Level 1 and 2 U.S. Navy Marine Corps casualty evacuated to a higher level of care. It can be seen from Table 4 that following gunshots, shrapnel, and RPGs, motor vehicle accidents, occurring in proximal support of an enemy engagement, are relatively high.

Table 4
WIA Mechanism of Injury for Navy-Marine Corps Casualties Evacuated from Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>Navy/Marines OIF-1</th>
<th>21 Mar - 15 May 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIA Mechanism of Injury</td>
<td>n</td>
</tr>
<tr>
<td>Gunshot Wound</td>
<td>76</td>
</tr>
<tr>
<td>Shrapnel/Fragmentation</td>
<td>65</td>
</tr>
<tr>
<td>RPG/grenade</td>
<td>39</td>
</tr>
<tr>
<td>Motor Vehicle Accident</td>
<td>28</td>
</tr>
<tr>
<td>Fall</td>
<td>17</td>
</tr>
<tr>
<td>Explosion</td>
<td>16</td>
</tr>
<tr>
<td>Unknown/Not Recorded</td>
<td>16</td>
</tr>
<tr>
<td>Landmine</td>
<td>14</td>
</tr>
<tr>
<td>Blast</td>
<td>11</td>
</tr>
<tr>
<td>Mechanical/Machinery</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
</tr>
<tr>
<td>Multiple (NOS)</td>
<td>4</td>
</tr>
<tr>
<td>Blunt</td>
<td>3</td>
</tr>
<tr>
<td>Debris</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>315</strong></td>
</tr>
</tbody>
</table>
Table 5 shows primary ICD-9 codes resulting from each of the WIA mechanisms of injury. An examination of these data shows that open wounds and fractures are the primary pathologies associated with the mechanisms. It should be noted, however, that because these classifications are considered primary in nature that a certain number of the open wound category could contain additional fractures that appear secondary to the open wounds.

Table 5
WIA Primary ICD-9 Diagnosis for Navy-Marine Corps Casualties Evacuated from Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>WIA Primary ICD-9Categories</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open wounds</td>
<td>162</td>
<td>51.4%</td>
</tr>
<tr>
<td>Fractures</td>
<td>55</td>
<td>17.5%</td>
</tr>
<tr>
<td>Sprains</td>
<td>24</td>
<td>7.6%</td>
</tr>
<tr>
<td>Multiple</td>
<td>14</td>
<td>4.4%</td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
<td>4.4%</td>
</tr>
<tr>
<td>Amputations</td>
<td>10</td>
<td>3.2%</td>
</tr>
<tr>
<td>Burns</td>
<td>7</td>
<td>2.2%</td>
</tr>
<tr>
<td>Contusions</td>
<td>7</td>
<td>2.2%</td>
</tr>
<tr>
<td>Intracranial injury</td>
<td>6</td>
<td>1.9%</td>
</tr>
<tr>
<td>Crushing</td>
<td>5</td>
<td>1.6%</td>
</tr>
<tr>
<td>Dislocations</td>
<td>5</td>
<td>1.6%</td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>1.6%</td>
</tr>
<tr>
<td>Effects</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>315</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 6 shows the primary anatomical site of injury for Navy-Marine Corps WIA casualties evacuated from Level 1B/2 MTFs. Consistent with the use of body armour is the relatively low incidence of back, chest, and abdomen injuries. Equally consistent is the high rate of injuries for traditionally unprotected areas of the extremities and face.
Table 6
WIA Primary Site of Injury for Navy-Marine Corps Casualties Evacuated from Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>WIA – Primary Site of Injury</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Extremities</td>
<td>106</td>
<td>33.7%</td>
</tr>
<tr>
<td>Upper Extremities</td>
<td>96</td>
<td>30.5%</td>
</tr>
<tr>
<td>Face</td>
<td>27</td>
<td>8.6%</td>
</tr>
<tr>
<td>Multiple</td>
<td>24</td>
<td>7.6%</td>
</tr>
<tr>
<td>Back</td>
<td>18</td>
<td>5.7%</td>
</tr>
<tr>
<td>Head</td>
<td>15</td>
<td>4.8%</td>
</tr>
<tr>
<td>Chest</td>
<td>13</td>
<td>4.1%</td>
</tr>
<tr>
<td>Abdomen</td>
<td>6</td>
<td>1.9%</td>
</tr>
<tr>
<td>Neck</td>
<td>5</td>
<td>1.6%</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>5</td>
<td>1.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>315</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Next, the incidences of non-battle injury (NBI) trends were examined in the population of Navy-Marine Corps casualties evacuated from Level 1B/2 MTFs. Analyses were conducted to reveal NBI trends for mechanism of injury, primary IDC-9 category, and primary site of injury. Table 7 shows the results of the examination of NBI mechanisms of injury. It is apparent from Table 7 that less emphasis was placed on identifying the mechanism of injury for NBI at the forward MTFs than was the case for the WIA population. The high incidence of a ‘non stated’ mechanism is unfortunate in this context as the further removed from the point of injury this assessment is made, the less likely that it will ever be determined. Of other interest in these finding is the high rate of motor vehicle accident injuries experienced by the deployed forces during the actual combat period. This population of motor vehicle accidents is distinct from the population reported in the WIA results. However, the reality of separating the WIA from NBI motor vehicle accidents is often a difficult distinction to make.
Table 7
NBI Mechanism of Injury for Navy-Marine Corps Casualties Evacuated from Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>NBI Mechanism of Injury</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Stated</td>
<td>52</td>
<td>22.1%</td>
</tr>
<tr>
<td>Motor Vehicle Accident</td>
<td>43</td>
<td>18.3%</td>
</tr>
<tr>
<td>Fall</td>
<td>35</td>
<td>14.9%</td>
</tr>
<tr>
<td>Blunt</td>
<td>18</td>
<td>7.7%</td>
</tr>
<tr>
<td>Other</td>
<td>16</td>
<td>6.8%</td>
</tr>
<tr>
<td>Accidental discharge</td>
<td>14</td>
<td>6.0%</td>
</tr>
<tr>
<td>Crush</td>
<td>12</td>
<td>5.1%</td>
</tr>
<tr>
<td>Sports</td>
<td>12</td>
<td>5.1%</td>
</tr>
<tr>
<td>Training</td>
<td>11</td>
<td>4.7%</td>
</tr>
<tr>
<td>Mechanical</td>
<td>10</td>
<td>4.3%</td>
</tr>
<tr>
<td>Burns</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td>Cut/Pierce</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>235</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 8 shows primary ICD-9 codes resulting from each of the NBI mechanisms of injury. It can be seen that musculoskeletal injuries predominate in this population with fractures and sprains accounting for a large proportion of evacuations from Level 1B/2 MTFs.

Table 8
NBI Primary ICD-9 Diagnosis for Navy-Marine Corps Casualties Evacuated from Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>NBI – ICD Categories</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractures</td>
<td>72</td>
<td>30.6%</td>
</tr>
<tr>
<td>Sprains</td>
<td>68</td>
<td>28.9%</td>
</tr>
<tr>
<td>Wounds</td>
<td>36</td>
<td>15.3%</td>
</tr>
<tr>
<td>Other</td>
<td>16</td>
<td>6.9%</td>
</tr>
<tr>
<td>Intracranial Injury</td>
<td>10</td>
<td>4.3%</td>
</tr>
<tr>
<td>Crushing</td>
<td>10</td>
<td>4.3%</td>
</tr>
<tr>
<td>Dislocations</td>
<td>8</td>
<td>3.4%</td>
</tr>
<tr>
<td>Burns</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td>Unknown/Not Recorded</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td>Amputations</td>
<td>3</td>
<td>1.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>235</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table 9 shows the primary anatomical site of injury for Navy-Marine Corps NBI casualties evacuated from Level 1B/2 MTFs. As was the case seen in the examination of injury site in the WIA population, extremity injuries predominate. Similarly high rates of back, face, and head injuries are consistent with the frequency with which casualties were evacuated due to involvement in motor vehicle accidents.

Table 9
NBI Primary Site of Injury for Navy-Marine Corps Casualties Evacuated from Level 1B/2

<table>
<thead>
<tr>
<th>NBI – Primary Site of Injury</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Extremities</td>
<td>93</td>
<td>39.6%</td>
</tr>
<tr>
<td>Upper Extremities</td>
<td>71</td>
<td>30.2%</td>
</tr>
<tr>
<td>Back</td>
<td>18</td>
<td>7.7%</td>
</tr>
<tr>
<td>Face</td>
<td>18</td>
<td>7.7%</td>
</tr>
<tr>
<td>Head</td>
<td>14</td>
<td>6.0%</td>
</tr>
<tr>
<td>Multiple</td>
<td>7</td>
<td>3.0%</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td>Neck</td>
<td>4</td>
<td>1.7%</td>
</tr>
<tr>
<td>Abdomen</td>
<td>2</td>
<td>0.9%</td>
</tr>
<tr>
<td>Chest</td>
<td>2</td>
<td>0.9%</td>
</tr>
<tr>
<td>Total</td>
<td>235</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Finally, the incidence of disease was examined in the population of casualties evacuated from Navy-Marine Corps Level 1B/2 MTFs during the official combat period. Table 10 presents these results. Table 10 shows that diseases of the digestive tract predominate in this population. Next, presumably due to the austere diagnostic capabilities of these MTFs, are ill-defined symptomologies. A preliminary examination of data currently in house suggests that many of these patients were transferred to one of the two Level three fleet hospitals operating in theater during this period. As the fleet hospital data is examined more thoroughly in the coming months, it is expected that more definitive diagnostic categories will be able to be assigned these patients with reported ill-defined symptomologies.
Table 10
Disease Primary ICD-9 Diagnosis for Navy-Marine Corps Casualties Evacuated form Level 1B/2 MTFs

<table>
<thead>
<tr>
<th>Primary ICD-9 Disease Categories</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive</td>
<td>44</td>
<td>17.4%</td>
</tr>
<tr>
<td>Symptoms Ill Defined</td>
<td>38</td>
<td>15.0%</td>
</tr>
<tr>
<td>Mental Disorders</td>
<td>29</td>
<td>11.5%</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>29</td>
<td>11.5%</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>21</td>
<td>8.3%</td>
</tr>
<tr>
<td>Nervous System Sense Organs</td>
<td>17</td>
<td>6.7%</td>
</tr>
<tr>
<td>Skin</td>
<td>15</td>
<td>5.9%</td>
</tr>
<tr>
<td>Supplemental</td>
<td>15</td>
<td>5.9%</td>
</tr>
<tr>
<td>Infectious and Parasitic</td>
<td>10</td>
<td>4.0%</td>
</tr>
<tr>
<td>Circulatory</td>
<td>10</td>
<td>4.0%</td>
</tr>
<tr>
<td>Endocrine, Nutritional</td>
<td>8</td>
<td>3.2%</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>6</td>
<td>2.4%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>5</td>
<td>2.0%</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td>Congenital</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>253</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Among the more comprehensive data sets describing Navy-Marine Corps Level 1B/2 casualties have been obtained from the three Level 2 surgical companies deployed in support of OIF-1. These data describe those patients seen by the Alpha, Bravo and Charlie surgical companies during the official combat period that were subsequently evacuated. Table 11 shows the mechanism of injury for all WIA patients seen at the Navy-Marine Corps Level 2 surgical companies during the official combat period. It is interesting to note that most WIA patients evacuated form Level 1B/2 (n =315) were seen at the surgical companies (n =205) prior to evacuation to the next level of care.
### Table 11
WIA Mechanism of Injury for Level 2 Surgical Company Patients

<table>
<thead>
<tr>
<th>WIA Mechanism of Injury</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunshot Wound</td>
<td>61</td>
<td>29.8%</td>
</tr>
<tr>
<td>Shrapnel/Fragmentation</td>
<td>34</td>
<td>16.6%</td>
</tr>
<tr>
<td>RPG/Grenade</td>
<td>27</td>
<td>13.2%</td>
</tr>
<tr>
<td>Motor Vehicle</td>
<td>17</td>
<td>8.3%</td>
</tr>
<tr>
<td>Landmine</td>
<td>13</td>
<td>6.3%</td>
</tr>
<tr>
<td>Fall</td>
<td>11</td>
<td>5.4%</td>
</tr>
<tr>
<td>Blast</td>
<td>7</td>
<td>3.4%</td>
</tr>
<tr>
<td>Explosion</td>
<td>7</td>
<td>3.4%</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>2.0%</td>
</tr>
<tr>
<td>Mechanical</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Blunt</td>
<td>2</td>
<td>1.0%</td>
</tr>
<tr>
<td>Machinery</td>
<td>2</td>
<td>1.0%</td>
</tr>
<tr>
<td>Crush</td>
<td>1</td>
<td>0.5%</td>
</tr>
<tr>
<td>Unknown/Not Recorded</td>
<td>16</td>
<td>7.8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>205</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 12 show the primary ICD-9 category for all Navy-Marine Corps patients seen at each of the three Level 2 surgical companies during the official combat period. Table 12 shows that the surgical companies saw primarily WIA and NBI patients during this reporting period.
Development and Preliminary Findings of a Combat Trauma Registry for the U.S. Navy-Marine Corps

Table 12
Navy-Marine Corps Level 2 Surgical Company Patients by Primary ICD-9 Category (WIA/NBI/Disease)

<table>
<thead>
<tr>
<th>Category</th>
<th>Alpha Co</th>
<th></th>
<th>Bravo Co</th>
<th></th>
<th>Charlie Co</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Circulatory</td>
<td>0</td>
<td>0.0%</td>
<td>5</td>
<td>2.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Congenital</td>
<td>0</td>
<td>0.0%</td>
<td>1</td>
<td>0.4%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Dental</td>
<td>0</td>
<td>0.0%</td>
<td>1</td>
<td>0.4%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Digestive</td>
<td>6</td>
<td>4.8%</td>
<td>10</td>
<td>4.0%</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>Endocrine, Nutritional, Metabolic</td>
<td>1</td>
<td>0.8%</td>
<td>1</td>
<td>0.4%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>3</td>
<td>2.4%</td>
<td>3</td>
<td>1.2%</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>Infectious and Parasitic</td>
<td>0</td>
<td>0.0%</td>
<td>5</td>
<td>2.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Injuries and Accidents</td>
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<td>62.4%</td>
<td>148</td>
<td>59.9%</td>
<td>27</td>
<td>60.0%</td>
</tr>
<tr>
<td>Mental Disorders</td>
<td>4</td>
<td>3.2%</td>
<td>2</td>
<td>0.8%</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>Miscellaneous</td>
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<td>1.6%</td>
<td>6</td>
<td>2.4%</td>
<td>3</td>
<td>6.7%</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>8</td>
<td>6.4%</td>
<td>19</td>
<td>7.7%</td>
<td>4</td>
<td>8.9%</td>
</tr>
<tr>
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<td>0.0%</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>Nervous System, Sense Organs</td>
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<td>0.8%</td>
<td>9</td>
<td>3.6%</td>
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<td>0.0%</td>
</tr>
<tr>
<td>No Information</td>
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<td>4.0%</td>
<td>1</td>
<td>0.4%</td>
<td>7</td>
<td>15.6%</td>
</tr>
<tr>
<td>Not Coded</td>
<td>9</td>
<td>7.2%</td>
<td>14</td>
<td>5.7%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Pregnancy, Puerperium</td>
<td>0</td>
<td>0.0%</td>
<td>2</td>
<td>0.8%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>3</td>
<td>2.4%</td>
<td>1</td>
<td>0.4%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Skin, Subcutaneous Tissue</td>
<td>1</td>
<td>0.8%</td>
<td>5</td>
<td>2.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Symptoms, Ill-Defined</td>
<td>4</td>
<td>3.2%</td>
<td>14</td>
<td>5.7%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>100.0%</td>
<td>247</td>
<td>100.0%</td>
<td>45</td>
<td>100.0%</td>
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</tbody>
</table>

5.0 Discussion

The retrospective capture of patient data from theater MTFs close to the point of injury did not result in sufficiently comprehensive data sets to allow for a thorough investigation into the nature of combat casualty care in the forward areas. Due to the chaotic nature of the forward combat casualty care environment, a more structured, prospective process for capturing these data is required. Not only must a structured process be instituted, but it must be facilitated throughout the operational period to ensure that it remains viable and is performed on as many combat casualties as feasible in the chaotic forward MTF environment. Furthermore, to ensure an adequate body of data are captured, the providers responsible for capturing these data must possess knowledge that the data they are collecting will prove useful in improving their ability to successful manage their patients.

5.1 OIF-2 Data Collection Plan

A systematic plan for capturing more robust data sets has been developed and implemented in all Navy-Marine Corps level 1-3 MTFs. The OIF-2 data collection plan includes various methodologies for capturing data that is matched to the operating realities of the individual MTFs.
5.1.1 The CTR Data Collection Form

The plan encompasses the use of four primary approaches to capturing data during OIF-2. The first is the use of the a combat casualty medical encounter form. The form used is a modified version of the Theater Trauma Registry form used by U.S. Army MTFs to capture CTR data on patients treated at their facilities. The original intent for the form was to simply fill it out and place it into the patient’s medical record and transfer it with the patient. Experience gain on the Navy-Marine Corps CTR program revealed that data captured in theater and sent with the patient through the medical chain of evacuation is generally lost enroute. To remedy this unfortunate reality, the Navy-Marine Corps version of the form was modified. Rather than send the only copy with the patient, the Navy-Marine Corps version of the form was printed to include a self-carboning copy. The providers fill the form out once, placing one copy in the patient medical record and retaining the second copy at the MTF. These second copies are forwarded to the Naval Health Research Center for analyses. This form has been placed in each of the 29 BASs, 3 STPs, 3 FRSSs, 3 surgical companies, and one EMF currently deployed in support of OIF-2.

5.1.2 Laptops

In addition to the CTR data collection forms, laptop personal computers have been placed in each of the forward Navy-Marine Corps MTFs. Among the tools loaded on the laptops is an electronic version of the CTR data collection form. This option is provided for MTF clinicians who prefer filling out the form electronically rather than the traditional paper and pencil method. A communications protocol is installed on each laptop permitting the transfer of the completed forms to the Naval Health Research Center whenever internet communications are available. In addition, an excel spreadsheet has been loaded onto the laptop to provide MTF clinicians with a means of recording a census of the patients seen at their MTF. Due to the nature of the combat environment, not all patients will have a form completed. In these instances, the spreadsheet is provided to record a minimum data set that at the very least captures information documenting that the patient was seen at the MTF. Laptops have been placed in the 29 BASs, 3 STPs, 3 FRSSs, 3 surgical companies, and one EMF.

5.1.3 Digital Voice Records

A third data capture methodology, digital voice recorders, have been placed in some of the forward Navy-Marine Corps MTFs. During OIF-1, some success was realized using voice recorders to capture clinical details of care at the FRSSs. This same approach is currently being utilized at each of the 29 BAS, 3 STPs, and 3 FRSSs. Providers at each of these MTFs have the option of recording a core set of CTR data elements on small, handheld digital voice recorders. A small laminated card describing the core data elements required is tethered to each voice recorder for review during the recording of each case. Periodically, the voice recorded files are to be downloaded to the laptops and using an installed communications protocol are transmitted to the Naval Health Research Center for extraction and analysis.

5.1.4 Portable Desktop Copiers

The fourth and final data capture methodology, desktop copiers, have been installed at the more stable forward MTFs including the 3 FRSSs, 3 surgical companies, and 1 EMF. Because the patient record generated at these type of MTFs exceeds the data capture capability of the CTR form, another approach was required to collected details of patient care such as operating room reports and nursing notes. In these more stable facilities, providers are asked to copy the patient record prior to evacuating the patient. Copies of the patient record are retained at the MTF and periodically forwarded to the Naval Health Research Center for analysis.
It is the expectation that by being more proactive and systematic in the capture of Navy-Marine Corps CTR data at the forward MTFs, a more comprehensive view of the events occurring to casualties as they move through the medical chain of evacuation can be assembled than has been historically possible.
An Examination of Surgical Skill Performance under Combat Conditions Using a Mannequin-Based Simulator in a Virtual Environment

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SUMMARY

The present study examined the performance of a surgical procedure under simulated combat conditions. Fifteen medical students were taught to perform a tube thoracostomy on a mannequin-based simulator in a traditional medical school setting under the direction of an ATLS® certified surgeon. The participants then performed the procedure in a fully immersive CAVE virtual environment running a combat simulation including gunfire, explosions, and a virtual sniper under both daylight and nighttime conditions. The results showed that completion times depended on the order of daylight and nighttime conditions with a slight disadvantage for the nighttime condition. However, the quality of the procedures performed by the students suffered in the simulation and particularly under the nighttime conditions. Further, there were nine instances in which the participants were killed by the virtual sniper before completing the procedure. Taken together, these results suggest that the surgical skills acquired by students in a traditional medical school setting may be compromised when they are called upon to perform them under hazardous conditions. Further, the findings from this study show that virtual environments can provide a safe environment for military medical personnel to train for dangerous duty.

1.0 INTRODUCTION

Simulators have been a standard component of military training for many years in a variety of contexts including aviation, ground operations, weapons training, and decision making in command and control operations. By contrast, the use of simulation technology for training medical procedures is relatively new. Although medical simulation devices have been around since the 1940s, most of them have been little more than physical models with limited functionality. However, the current breed of medical simulators is quite sophisticated and many have impressive levels of realism. In addition, the number and variety of systems commercially available has increased dramatically over the last decade (Dawson, 2002; Satava, 2001). Further, medical schools are beginning to incorporate simulation technology into training curricula as they face increasing pressure to train physicians and surgeons to higher levels of competency, in shorter periods of time, while simultaneously improving safety (Healy, 2002).

The advantages of training with simulators are well documented. They provide an environment to train specific skills in the absence of uncontrollable influences, an unlimited number of trials to acquire skills, immediate performance feedback, and an opportunity for trainees to diagnose and treat rare or infrequent conditions. Perhaps, the most important advantage is that they permit the opportunity to train under conditions that would be too dangerous in actual operational settings. Although this last area represents a standard use of
An Examination of Surgical Skill Performance under Combat Conditions Using a Mannequin-Based Simulator in a Virtual Environment

simulation for training many different skills in military contexts, it has been largely overlooked in the medical arena.

The goal of the present study was to examine the performance of surgical skills in a virtual environment (VE) under simulated combat conditions. Military medical personnel who have been in war often acknowledge that the training they receive in traditional medical schools does not always transfer to combat situations (see for example Miller, 2003). Thus, the specific purpose of this study was to determine the extent to which surgical skills acquired in a traditional medical school might be compromised in a simulated combat scenario. Toward this end, a common emergency surgical procedure, tube thoracostomy (chest tube insertion) was selected for study.

Tube thoracostomy involves decompressing the chest cavity to release a pneumothorax or hemothorax. The procedure entails making a 1 – 2 cm transverse incision in the skin near the pectoralis major muscle lateral margin, spreading the chest wall musculature, puncturing the pleural space, and guiding a tube into the opened pleural space to permit drainage.

For this study, medical students performed a thoracostomy on a mannequin-based simulator in a CAVE virtual environment under simulated combat conditions. These conditions included visual and auditory depictions of munitions fire, gunfire, and a virtual sniper who would shoot at the participants if they did not take proper cover. Thus, the battle scenario was designed to provide a heightened sense of realism in which to examine performance of the thoracostomy procedure. The participants performed the procedure under two different lighting conditions: daytime and nighttime. The two lighting conditions were included to create different levels of workload and stress within the combat scenario. In particular, the nighttime condition was included because military medical personnel might not always have control over the visibility conditions in which they must perform. It was expected that if performance were compromised under the simulated combat scenario, it would suffer more under the nighttime visibility conditions.

2.0 METHOD

2.1 Participants

Fifteen medical students from the Eastern Virginia Medical School in Norfolk, VA participated in the study. All students were in their second or third year of training and none had prior experience with the thoracostomy procedure or the simulators used in this study. The participants received $30 as compensation for their time.

2.2 The Training System

The method used to teach tube thoracostomy was based on the ATLS® course curriculum as described in the ATLS® Instructor Manual 1997 edition. The procedure was taught using the TraumaMan® system by Simulab, Inc. TraumaMan® is a mannequin-based simulator used throughout the world for surgery education and is the only simulator approved for the ATLS® Surgical Skills Practicum by the American College of Surgeons. The TraumaMan® system is the standard training device for ATLS® courses at Eastern Virginia Medical School. The simulator includes a realistic anatomical model of the neck, chest, and abdomen with replaceable tissue components and fluid reservoirs that permit instruction on six surgical procedures. Only the thoracostomy procedure was used in the present study.
2.3 Training

Students were trained in two separate sessions and worked in groups of three or four. All students received a didactic session reviewing the indications for the thoracostomy procedure, the technical aspects of the procedure, and the potential complications that could be encountered. Following this, the students were shown how to perform the procedure on the mannequin. Critical determinants of successful performance on the task included correct topographic anatomic landmark identification as well as whether the tube entered the pleural space. Students worked in teams of two and were allowed approximately 90 minutes of practice under the supervision of an attending surgeon qualified to teach ATLS®. Each student was required to perform a successful procedure (see below) for the instructor in less than two minutes in order to move on to the simulation session.

2.4 Virtual Environment Implementation

The VE used in the study was the CAVE (CAVE Automatic Virtual Environment). The system consisted of two main computers connected through a 100-mbps network switch. An SGI ONYX 2 computer was used to display the application in the CAVE, provide the sound playback, and read the information from the tracking device. This computer used VEGA, and IRIX 6.5. An SGI O2 computer served as the main console and was used to launch the application and issue command overrides controls during the simulation. This computer used IRIX 6.5, Motif, and Buttonfly. Images were presented on three 10x10 ft walls of the CAVE with a resolution of 1024x768.

A Radio Shack electronic beam was fixed to the top of the boxes (approximately 3 ft. above the ground). The electronic beam was used to engage the virtual sniper (see below).

2.5 Combat Simulation

The combat simulation depicted a small town under fire. One building was in flames, but most of the other combat cues were auditory in nature. Combat was simulated using the VEGA special effects module to trigger visual and auditory explosion events as well as background gunfire at specific times. The events were timed to repeat at specific intervals. The entire scenario was run in a continuous loop until the participant finished the procedure.

Day and nighttime conditions were created by adjusting the luminance intensity of the image with the time-of-day feature in the VEGA software. Under the daytime conditions, there was enough ambient illumination emanating from the walls of the CAVE to make the barricade, mannequin, and instruments easily visible. Under the nighttime conditions, however, there was very little illumination provided by the CAVE walls. Thus, the participants performed the procedure in near total darkness except for the occasional explosions that provided temporary increases in illumination.

The audio track was created using Sound Forge software. Sound samples from unrestricted sources on the internet were downloaded and filtered. Voice samples were saved in mono at a 22.1 kHz sampling rate. Background and other supplemental audio sounds included gunfire, explosions, machine gun fire, and some M1 tank fire. The files were converted to Audio Interchange File Format Version C (.AIFFC) for final presentation in the CAVE environment.
The audio files were presented over two channels. The left and right speakers were placed at approximately 225 and 315 degrees, respectively. The speakers were mounted on speaker stands at an elevation of approximately five feet. None of the audio sounds exceeded 90dB during the session.

A virtual sniper was included in the combat scenario as well. If the participant disrupted the electronic beam, an audio file would be played that provided either a warning or informed the participant that they had been killed.

2.6 General Procedure

Participants reported to the CAVE facility within two hours of training. All participants were scheduled in 20-min increments and were run individually. Participants were told they were going to play the role of an Army medic with a team of soldiers under fire. A member of the team had been injured and required a thoracostomy. Their goal was to get to the patient and perform the procedure to save his life.

They were handed a kit that contained a knife, chest tube, and clamps and were escorted into the CAVE. They were told that they would perform the procedure twice: once under daylight and once under nighttime conditions. Each attempt began with the participant standing at a starting point marked with tape on the floor. They were instructed to listen for a call for a medic. As soon as they heard the call, they were to get to the patient and perform the procedure as quickly as possible. They were not required to assess the need for the procedure. Further, they were not required to anesthetize the patient or suture/tape the tube to the patient after placing it in the pleural space. When they finished, they were told to return to the starting mark on the floor. Figure 1 shows the configuration of the CAVE facility and a participant performing the procedure.

![Figure 1: Participant performing the procedure under daylight combat conditions.](image-url)
The participants were also told that there was a sniper in one of the nearby buildings and that they had to take cover behind the barricade. Thus, the participants needed to perform the procedure while kneeling or lying on their stomachs. Further, if the sniper got them in his sights he would shoot to kill and they would hear a loud rifle shot. If the sniper missed, they would hear someone say “Get down.” If they were hit, they would hear the phrase, “Hasta la vista, baby.” At that point, they were considered dead; however, they were instructed to continue and finish the procedure. They were not fired upon again.

In actuality, all participants received one warning shot if they disrupted the electronic beam. If they disrupted the beam a second time, they would be killed.

The participants each performed two sessions and the order of day and nighttime conditions was counterbalanced across participants. After the first attempt, the simulation was stopped, the mannequin was rotated 180 degrees, and the participant performed the subsequent procedure on the opposite side of the mannequin. After their second attempt, the participants were escorted out of the CAVE and asked to complete a brief survey. They were then debriefed and allowed to offer comments or ask questions about the study. During this interval, the surgeon who had conducted the initial training session examined the mannequin and rated the participant’s performance based on the criteria listed below.

2.7 Dependent Measures

There were two dependent measures: completion time and performance ratings. The total time to complete the procedure was recorded from the initial call for the medic until the participant returned to the starting mark. The performance ratings were based on three criteria: topographical location for the skin incision, tube placement in the pleural space, and posterior angulation to ensure the tube was in a dependent portion of the thorax. Ratings for each were divided into three categories: good, adequate, and poor. The following was used to assess performance:

GOOD: tube inserted at the nipple line, 4th intercostal space, between anterior and posterior axillary line, with 45 degree posterior angulation. Tube entered in the pleural space.

ADEQUATE: tube placement was within 1 cm of the criteria specified above without angulation. Tube entered the pleural space.

POOR: tube outside above 1 cm radius without angulation and/or did not enter the pleural space.

3.0 RESULTS

3.1 Performance

The mean completion times for each attempt and the day and nighttime conditions are shown in Table 1. The completion times were analyzed with a mixed ANOVA for the factorial combination of attempt (first and second, the within-subjects variable) and order (groups 1 and 2, the between-subjects variable). The results revealed a significant main effect for attempt, $F(1,13) = 4.99, p < .05$, indicating a decrease in mean completion time from the first attempt ($M=92.60, SD=24.73$) to the second attempt ($M=75.13, SD=20.46$). However, neither the main effect of order nor the interaction between attempt and order was significant. A second
mixed ANOVA was performed on day and night conditions (the within-subjects variable) and order (groups 1 and 2, the between-subjects variable). None of the main effects was significant; however, the interaction between day and night conditions and order was significant $F(1,13) = 2.54, p<.05$.

Table 1: Mean Completion Times for Attempts and Day/Night Conditions (standard deviations in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>All Participants</th>
<th>Participants Who Were Not Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempt 1</td>
<td>92.60 (24.73)</td>
<td>99 (14.76)</td>
</tr>
<tr>
<td>Attempt 2</td>
<td>75.13 (20.46)</td>
<td>79.57 (17.27)</td>
</tr>
<tr>
<td>Day</td>
<td>77.87 (20.26)</td>
<td>86.29 (17.21)</td>
</tr>
<tr>
<td>Night</td>
<td>89.87 (26.57)</td>
<td>92.29 (20.48)</td>
</tr>
</tbody>
</table>

The nature of the interaction is shown in Figure 2 as a function of attempt. As can be seen in the figure, completion times were longer on the first attempt, but were clearly tied to the order of day and nighttime conditions. As would be expected, those who operated under nighttime conditions on the first attempt performed slowly. However, when this group performed their second attempt under daytime conditions they were much quicker. On the other hand, the participants who began with the daytime condition performed more quickly on their first attempt than the other group who began with the nighttime condition. However, when this second group moved onto their next attempt under nighttime conditions, their completion times improved, but not nearly as much as those of the other group.

![Figure 2. Mean completion times for order of day and night conditions plotted for each attempt.](image)
The analysis of completion times included data from all participants because the sample size in this study was fairly small. However, there were several instances in which participants were “killed” by the virtual sniper. These data are shown in Table 2. As can be seen in the table, most of the participants who were killed were shot during their first attempt. Although participants in this study who were killed were allowed to continue and complete the procedure, one could argue that these data should not be included in the overall means. Thus, the mean completion times were recalculated excluding data from participants who were killed. These recalculated means are also presented in Table 1 and show that completion times increased when the scores for those who were killed are removed from the data.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Attempt 1</th>
<th>Attempt 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Night</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

The performance ratings are shown in Table 3. The number of participants who received good, adequate, and poor ratings is shown for all three criteria. The ratings show that performance suffered in the simulation and particularly so for tube placement. There are no other consistent patterns among the frequencies except for a slight disadvantage for the night condition.

<table>
<thead>
<tr>
<th>Location</th>
<th>Angulation</th>
<th>Tube Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Adequate</td>
</tr>
<tr>
<td>Day</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Night</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2 Participant Responses

Participants completed a nine-item opinion questionnaire following the experimental session. The first item required participants to rate the realism of the thoracostomy procedure under simulated combat conditions. Although 9 participants (60%) reported that the simulation was either “somewhat” or “moderately” realistic, 40% reported that it was “extremely” or “quite” realistic. Participants were also asked to report whether the noise in the simulated combat environment distracted them. Despite the loud gunshots and explosions, 12 participants (80%) reported that the noise was not distracting or “slightly” distracting. Only 3 participants...
found the noise to be “moderately” or “extremely” distracting. The participants also indicated whether the lighting conditions and their physical position complicated the procedure. Twelve participants (80%) reported that the procedure was either “slightly” or “moderately” complicated under nighttime conditions, but the remaining 3 indicated that the nighttime conditions “significantly” complicated the procedure. Likewise, most of the participants reported that their physical position did not complicate the procedure. In fact, none of the participants believed that it had a “significant” or “extreme” impact on their performance.

The participants were also asked to describe any strategies they adopted for performing the procedure in the day and nighttime conditions. In the daylight condition, 57% of the respondents indicated that their main strategy was to remain low to the ground while performing the procedure. Several participants also reported that they attempted to “block out” the loud noises and focus only on the task at hand. Under nighttime conditions, approximately half of the participants (46.7%) followed the same strategy as in the daylight conditions, but the remaining participants reported that they relied more on anatomical landmarks and tactile feedback. Finally, the participants were asked to report the easiest and most difficult aspects of the experiment. Most participants agreed that the actual thoracostomy was the easiest part of the experience. On the other hand, they identified several portions of the experiment that were extremely difficult including: (1) knowing how low to remain to the ground, (2) trying to remain calm under pressure, (3) inserting the chest tube while lying down, and (4) feeling around for the equipment in the dark. In particular, participants commented that it was very difficult to remove the equipment from the bag and to place the cover back on the scalpel.

4.0 DISCUSSION

The primary goal of the present study was to examine the extent to which training in a typical medical school environment would generalize to simulated combat conditions. Students were taught how to perform a thoracostomy on the standard simulator used in ATLS® courses. In fact, they were given nearly twice as much time to practice the procedure as they would in a typical ATLS® course. By the end of the training session, each student performed the procedure to the satisfaction of the instructor, in under two minutes. They were then asked to perform the procedure in a fully immersive VE under simulated combat in daylight and nighttime conditions.

The findings were mixed. On the one hand, the results for completion times suggest that the participants were fairly efficient at performing the procedure. Overall, the mean time to complete the procedure was 84 sec ($sd = 22$) and the completion times dropped significantly from a mean of 93 sec ($sd = 25$) in the first attempt to 75 sec ($sd = 20$) in the second attempt. As expected, the completion times were affected by lighting conditions, but the results were tied to order. Specifically, the participants took 24 sec longer on average to perform the procedure under nighttime conditions if it was their first attempt, but if the nighttime conditions occurred on their second attempt, it increased completion times by only 13 sec over the daytime conditions. Thus, the participants required less time to perform the procedure on their second attempt and the effects of low visibility were less severe on their second attempt. Further, the overall completion times were not dramatically different from what they achieved at the end of their training session. Although the findings for completion times were encouraging, the results for the quality of performance were less so.

An analysis of the performance ratings showed that the ability of most medical students to perform the procedure was compromised in the simulation. Only 23% of the tube placements were judged as good and only one placement was judged good under nighttime conditions. Seventeen percent of the tubes were poorly placed. In addition, the topographical placement was judged to be poor on 13% of the attempts and the angle of placement was poor on 20% of the attempts. Thus, even though participants were able to achieve
completion times in the simulation comparable to those from training, they did so by sacrificing the quality of their performance.

It is important to understand that these results present an optimistic picture of performance. There are several reasons for this. First, the results must be viewed within the context of the combat scenario. There were 9 instances where the participants failed to heed the warning shot and were “killed” before they could complete the procedure. A finding such as this is indeed troubling because it suggests a potential loss of critical medical personnel in addition to jeopardizing the safety of the patient. Further, the results in Table 1 clearly show that if the data from participants who were killed are excluded from the means, the completion times for the remaining participants are noticeably poorer.

Second, the participants performed the procedure within two hours of their initial training session. Under traditional medical school training paradigms, months or years could elapse before a student or resident would have the opportunity to perform the procedure on a genuine patient. Thus, the levels of performance obtained in this study likely represent a “best case” scenario. One might expect the performance levels seen here to become progressively worse as the interval between training and initial attempt increases.

Last, the results show that the ability to perform a newly acquired emergency surgical procedure is significantly degraded even under simulated combat conditions. It is quite likely that the performance problems observed in this study would be exacerbated under genuine combat conditions.

Subjective reports indicated that most participants felt the experience was fairly realistic. Several students commented that they had to make a conscious effort to remain calm and focus their attention on the task. Although not every participant found the nighttime visibility conditions to be problematic, those who did attempted to perform the procedure by relying on tactile information. Further, some students commented that they found it challenging to perform the procedure while lying down. Collectively, these comments suggest that the students took the experience seriously and recognized the value of training outside of traditional environments.

One criticism of the present study may lie with the choice of thoracostomy as the procedure of interest. One could argue that it is unlikely this procedure would be performed in the field. That is, the injured patient normally would be moved to a safer location before performing the procedure. However, it is important to remember that the primary goal of this study was examine how skills acquired in a traditional medical school setting would hold up under stressful conditions simulated in a VE. Toward that end, we chose thoracostomy as a representative emergency procedure. Moreover, even though standard practice might dictate moving the patient to a safer environment before performing the procedure, it does not preclude the possibility that transporting the patient would be unfeasible in some situations. Thus, emergency medical personnel might be called upon to perform such a procedure to prolong a patient’s life until he or she could be moved at later time.

5.0 CONCLUSION

The present study was designed to examine how the ability to perform a surgical procedure would be affected in an immersive VE. The results showed that performance was significantly degraded under the simulated combat conditions. Moreover, the levels of performance seen here are probably better than what would be expected under more realistic conditions.
To our knowledge, the present study represents the first time that performance with a standard mannequin-based medical simulator has been studied within a fully immersive VE. From this perspective, our results show that VEs can be a valuable tool for medical training because they provide a rich context in which to examine performance. The benefits of this approach are numerous. First, VEs provide a safe environment for training medical personnel on a wide range of scenarios under a variety of stressful conditions. It is no longer necessary to rely on the reports of medics or corpsmen who have been to war as the sole source of data concerning the adequacy of their training and level of preparedness for practicing medicine in combat zones. It is now possible to address specific medical training needs before personnel are deployed.

Second, VEs extend the range of applications for current medical simulators. For example, the TraumaMan® system used in this study was designed primarily as an emergency medicine training device. However, we have shown that the simulator can also be used as a research tool to study performance. Obviously, other mannequin-based or even VR simulators can be used in a similar fashion.

Last, VEs provide a safe environment for studying performance under simulated hazardous conditions. Virtual environments open up the possibility of examining a wider variety of medical procedures performed under an unlimited number of conditions. More important, however, they offer a laboratory in which to study new training techniques and countermeasures for medical personnel who must perform in dangerous situations.

6.0 ACKNOWLEDGEMENT

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7.0 REFERENCES

Experience and Consequences on the Deployments of the Medical Services of the German Army in Foreign Countries – Surgical Aspects

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ABSTRACT

Several deployments supported by the German army medical services lead to new experiences concerning personal, training, preparation, support, equipment and standardisation.

The consequences are not only important for the surgical work but also for anaesthesiology, intensive care, internal medicine and neurology and psychiatry.

The challenge for our medical services is the fast and complete facilitation of all purposes necessary for the adequate and modern care for our soldiers, the soldiers of the UN-nations and nevertheless for local people in the sense of humanitarian help.

INTRODUCTION

The military surgeon is always in a crucial situation: in case of war he has to provide the quickest and most competent care for the casualty. What we learnt from the past is the phenomena that all well known experience from the surgical care has to be learnt again because young surgeons are not well prepared for their task in the peaceful times at home.

What about the training of military surgeons nowadays? Only few of them got a fellowship for trauma care after their general training: most of them do oncology, transplantation or merely general surgery. In the USA only a few civil centers in a region are allowed to treat the major trauma cases. Normally military hospitals are not involved in daily care of major trauma cases. So it is no wonder, that in case of deployment the military surgeon is not prepared in the surgical management and operation procedures of war wounded.

Another disadvantage seems to be the high specialisation in the US within a small field of surgery. So the expression “general” surgeon seems to be wrong concerning the fact that for example he does not care for the extremities! This is hard to understand for the military surgeons from Europe because 80 to 85 per cent of the casualty which survive in a war environment are only wounded at the extremities.

So, what would be a rational for the training of military surgery? The authors wish to emerge a discussion about the question how and where military surgeons have to be trained in a practical way to fulfill the requirement of special expertise in a hostile environment.

THE SITUATION

In all civilized countries specialization increases. That means that a nearly complete overview about surgery is no longer possible. Surgical science has reached a level that makes a skillful handling of the different specialties obviously not longer possible.

On the other hand there is sometimes a need for an utmost complete overview to understand and treat trauma within its complexity. So the question arises whether there have to exist surgeons who are allowed and competent to treat the different facets of trauma. It is e.g. not understandable why in the US a trauma surgeon should not be able to perform an external fixation procedure before a necessary reconstruction of vessels. External fixation is such a quick and simple procedure that in our opinion every trauma surgeon has to know and practice it. As long as legal restrictions block the development of broad surgical training no improvement of care can be possible.

Concerning the military environment not always war is the situation military surgeons find them in. War is the most extreme circumstance under which surgical procedures have to be performed: Risk of own wounding, operations under time pressure with limited resources, psychological stress by sorting and so on do not always occur. In most cases, especially in the modern peace keeping missions, time, facilities, logistics and the number of casualty are not very different than at home. What differs is the type and the amount of special war wounds and the limitation of facilities, personnel and known instruments. This special situation is in Germany called Einsatz, what means that the military surgeon has to provide a surgical work that in its result has to be comparable with that at home. This special requirement needs a surgeon who is trained in all the main fields of surgery! He is the responsible doctor for the applied procedures and for the outcome. It is well known, that most of the wounds which could be survived normally are the wounds and fractures on the extremities. They amount in most of the statistics up to 80 - 85 per cent of the casualty. The necessary surgical procedures can be trained in a relatively short time and need only little practice. On the other hand, the wounds of the cavities are more life-threatening and need more knowledge and manually expertise. So every military surgeon must be trained in craniotomy, thoracotomy and laparatomy to stop fatal bleedings and repair organs and intestines.

What means military surgical expertise?

Obviously there is a difference between civil and military surgery: Some are the opinion that military surgery is worse than civil surgery and the military surgeon is therefore worse than a civil surgeon.

When we look to the imaginable different environments where a military surgeon works something will become more clear:

Comparing the environments in normal surgery, deployment surgery and war surgery, there are different emerging problems. But all basic principles in every environment are the same: to do the best under the given situation: but to do the best means to be the best! How military surgeons can achieve the excellency to be the best? Nobody can provide the best care for casualty during deployment or war if he is not well prepared in the normal situation. That means in other words: if a military surgeon is not trained in trauma care, he is not prepared for an adequate therapy in case of deployment or war. The military surgeon must be as good as his civil colleges in trauma care plus a lot of special training for the purpose of working under special conditions.

That means not that he has to be a generalist! Generalist or specialist? What do these expressions mean?

In general one may think, that the Generalist knows everything in all the fields and the Specialist knows everything in one field.

But when you know everything in one field you are a specialist by a curious way of automatism.

To be a specialist is nowadays honorable and successful, because the specialist can not be attacked in any way: He is the last judge in a small field - and he is only, and only there responsible. But this is also the problem of this fact. Our doing becomes more and more complex. Surgery is divided into many pieces and it seems not longer to be manageable.
Because the whole thing is more than the sum of its parts.

The competence of surgeons in different specialties grows more and more, but the orientation to care for the whole patient decreases at the same time.

It is questionable whether a specialist is the answer to the challenge of a military surgeon. Nevertheless the generalist is also not the answer to this dilemma. The problem would only shift from a doctor who knows only little from whole field to a doctor who knows all on a small field.

Surgical skills that are divided into skills of the specialist and the skills of the generalist cannot solve the problem.

In our opinion, there are not too few specialists of generalists - no - what lacks is the surgical expert for the genuine purpose of military surgery. This expert is a kind of surgeon who always runs on a borderline of multiple surgical specialties, able to keep in mind and hands the knowledge to act in a interdisciplinary -or better - in a trans-disciplinary manner.

The wounded victim as a whole cannot be divided in several parts to deal with - a responsible military surgeon must be an expert in overlooking the whole problem and must have the ability to act on the life-threatening injuries first.

What are the problems concerning the training of such an expert?

Not the separation between specialist and generalist, not the frustrate discussion who is the better surgeon of both - no, it is the inability to deal in a reasonable manner with this issue. The question is not:

How to train the super-all-expert? It is the question of judgement what kind of surgical skills combined with knowledge is feasible for this expert in fact. That means, how can we integrate our theoretical knowledge with a concept of orientation.

We are responsible for the future military surgeons experts: we must commit: what they have to know, where they have to go, and how they have to be trained. But this is a hard job to do, for the teachers and the young surgeons as well.

How to gain expertise in military surgery? A proposal.

Military surgeons and responsible politicians must be aware that this special expertise will take a long time of training, learning, exercise and permanent work in trauma. In Europe it is a matter of fact that military surgeons work all on trauma. They compete with their civil colleges in treatment and science of major trauma cases. In Germany a special schedule for training to the endpoint of military surgical expertise will be the following in the future. The authors believe that only a surgeon who run through this training for at least 8 years can be responsible for what he is required.

This training program must include a training of the basic surgical skills within 24 month. During this time he is a non commissioned surgeon (NCS). 6 month training in an ICU, 6 month on a surgical ward, and 12 month in one of the specialties. During the basic training the surgeon must be familiar with the ultrasound of trauma. He has to fix up to 600 sonographies. Radiology has also to be learnt, especially the diagnosis in angiography, CT and MRI. After 2 Years he is specialised as for 4 years as a general surgeon: that means 1 year visceral-, 1 year thoracic, 1 year vascular and 1 year otho-/trauma rotation.

After the curriculum of training he will get the qualification of a limited commissioned surgeon (LCS).
Now the special training to become a full commissioned surgeon (FCS) can start. It lasts another 2 to 3 years in which the LCS has to train for at least 24 months on in his specialty (visceral-, thoracic, vascular, ortho-/trauma) surgery. He must be after this time familiar with all kinds of specialised procedures. After this training the military surgeon is in our opinion competent and fully responsible for deployment or war. Otherwise all the lessons which had been learnt by our predecessors must be learnt by us again.

Sir K.R. Popper is known for his statement: Those, who did not learn from the past, are condemned to repeat it.
Prehospital Data Collection and Analysis for Combat Algorithm Design and Remote Triage

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ABSTRACT

Few data warehousing systems provide a complete and continuous history of trauma patients from the outset of prehospital care to the point of hospital discharge. Typical prehospital database systems are limited to data recorded by emergency personnel during patient transport and contain a minimal number of data points. This incomplete information provides a snapshot of patient status during the course of treatment, but does not present a complete picture. Furthermore, the lack of patient outcome information in prehospital trauma data repositories severely limits meaningful correlation analyses. This lack of data has consequently led to the development of treatment algorithms based on anecdotal evidence rather than proven statistical methodologies. Therefore, in order to develop more accurate prehospital protocols and algorithms for remote triage, we created a robust system for recording patient injuries, vital signs, interventions, and outcome. This paper describes the Trauma Vitals warehousing system which was designed to provide researchers with a comprehensive database for the continuous capture, storage and analysis of trauma patient data during all phases of prehospital and hospital critical care. Patient prehospital vital signs are recorded automatically to ensure comprehensive data collection. Automatically collected prehospital data is combined with manually entered prehospital and emergency department (ED) hospital data. The system incorporates a web-based approach using a Java applet interface for handling user requests and data management commands to an underlying real time database.

1.0 INTRODUCTION

Prehospital treatment of trauma patients is a critical aspect of emergency medical practice in both civilian and battlefield environments. Initiation of early and effective life saving interventions (LSIs) after severe trauma injuries directly affect patient morbidity and mortality rates. Historically, due to a lack of prehospital critical information, such as fluctuations in patient vital signs during transport, treatment decisions have relied on the experience of medical personnel, not on empirical data. Typical physiological monitoring of injured patients involves a single data point (one blood pressure, pulse, respiratory rate and mental status) or at best two such measurements during the prehospital care phase. This severely restricted and often imprecise physiological information serves as the basis for critical decisions regarding appropriate interventions. However, in order to effectively develop new emergency protocols and treatments or to validate current procedures a statistically

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1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

valid model is needed. Design of an automated prehospital data collection system is a critical step for future critical care options. Such a system will provide tools for making triage decisions for multiple patients in the field. Combined with patient outcome (dead or alive) information, accurate trauma injury models can be designed to more effectively prioritize patients and create a better patient flow to the different available facilities.

In the past, database technologies for capturing disparate patient information from multiple heterogeneous sources have taken several approaches based on a basic relational model [1-5]. However, these approaches do not address high resolution vital signs recorded from patient monitoring devices and do not cover the entire critical care phase that is required for effective analysis of trauma patients. The U.S. Army Institute of Surgical Research, in collaboration with the University of Texas Health Science Center in Houston, TX, has developed the Trauma Vitals system for the capture and analysis of prehospital patient vitals signs and to address the lack of prehospital trauma data available to researchers. This unique system permits warehousing of patient prehospital and hospital information, treatments and outcomes for use in the verification and validation of trauma injury models. The system has the capability to automatically record real time numeric as well as waveform data from Code 3 (severely injured) trauma patients during the prehospital transport phase. Using a web-enabled client, (a Java program downloaded to a local computer), a research nurse can create a patient record that includes a complete representation of a patient’s physiological status during the prehospital phase in addition to ED treatments, and eventual patient outcome. Similarly, using a web-enabled client, researchers can query the system for particular data sets which can be imported into statistical analysis packages.

The Trauma Vitals system is currently operating in the Houston Life Flight service to record trauma patient information from Code 3 helicopter transports in the Houston metropolitan area to the Level 1 trauma center at the Memorial Hermann Hospital. The system already contains data for more than 850 patients available for analysis through a web-enabled front end client and is expected to grow significantly as additional data collection sites are added at other Level 1 centers.

2.0 SYSTEM OVERVIEW

The Trauma Vitals system is composed of two modules that work symbiotically to provide patient prehospital and ER data storage capability. An automated data recording module records prehospital vital signs en route to the critical care facility. The second module consists of a web-enabled warehousing system designed for use by trauma researchers and patient data managers. Data from all recorded incidents is stored and correlated using a relational database engine that provides the data management tools for storage and warehousing the records in addition to providing the querying engine for data analysis and algorithm testing.

- The system was designed with the following characteristics in mind:
  - Web-based client data management and querying design to provide ubiquitous access capabilities.
  - Relational database and warehouse server technology to enable high performance and availability implementation of the underlying data warehouse.
  - Standards-based formats and communication protocols; XML data representations and formats supply seamless information exchange and exporting from the system.
3.0 AUTOMATED DATA CAPTURE AND PROCESSING

The data recording system includes the capture of data from multiple simultaneous sources through the use of an automated data recording system. The system consists of a personal digital assistant (PDA) attached to a Propaq model 206EL vital signs monitor via a serial RS-232 cable. Each unit is deployed on every emergency vehicle involved in patient transports to the receiving hospital. The collected data is stored in a removable storage card loaded onto the PDA through a built-in expansion slot. Figure 1a shows the Propaq monitor with the original PDA used for the project. Initial deployment of the data collection unit was done using the commercially available HP Jornada PDA. However, due to reliability issues, this unit was subsequently replaced by a rugged device from the Talla-Tech corporation. Figure 1(b) shows the new PDA (Tacter R-PDA, Talla-Tech, Inc., Tallahassee, FL) used by the collection unit.

![Figure 1](image1.png)

(a) Original Propaq monitor and PDA data collection unit
(b) upgraded rugged PDA

Collection of prehospital data requires a system capable of capturing patient data during transport from the incident scene to the receiving hospital. Monitors are placed on the patient during transport for evaluation of conditions as dictated by the emergency protocols of the responding emergency personnel. Collection of this data, therefore, has to be implemented such a manner as not to interfere with the normal duties of EMS personnel. Transparency of the data collection process has been a major objective in the implementation of the system.

The Tacter R-PDA Type B data collection unit uses a custom-built RS-232 cable for interfacing between the rugged connector on the PDA and the RS-232 Acuity port on the Propaq monitor. Communication between the PDA and the device is done through the PDA serial port using proprietary software written for the PocketPC operating system based on the Microsoft Embedded Visual tools development environment. This software module implements the required device management functions for activating the data collection sessions, saving and retrieving recorded streams, and managing stream inputs from the monitor.
The data capture unit had several operational requirements which needed to be met before deployment:

- **Transparent operation.** The data collection system had to operate without interfering in the routine operations of emergency personnel. To this end, a software daemon in the PDA monitors the power activity of the Propaq monitors continuously. Using a cyclic packet, the daemon interrogates the communications port of the monitor using a status packet. During power-off phases, the monitor does respond and the PDA is kept in sleep mode. If an acknowledgement is received by the PDA in response to the status packet, the PDA is set to capture mode and a new patient record is created. In this manner, all activities of the monitor are managed by the PDA transparently from the emergency personnel.

- **Text-based storage of recorded vital signs.** In order to verify the quality of recorded data packets, an ASCII (text) based format was developed to store the data streams generated by the monitor. Using a text format simplifies the verification and validation of captured data without requiring specific reader/writer tools to accommodate the chosen file format.

- **Extended data capture capability.** Units in the field require a data storage and battery capacity of at least 48 hours to accommodate weekend shifts. This necessitated storage and battery capacity for recording all required vital signs during these extended deployment shifts. Memory requirements were met by using a PCMCIA card with a 32 MB storage capacity that allows continuous recording time of approximately 5 hours. The battery life of the device was extended by using two power saving daemons to conserve the software power usage during operation. A screen manager daemon implements a screen blanking algorithm for turning off power to the screen when not in use. Similarly, a file manager daemon is used to transfer data files to the local storage card only during file closing procedures. These measures enable the data collection unit to operate up to 72 hours unattended.

- **Required numeric vital signs (from monitor)**
  
  a. Heart Rate (from echocardiogram (ECG), oxyhemoglobin saturation (SpO2), and non-invasive blood pressure (NIBP))
  
  b. NIBP (Systolic, Diastolic, Mean)
  
  c. SpO2
  
  d. End tidal carbon dioxide (EtCO2)
  
  e. Respiration rate

- **Required waveform vital signs (from monitor)**
  
  a. ECG
  
  b. SpO2
  
  c. Respiration

The collected data is uploaded to a centralized SQL-based server via a web-enabled Java client application by a project research nurse. Data stored in the server includes the recorded vital signs along with the written patient run sheet, chart information, and eventual patient outcome. Communication protocols and data exchange transactions are handled by a server side Java module which manages communications between the Java clients and the underlying database system.
4.0 WEB ENABLED DATA MANAGEMENT

In order to create a complete record of the trauma patient, data is collected from all critical care treatment phases. This includes obtaining data for treatments, diagnosis, status, and medications given during the transport phase and during the ED visit. Additionally, the patient outcome is also obtained as part of the incident record. Treatment and diagnosis data is divided into the prehospital and hospital (ED) phases. Prehospital data includes:

- Incident description. Estimated time of incident and additional scene times as an offset of the time of incident. No dates, names, or other identifying information is included due to HIPAA restrictions.
- Prehospital interventions. All interventions performed by emergency personnel, entered manually via the client interface.
- Manual pulse characters for the radial, femoral, and carotid arteries.
- Patient injuries and method of injury. Recorded manually by paramedics.
- Fluids given. Fluid types and volume.
- Glasgow Coma Score at the incident scene.
- Manual respiration rates and blood pressures. Taken directly by emergency personnel.
- Automatic numeric and waveform vital signs.
- Hospital data recorded by the system:
  - Hospital interventions. All interventions performed in the ED after arrival.
  - Diagnosis and Treatments. ICD9 codes for the treatments and diagnosis in the ED.
- Fluids. Fluids given in the ED in the first 24 hours.

Other data:

- Post 24 fluids. Fluids given after the first day of treatment.
- Patient outcome. Alive or dead at discharge with corresponding time.

In order to facilitate the storage of all these disparate sets of data items, a client/server system is used to correlate and manage both automatic and manually collected data sets. The system client is deployed via a Java applet which is deployed on a web site which users visit to log into the system. The applet uses a Java interface to allow the users to manage the current set of data, upload new records to the system, or query the system for patterns of interest.

Communication between the client and the database server is implemented in a two-phase approach. Phase 1 consists of a user login into a web page containing the client Java applet needed by the system. An Apache web server is used to deploy the content of the web page in addition to sending the client applet up to the requesting user via the standard HTTP web protocol. Figure 2(a) shows the communication path for the initial client download. The client applet is contained within a signed Java Archive (JAR) file that the user must accept through the standard web security protocol in order for the application to execute on the user’s machine. Once the applet has been fully downloaded and accepted by the user, the Java Run Time (JRE) environment on the client machine will execute the applet code to initialize and establish the second phase connection back to the database server. Using a standard Remote Method Invocation (RMI) protocol, the
client applet will establish a connection to an RMI server process on the database machine. Database access commands are executed by wrapping the client SQL commands with RMI procedure calls. Calls to the system meet the current standard SQL format [6]. The RMI server attaches to the underlying database and data repository using a standard JDBC driver. Figure 2(b) shows the send phase connection between the client and the RMI server. Arriving RMI procedure calls are converted to SQL commands by the RMI server and executed on the attached database. Results are returned via RMI string packets. The system currently uses the MySQL database engine for maintaining the data repository. Figures 3(a) and (b) show some sample screenshots from the client application.

![Diagram](Diagram.png)

(a) Initial client applet deployment  
(b) Established communication pattern

**Figure 2**

Use and administration of the system is governed by a set of operational levels which define the role of each user who has access to the system. These include read only access, read/write access, and administration. Read only access users can query the system and observe results, but cannot create new records. Similarly, read/write access is given to data managers who will collect the needed data and upload it to the system as new records. Finally, an administrator level is used for managing system resources and accounts.

### 5.0 ONLINE QUERYING SYSTEM

Once a patient record has been loaded and verified by the system data managers, the record becomes part of the data warehouse and is available for queries by system users. These queries are performed by the users through the use of selection and range operators which allow researchers to query every data item on the incident record. Several approaches have been developed to effectively query and retrieve medical records from a relational database or develop new querying languages specific to medical record requirements [7-14].

Elements which are either present or absent from the incident record are queried through a selection operator using a drop down approach for each item on the system. For example, if a patient was intubated in the prehospital phase, then the user can select the intubation intervention from a pull down list and add it to the query. If a single item is added to the list, all records which have the item present will be retrieved by the
system. Range operators are used for selection of data items which have numeric ranges. Items can be queried based on less than (<), greater than (>), equal to (=), or in between (< x <). Multiple selections within a particular data item (such as prehospital LSI) are chained as OR queries, whereas multiple selections across different data items (such as prehospital LSI and prehospital NIBP) are treated as AND queries. Items stored as free text (such as descriptions) can be queried using a “*” operator for matching one or more text patterns in the field.

Query results are returned to the user as a list of patient records that match the query inputs. Figure 4 shows an example query results page. Query results can be further limited by selecting/unselecting individual patient records from the results page. The remaining selected records can then be exported to the local user’s computer for further analysis and validation.

Query records can be exported in both XML and character delimited formats. Due to the possible large sizes of the associated captured vital signs, the user has the ability to select if captured data streams should be exported to the local machine with the rest of the records. This format is useful for exchanging data files between systems by creating a self describing file format which can easily be exchanged across heterogeneous systems [15-17]. A character delimited file export can generate the selected records as a text file which can be imported into commercially available data tools.

Figure 3a: Example screenshot of fluids page
6.0 CURRENT STATUS

The Trauma Vitals system is in use at the Memorial Hermann Hospital Life Flight system in Houston, Texas. The Life Flight service has three helicopter transport vehicles for the Houston metropolitan and surrounding areas and averages 10 trauma patient transports per day. All helicopters in the service have been deployed with a data collection unit for complete coverage of all helicopter critical patient transports. A full time research nurse manages the data collection and uploading of patients to the database from his/her workstation. The current system contains over 850 fully correlated incident records with prehospital, hospital, and outcome data.

7.0 FUTURE DIRECTIONS

Future directions will concentrate on improvement of the data collection process, expansion of the data collection sites, better data management approaches, and improved data warehousing functionality. The current system has been limited to capturing data streams from a Propaq monitor through a PDA. However, we are testing new air-certified monitors that can collect data using internal and/or removable storage devices, for possible replacement of the current system. Work will also focus on expansion of the data collection
project beyond the current deployment in Houston, TX. The addition of new data collection sites will provide larger population samples and create a more statistically robust data representation for trauma and critical care patients.

Currently, the warehousing system is implemented using a MySQL database backend server. A new schema has been designed to accommodate additional database servers including Oracle and SQL Server. These new architectures will improve database capabilities for expansion of the system as new data collection sites are created. A new schema has been designed for more accurate tracking of records, and to provide increased performance for users through new Java and web-enabled technologies.

**8.0 SUMMARY**

The Trauma Vitals system has been designed to provide a means for collection, storage, and analysis of prehospital and emergency patient data. Using a set of automated data recording units, the system is able to capture a patient’s real time vital signs from the time of incident pickup until hospital delivery. When combined with the manually digitized incident information and patient outcome, the system is able to provide a complete picture of the critical care patient.

Using web-enabled techniques, the system provides an online accessible warehouse of critical care variables which can be used by researchers for verification and validation of current and future emergency and prehospital protocols. The system is built using standard commercial off-the-shelf database and client/server software components to provide a system which can be accessed via any standard HTML and Java compliant browser. Using client/server communication techniques between a Java applet executing on the client machine and the centralized server system, users and administrators can manage all stored patient records and provide a means for querying patient records on any prehospital or ER item warehoused by the system.
9.0 REFERENCES


Rheoencephalography (REG) as a Non-Invasive Monitoring Alternative for the Assessment of Brain Blood Flow

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ABSTRACT

This work focuses on exploration of the potential use of pulsatile brain bioimpedance (rheoencephalography - REG) measurement as a non-invasive, continuous method for assessing the status of cerebral blood flow (CBF) in combat casualties.

1.0. INTRODUCTION

1.1. Relevance

An important goal of the Army's Medical Department is to develop physiological monitoring of parameters that will aid in the assessment and treatment decisions of combat casualties. The two most frequent causes of death in combat are exsanguination (44 %) and central nervous system injury (31 %) [1]. Development of devices for early noninvasive monitoring of multiple parameters in the field is required for expedient and effective triage and treatment decisions [2]. The superiority of a resuscitation strategy that targets maintenance of CBF and function in the context of cardio-pulmonary resuscitation has been demonstrated by P. Safar and used in the emergency medical practice [3].

1.2. Rationale - Pathophysiology

Systemic hypotension, brain ischemia and hypoxia can cause brain damage mediated by microvascular changes. Monitoring CBF or cerebrovascular reactivity is applied in neurosurgical clinical practice in order to

evaluate the status of the patient after brain injury or operation. The concept of CBF thresholds of ischemia was introduced in the seventies when it was observed that a more dense zone of ischemia exists within the central core of the ischemic zone, but in the peripheral zones, where electrical silence may pertain, there is a zone of intact ionic homeostasis, which has been termed the “ischemic penumbra”. The flow threshold for maintenance of electrical activity in the cortex is 15-20 ml/100g/min [4,5] and an ultimate goal for resuscitation from hemorrhagic hypotension is to maintain the CBF above this level.

1.3. Broader View

The application of REG for potential military applications began at the Walter Reed Army Institute of Research over 40 years ago [6,7]. Authors found characteristic changes in the REG wave with increased intracranial pressure in humans and in animals, furthermore complained about the manual control of REG amplifier. Since that time advances in the development of microprocessors and signal processing techniques has presented the possibility to reconsider the feasibility of implementing a portable or even wearable version of the rheoencephalography monitoring technique to evaluate the adequacy of brain blood flow [8]. Our goal is to develop a non-invasive approach to monitoring brain blood flow that would serve as an important component of assessing the severity of injury and as an end-point for resuscitation. Use of REG as a wearable monitoring system would allow life-saving assessment and treatment during the first critical minutes after injury during the transport of a wounded soldier.

1.4. This Study

In the animal studies reported here, we monitored and compared global CBF using REG, local CBF by laser Doppler flow, and carotid flow by Doppler ultrasound during physiological stimuli known to produce predictable changes in CBF.

2.0. METHODS

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition.

2.1. Rats (Group 1)

In this group two studies were undertaken in anesthetized rats to study the changes CBF using two standard perturbations: 1) CO2 and O2 inhalation and 2) electrical stimulation of the brain.

Brain electrical impedance was recorded in 8 male Wistar rats anesthetized with pentobarbital and ventilated with 30% O2 (balance N2). Stainless steel needle electrodes were implanted into the brain and fixed with dental cement. These REG recordings were made by monitoring impedance shifts with bipolar (intrahemispheric) or tetrapolar (interhemispheric) techniques with an excitation frequency of 50 kHz (Model 2991 and 2994, UFI, Inc. Morro Bay, CA). CBF levels were altered by changing the inspired gas mixture for one minute (one mixture for one minute; 100% O2, 5% CO2, and 20% CO2) or by applying electrical pulses (20 Hz, 0.5 msec pulse duration, 2 sec pulse trains with intensity varied to produce an effect, i.e. max 15 sec, ramped up by 10 V for each trial until CBF increased, max 60 V, and 3 trials) directly to the exposed cerebral surface of one hemisphere. The stimulating electrodes were covered with dental cement, also. REG measurements and DC impedance (Ro) were recorded simultaneously on a portable IBM compatible computer using CODAS (DATAQ, Inc., Akron, OH) data acquisition system. Data collection for
analysis lasted 4 min, covering a baseline, gas administration or electrical stimulation and recovery. Each of the recorded signals was digitized at a rate of 250 Hz using a 12-bit A/D conversion board. The signals were processed by Rheosys software. Data were quantified using the recorded pulsatile and basal resistance values, as described earlier [9].

2.2. Rats (Group 2)

In this study in anesthetized rats, various CO2 concentrations were used to study CBF reactivity.

REG signals were recorded in female Wistar rats anesthetized with pentobarbital (N = 5, 13 measurements; some rats were used more than once). Stainless steel needle electrodes were implanted into the brain and fixed with dental cement. These measurements were made prior to, during and after 2-min substitutions of varying fractions of CO2 (4%, 6%, 8% or 10%) for N2 in the inspired gas mixture. The REG measurements were made using a rheograph (ReoRon-61; Mikromed, Esztergom, Hungary). The measuring frequency was: 160 kHz, sensitivity: 0.03 ohm. The REG and electrocardiogram signals were connected to the analog memory recorder (MCAM-4, Roliutron, Budapest, Hungary). The data processing of the above-mentioned modalities was signal averaging (n = 40 pulses in about 10 sec), triggered by the R-peak of the EKG. The basis of comparison was the change of amplitude, integral and ascending portion of the REG curve during different concentrations of CO2 inhalation compared to baseline.

2.3. Rat (Group 3)

In this group three correlative studies were undertaken on anesthetized rats to study CBF responses to the following perturbations: 1) CO2 inhalation, 2) carotid clamping, and 3) hemorrhage to 40 mmHg.

Sprague-Dawley rats (250-350 g) were anesthetized with sodium pentobarbital IP (50 mg/kg), heparinized (50 IU/100g, IV), a tracheostomy performed and body temperature maintained with a closed loop heating pad-rectal thermometer system (Homeostatic Blanket Control Unit, Harvard Apparatus, Edenbridge, KT). One femoral artery was used for heart rate and blood pressure, and the other was attached to a peristaltic pump for hemorrhaging the animal. The controlled hemorrhage model was a modified Wiggers isobaric shock model in which the animal’s mean arterial blood pressure is reduced at a precise rate, under computer control, to a target mean arterial pressure of 40 mmHg, and then held there until experimental intervention, resuscitation, decompensation or death occurred. The experiment was performed using a computer-based data acquisition system, running a program written in LabView (National Instruments, Houston, TX). REG was measured with intracranial electrodes (Plastics One, Roanoke, VA) with 5 mm uninsulated surface in intrahemispherial derivation (left or right side) (see Fig. 1). The composition of the CO2 inhaled gas used for the challenge was 10% CO2 (Calibrating Gas, Nova Biomedical, Waltham, MA; 10% CO2, 0% oxygen, 90% N2) for 1 s. The total number of rats measured by REG during CO2 inhalation was n=11, in 63 trials. Here we present results of two subgroups (A: n = 4, trials = 32, B: n=5, 17 trials). Occlusion of the common carotid arteries was accomplished using aneurism clips or carotid ligatures, while the rat was in the supine position (n=5, 13 trials). Readings from the Doppler flow probes verified the lack of blood flow through the carotid arteries, Fig. 2. During hemorrhage mean blood pressure was 40 mmHg. Measurement of CBF was taken with REG (n=14), laser Doppler flowmetry (Integrating probe, Periflux System 4001, Perimed Sweden), (LDF; n=4), and carotid flow by Doppler ultrasound (T201 Ultrasonic Bloodflow Meter, Transonic Systems, Ithaca, NY), (n=11).
2.4. Pig

A 22 kg pig was anesthetized with ketamine and acepromazine (iv). The pig was instrumented with a LDF probe over the parietal cortex (19 mm AP, 10 mm Lat.). The probe was inserted through a hollow cranial bolt, and contacted bone was thinned to translucency. The local CBF was measured by a LDF monitor.
(VasaMedics, St. Paul, MN) with 3 second averaging. Arterial blood pressure (BP) was also measured via catheterization of the femoral artery. BP was measured using a pressure transducer connected to a pressure amplifier ((23XL, Gould Instrument Systems, Valley View, OH). Baseline mean BP was 62 mmHg; this value was considered as 100 percent. The REG was measured by placing 9 mm diameter stainless steel disc electrodes on the skull over the parietal cortex. The probes were held in place by 0-80 x 3/16” stainless steel screws. A conductive gel was used to decrease resistance between the electrode and the skull. To investigate autoregulation of CBF with varying SAP a series of temporary (5 min) aortic occlusions (n = 4) were performed proximal to the kidneys. SAP, LD and REG were continuously collected before, during, and after each occlusion.

2.5. Challenges

CBF was altered with the following manipulations: CO₂ inhalation [10,11], brain electrical stimulation [12], ligature of the common carotid arteries [13,14], and hemorrhage in rats, furthermore aorta compression [15] in swine.

2.6. Data Acquisition

Unless noted otherwise, the REG amplifier was operated with 46 kHz (KR-Rheo Preamp, Galileo, Italy). An IBM compatible PC performed the data collection with a PC-LPM-16 (National Instruments, Austin, TX), or PCL-718 AD card (Advantech, CA). The A/D sampling rate was 100-500 Hz. For analog physiological data acquisition, proprietary software (Analyze, Chart, Extract, Gral [16], Redirec [17], DataLyser) was used on a PC and the data processed off line. The REG, LDF and carotid flow calculation and comparison was analyzed quantitatively, based on amplitude (minimum - maximum distance of a REG pulse) and integral (area under the pulse curve) measurement of 5 to 10-sec time-windows. Since neither LDF nor REG is an absolute flow measurement, a control segment of recording was chosen from the pretreatment period (baseline), and the changes were expressed as a percentage of baseline. In order to decrease the respiratory interference with REG, the data was digitally filtered (Butterworth band pass, 0.3-60 Hz, 512 point, 60 dB; with a software module integrated into the DataLyser software (Baranyi, WRAIR).

2.6.1. Statistical Analysis

Student t-test, Pearson’s linear correlation, and Spearman’s rank correlation were utilized within the Minitab software for data analysis. Pearson's correlation coefficient indicates the strength of a linear relationship, and Spearman's rank correlation indicates the strength of a monotonic relationship. The P-values indicate how likely it is that the coefficients are equal to zero (i.e. the null hypothesis tested is that each coefficient is equal to zero). P < 0.05 was considered significant.

3.0. RESULTS

3.1. Rat/1

3.1.1. Brain Electrostimulation

Electrical stimulation caused a REG amplitude increase in the ipsilateral (but not the contralateral) hemisphere Fig. 3.
Figure 3. Typical maximal REG amplitude responses to electrical stimulation of the right hemisphere. Stimulation produced a marked increase in REG pulse amplitude of the stimulated hemisphere and a possible decrease in pulse amplitude of the non-stimulated left hemisphere. These responses would not likely be detected by monitoring the total CBF. The time of the stimulation is indicated by downward arrow. Y-axis units are in percentage of baseline. Legend: left (oL), right (-R), and combined left and right (VLR); mean of 3 runs on one rat ([18] with permission).

3.1.2. CO₂ Inhalation

When 5% CO₂ was substituted for the equivalent fraction of N₂ in the inspired gas mixture, there were no significant changes in the REG amplitude, indicating that this treatment was without effect on CBF. The substitution of 20% CO₂ for the equivalent fraction of N₂, however, markedly increased the REG signal amplitude, indicating increased CBF, Fig. 4.

Figure 4. REG amplitude prior to, during, and following 2 min of 100% O₂, 5% CO₂, and 20% CO₂ inhalation. The time of the start and stop of the gas administration is indicated by downward and upward arrows, respectively. Inspiration of 100% oxygen (o) produced a slight decrease in REG amplitude. Inspiration of 5% CO₂ (-) with 30% O₂ did not affect REG amplitude. Breathing 20% CO₂ (△) with 30% O₂ produced a 50% increase in REG amplitude. Y-axis units are in percentage of baseline ([18] with permission).
3.2. Rat/2

During CO₂ inhalation a linear relationship was established between CO₂ concentration and REG peak amplitude (correlation coefficient: 0.88, p = 0.05), and the rise time (anacrotic portion) of the curve (0.88, p = 0.05), Fig. 5.

![Graph of REG Amplitude and Integral during CO₂ inhalation]

**Figure 5.** Increase of REG amplitude (upper) and integral (lower) during inhalation of various CO₂ concentrations. Y-axis values are percentage of baseline. Data are mean ± SD. Statistical significance (compared to baseline) is expressed as: * = p<0.05, and ** = p<0.01 ([18] with permission).

3.3. Rat/3

3.3.1. CO₂ inhalation

During CO₂ inhalation increases in REG and LDF were significant, while carotid flow and systemic arterial pressure decreased. The transient increases in REG pulse amplitude (69 % ± 2.6) and LDF (78.1 %, 4.4) were highly significant (p<0.001), Fig. 6 and Table 1 and 2.
Rheoencephalography (REG) as a Non-Invasive Monitoring Alternative for the Assessment of Brain Blood Flow

Figure 6. REG pulse amplitude increases during CO$_2$ inhalation (subgroup A). Filtered REG: rheoencephalograph after removal of the respiratory subharmonic, Carotid L and R: left and right carotid arterial flow, SAP: systemic arterial pressure, CO$_2$: exhaled carbon dioxide and at the arrow: 10 % inhaled CO$_2$ during 1 s. Time window: 60 s. The rat/file ID was: 157 – 3.

Table 1. REG integral increase during CO$_2$ inhalation (subgroup B).

<table>
<thead>
<tr>
<th>Rat ID</th>
<th>BL</th>
<th>MAX</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>158-1</td>
<td>0.071</td>
<td>0.196</td>
<td>176.06</td>
</tr>
<tr>
<td>158-2</td>
<td>0.097</td>
<td>0.278</td>
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<td>158-3</td>
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<tr>
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<td>0.1</td>
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<tr>
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<td>0.586</td>
<td>641.77</td>
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<tr>
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<td>0.454</td>
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<td>0.508</td>
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<tr>
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<td>620.00</td>
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<td>8-6-4</td>
<td>0.01</td>
<td>0.063</td>
<td>530.00</td>
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<tr>
<td>8-6-5</td>
<td>0.011</td>
<td>0.059</td>
<td>436.36</td>
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</tbody>
</table>
Rheoencephalography (REG) as a Non-Invasive Monitoring Alternative for the Assessment of Brain Blood Flow

Rat ID: identification of a rat/trial; BL: value of 5-second baseline REG integral; MAX: value of 5-second integral during maximal REG amplitude; Increase: value expressed as percentage of BL. The group average increase was 417.83 ± 366.74. Total number of rats was 5; total number of trials was 17. The increase was expressed as percentage of BL; it was significant (p = 0.0048). The Pearson correlation coefficient was 0.79, and the Spearman correlation coefficient was 0.88. The p-value associated with each correlation was less than 0.0001.

<table>
<thead>
<tr>
<th>Rat ID</th>
<th>Number of time points</th>
<th>Pearson's (r)</th>
<th>Spearman's (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/11/02</td>
<td>29</td>
<td>0.63</td>
<td>0.73</td>
</tr>
<tr>
<td>7/18/02</td>
<td>29</td>
<td>0.80</td>
<td>0.74</td>
</tr>
<tr>
<td>7/23/02</td>
<td>35</td>
<td>0.71</td>
<td>0.66</td>
</tr>
<tr>
<td>7/25/02</td>
<td>24</td>
<td>0.63</td>
<td>0.81</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>2.77</td>
<td>2.94</td>
</tr>
<tr>
<td>Mean</td>
<td>29.25</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
<td>SD</td>
<td>4.50</td>
<td>0.08</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 2. Statistical summary of the CO₂ inhalation results in REG/LDF group. The 10 sec time window of the analyzed sample was characterized by 3 or 4 values. Pearson's (r) for all 117 time points = 0.80, Spearman's (r) for all 117 time points = 0.73. All correlations are statistically different from zero with a p-value less than 0.01. Rat ID: identification of a rat.

3.3.2. Carotid Clamping

Figures 7 and 8 show the effect of carotid artery clamping on REG amplitude and integral. During carotid artery clamping, the decrease in REG amplitude and integral were both highly significant (P < 0.0001).

Figure 7. Effect of clamping of common carotid arteries. Following the first clip placement on the left carotid artery, the rheoencephalogram (REG) amplitude (Filtered REG) decreased; the placement of the second clip right carotid artery had no further decrease. During the clamping period there was no pulse amplitude observed in either carotid trace. For better visibility the recording traces were blown up; the real flow calibration values appear on the right side. Amplification of left and right carotid flow differed. A few
minutes after removal of one clip from the right carotid artery, REG amplitude moderately increased, and after removal of the second clip from the left carotid artery, the REG amplitude returned to slightly above the baseline level. Similarly, both carotids showed a slight hyperemic reaction. The baseline systemic arterial pressure (SAP) value was 135/80 mm Hg; during clamping, the minimal value was 80/40 mm Hg. REG was an intrahemispherial (left side) derivation. The time window was 10 min; REG Filter was 3-100 Hz. ECG: Electrocardiogram. The rat/file ID was 5-23-02/11.

**Figure 8. REG integral before and during carotid clamping.** Data are expressed as a percentage of baseline. Mean decrease: 27.53 %, SEM: 0.05, SD: 0.1804.

### 3.3.3. Hemorrhage

During hemorrhage, REG transiently increased (147% ± 44; p=0.037), while cortical flow (measured by laser Doppler) (78 % ± 45; p=0.046) and carotid flow (52 ± 7.5; p=0.005) decreased and correlated with systemic arterial pressure, Fig. 9.

**Figure 9. REG pulse amplitude increase during hemorrhage.**
Arrows indicate start and end of SAP decrease to 40 mmHg. Carotid flow decreased similarly to SAP without showing any sign of CBF autoregulation. REG amplitude transiently increased than decreased, demonstrating CBF autoregulation and indicating its lower limit before 40 mmHg SAP. The EEG amplitude decrease coincides with the disruption in brain activity coincident with the diminished CBF status. EEG: electroencephalogram, Filtered REG: left side, after removal of respiratory subharmonic, Carotid Flow: left side, SAP: systemic arterial pressure, CO₂: exhaled carbon dioxide. Time window: 24 m. The rat/file ID was: 174 –CO3.

3.4 Pig

During aortic occlusion (caused by abdominal compression) the systemic arterial blood pressure increased 59.67 ± 11.92 percent (mean ± SD; p = 0.008), LDF increase was non-significant 10.75 ± 2.21 % (mean ± SD; p = 0.089), and REG decreased 23.75 ± 8.18 % (mean ± SD; p = 0.01), Fig. 10.

**Figure 10. A typical effect of the compression of abdominal aorta in a pig.** During the 5-min compression (start and end indicated by arrows), mean arterial blood pressure (MABP) increased; local CBF measured by LDF (LD CBF) increased; and global CBF measured by REG amplitude (REG), decreased. This global CBF decrease is the demonstration of CBF autoregulation. MABP Y-axis is mmHg, LD CBF; REG values are expressed as a percentage of the baseline (%C) ([18] with permission).

4.0 DISCUSSION

4.1 Challenges

CBF was altered with stimuli known to cause increases in CBF: CO₂ inhalation and brain electrical stimulation. Clamping of the common carotid arteries decreases CBF. The CBF autoregulation was additionally tested by hemorrhage causing blood pressure decrease, resulting brain vasodilatation, and aorta compression causing blood pressure increase resulting brain vasoconstriction. For details see 4.2.1.
4.2. CBF

The brain has ongoing, substantial energy requirements but minimal stores of energy-generating substrates. As a result, it is completely dependent on a continuous, uninterrupted supply of substrate (oxygen, glucose). Although the demand by the brain for energy-generating substrates is substantial (the central nervous system consumes 20% of the oxygen or 170 mmol/100 g per min or 3-5 ml O_2/100 g brain tissue per mm or, approximately, 40-70 ml O_2/min) and 25% of the glucose (31 mmol/100 g per mm) utilized by the resting individual under physiological conditions, this is met more than adequately by the 15% of the resting cardiac output (750 ml/mm) which perfuses the brain (mean global CBF) = 50 ml/100 g brain tissue per mm (range 45-55 ml/100 g per mm) approximately 80% to grey matter and 20% to white matter). Indeed, normally, the supply of oxygen (approximately 150 ml O_2/min) is considerably in excess of requirements (around 40-70 ml O_2/min) such that the brain extracts only 25-30% of that supplied. In addition, the brain can conserve energy and, hence, decrease demand by switching off many of its metabolic processes before its reserves have been compromised when the delivery of substrate reaches 'critical' values. However, the flip side of this argument is that, paradoxically, the brain cannot tolerate significant increases in the volume of the contents of the rigid container in which it is enclosed. Moreover, because the brain’s own store of energy-generating substances (glycogen, glucose, oxygen) is small (so small that, at normal rates of adenosine triphosphate production, the stores of glycogen in the brain would be exhausted in less than 3 min) it is uniquely dependent on a continuing, and adequate, supply of substrate [19].

4.2.1. CBF Autoregulation

Autoregulation in the cerebral circulation may be defined more pragmatically as the mechanism that protects the brain against the dangers of hypoxia at low perfusion pressures and against the risks of brain edema at high arterial pressures. Based on this definition, cerebral autoregulation may be thought of as a homeostatic mechanism that is superimposed over and above the baroreceptor reflexes. The baroreceptors, strategically located at the most proximal locations in the cerebral circulation, provide the first line of defense against acute ranges in arterial pressure. Autoregulation then serves as the next line of defense by helping to maintain constant cerebral capillary pressure, thus assuring a steady supply of essential metabolites and simultaneously protecting the blood-brain barrier. Several hypotheses (myogenic, neurogenic, and metabolic) have been proposed to account for the mechanisms that underlie autoregulation, detailed elsewhere [20].

The anatomical foundation of CBF autoregulation is the arteriole. The arterioles are the last small branches of the arterial system, and they act as control valves through which blood is released into the capillaries. The arteriole has a strong muscular wall that is capable of closing the arteriole completely or of allowing it to be dilated several fold, thus having the capability of vastly altering blood flow to the capillaries in response to the needs of tissue [21,22]. This arteriolar functioning is visualized by functional MRI in brain imaging [23].

REG showed the classical CBF autoregulatory response, indicating its close relationship to arteriolar changes. Early CBF-REG studies did not focus on this topic [24-27].

4.2.2. CBF and CO_2

In man, 5% and 7 % CO_2 inhalation raises CBF by approximately 50 and 100 %, respectively [10]. Cerebral vasodilatory responses to hypercapnia and hypoxia are consistent, reproducible and reversible. Accordingly, changes in systemic gas tensions have been frequently employed as a test of essential cerebrovascular reactivity under normal and pathophysiologic conditions. The mathematical expressions that govern the relationship between CBF and partial pressure of carbon dioxide have been described [11, 28]. In these
experiments we demonstrated that REG detects CBF (and/or volume) increase during CO₂ inhalation, similarly to other quantitative CBF techniques.

4.2.3. CBF and Hemorrhage

The vital functions of the brain, in spite of its well-developed autoregulation, are impaired during prolonged hypovolemic conditions as the CBF autoregulatory reserve is exhausted. Afferent neural input to the brain seems to be elevated during shock. It may be presumed that this leads to increased tissue metabolism and the accumulation of metabolites. The low flow combined with elevated neuronal activity and cellular metabolism produces an imbalance between oxygen delivery and oxygen utilization [29] leading to neuronal damage.

Cerebrovascular responses to hemorrhage reflect the balance between autoregulatory vasodilatation and sympathetic vasoconstriction [30]. During hemorrhage CBF heterogeneity [31] and hypovolemic cerebral hyperemia was observed [32]. In hemorrhagic hypotension the shift of CBF autoregulation is described [20]. In our experiments during hemorrhage only REG showed the classical CBF autoregulation: LDF and carotid flow followed SAP decrease during hemorrhage. It would appear from these responses that the REG responses may best be explained if the REG signal is a vascular volume measurement as opposed to a measure of blood flow. This would explain why the REG and LDF measures may change in apparently opposite directions, such as that encountered in the case of hemorrhagic hypotension.

4.3. REG

The physical basis of the electrical impedance method is based on the fact that blood or cerebrospinal fluid are better conductors than the brain or other 'dry' tissue. The electrical impedance method (measuring blood flow by alternating current) is known in clinical practice, however it is used mostly in cardiology and for measuring peripheral circulation. When used on the head, it is called rheoencephalography, REG [26]. REG is based on monitoring pulse synchronous variations in cranial electrical impedance over time. Various correlations were established between REG and CBF (volume, flow or pressure, detailed by [26]). REG pulse amplitude is quantified most frequently using its derivative [9,24,25] or integral. Both variables detect the applied CBF manipulations. The application of the REG derivative and integral has an advantage using computer data processing. Other potential REG processing methods [33] have resulted in no practical improvement. According to an earlier WRAIR publication, REG can be used for ICP monitoring [6]. Additionally, recent results suggest that noninvasive cerebral impedance measurements do reflect intracranial events, and are able to detect cerebral edema following hypoxia-ischemia [34].

One technical problem with the REG instrumentation used in this study is with regard to the fact that the REG device (except in Rat/1 group) with bipolar derivation is able to measure AC, i.e. pulsatile impedance only. The basic impedance is compensated at the beginning of the measurement in such type of impedance amplifiers, so they are unable to record the DC component as a signal. This prevents detection of changes in the DC portion of the signal, which are related to the overall brain volume. The tetrapolar devices are able to record basic impedance and consequently detect the absolute volume changes, as they are used in clinical practice for venous outflow phlebography or impedance cardiography.

In order to avoid potential interference with extracranial circulation here we used intracerebral REG derivations, only. We plan to study the relationship of intracerebral and surface derivations.

4.4. Monitoring

The primary aim of managing patients with acute brain injury in the intensive care unit is to minimize secondary injury by maintaining cerebral perfusion and oxygenation. The mechanisms of secondary injury are
frequently triggered by secondary insults, which may be subtle and remain undetected by the usual systemic physiological monitoring. Continuous monitoring of the central nervous system in the intensive care unit can serve two functions. Firstly it will help early detection of these secondary cerebral insults so that appropriate interventions can be instituted. Secondly, it can help to monitor therapeutic interventions and provide online feedback [35]. On the battlefield the Doppler CBF test (or other clinically used CBF measurement, such as fMRI, SPECT, PET scan) is not realistic due to size and weight considerations.

From our previous experiment [36] it is known that the information content of REG can be different, depending on the physiological or pathophysiologic range of CBF autoregulation. The REG signal reflects the electrical conductivity within the cranial cavity. Pathophysiologic changes influence the normal conductivity [27]. Our recent data obtained during CO₂ inhalation were collected in the physiological range, consequently, one cannot draw any conclusions regarding what will be shown under pathophysiological conditions such as hemorrhage or elevated ICP. Similarly, there is no comparative data examining the difference between local and global CBF and carotid flow during the above conditions. For noninvasive blood flow monitoring purposes, there is a need to clarify the potentially useful CBF monitoring modality (possibly global flow - REG) and examine the relationship between CBF, SAP, ICP, and EEG. Non-invasive monitoring, including cardiac impedance measurement, revealed low-flow and poor tissue perfusion that was worse in the non-survivors (on high-risk elective surgery patients, [37]).

4.5. Technical Remarks

In clinical practice the CBF autoregulation is measured as a routine test using Doppler ultrasound [38]. Doppler technique, as it is used in today's clinical practice, is not applicable in military environments. The REG CNS monitoring method is more suited for military environments because of its non-invasiveness, good time resolution and potential for miniaturization. Although several CBF-REG correlative studies have shown that it is possible to quantify CBF measured by REG [24-27], the studies reported here suggest that its utility would appear to be more related to its relationship to cerebrovascular reactivity (CBF autoregulation). Therefore, interpretation of the REG signal alone, without knowledge of the perfusion pressure or SAP, is difficult. However, under normotensive conditions, or steady state blood pressure conditions, changes in REG amplitude would be expected to correlate with changes in cerebral blood flow.

The key question then is whether REG measures volume or flow. The definition invoked by the FDA [39] states: Sec. 882.1825. “Rheoencephalograph: (a) Identification. A rheoencephalograph is a device used to estimate a patient's cerebral circulation (blood flow in the brain) by electrical impedance methods with direct electrical connections to the scalp or neck area.” In other words it specifically defines REG as a measure of flow and this is the reason why we used flow in the text. On the basis of previous and recent data [40-42], however, a more practical definition would use volume rather than flow. This statement is supported by human and animal measurements as well [36].

In the potentially applicable REG electronics and software used in the Cerberus system [8], the EKG signal is synthesized from impedance electrodes located on the upper arms and legs. In this case, the electrodes (conductive elastic fabrics sewn into a uniform) are multifunctional sensors; a 2-channel bio-impedance signal can be used to generate a pulse wave, an EKG, a respiratory trace and a pulse wave transmission time, which could potentially provide noninvasive blood pressure information [43].

5.0. CONCLUSIONS

Animal studies confirmed that REG qualitatively reflects changes in CBF during physiological perturbations known to alter CBF. The results are consistent with REG being a reflection of cerebrovascular responsiveness as opposed to the local CBF changes shown with LDF or carotid flow. Because of the CBF heterogeneity, only global CBF would be suitable for monitoring purposes.
REG monitoring may be a useful, non-invasive, continuous monitoring method of assessing global CBF and cerebrovascular reactivity or capacitance for combat casualty care of victims suffering from hemorrhage and may have applicability for maintaining cerebral blood flow in non-combat medicine as well. Further work will focus on correlating brain function: EEG and CBF/REG assessments [44-48] during hemorrhage and resuscitation, using various resuscitation fluids. Further studies are needed to test the CBF autoregulation with graded increases in intracranial pressure, a common consequence of traumatic brain injury [49-54].

6.0. ACKNOWLEDGEMENTS


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7.0. REFERENCES


Advanced Capabilities for Combat Medics

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ABSTRACT
The US Army Institute of Surgical Research (USAISR) has the lead for directing the Research Program Area for Advanced Triage Capabilities for Combat Medics in the Medical Research and Materiel Command (MRMC) research program in Combat Casualty Care. The objective of this Program Area is to develop and demonstrate a semi-automated trauma triage capability that provides critical casualty information remotely to the battlefield medic. When this goal is met, the medic will possess a greater decision making capability for prioritizing casualty care based on continuous information about live/dead status and severity and progression of the injury and which injuries require life saving interventions (LSI). Since hemorrhagic shock remains a leading cause of death on the battlefield, the research activities in the task area for advanced capabilities for remote triage are designed to focus on the identification and care of wounded soldiers with severe hemorrhage. This Research Program Area is founded on the fundamental premise that meeting this goal will save lives on the battlefield. The purpose of this paper is to describe the Program Area plan for conducting research that will lead to advanced diagnosis and triage capabilities for combat medics by developing an algorithm for clinical assessment of wounded soldiers.

1.0 BACKGROUND
Acute hemorrhage and subsequent circulatory collapse (shock) account for about 50% of the deaths on the battlefield and the forward operating table, a statistic that has remained relatively unchanged since World War I [1]. In addition, hemorrhage is the primary cause of death in about 30% of the injured soldiers who die from wounds. Likewise, uncontrolled hemorrhage accounts for up to 82% of the early operative deaths from trauma in the civilian arena. However, the mortality rate in combat casualties drops to between 2% and 4% if the trauma patient is stabilized through surgery [1,2]. It is therefore clear that the ability to significantly reduce the mortality and morbidity associated with hemorrhagic shock on the battlefield will depend heavily on improving the capability of first level responders (i.e., medics) to apply early LSI.

Hemorrhagic shock is typically identified by the degree of hypotension and nonspecific signs and subjective symptoms such as cold clammy skin, pallor, weak thready pulse, unstable vital signs, and diminished mentation that develop as a result of blood loss [3]. There are several physiological measures that predict circulatory shock and subsequent poor outcome. Of these, the battlefield medic is currently limited to the

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1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

assessment of mental status, pulse character and pulse rate measurements for diagnosis of wounded soldiers. In special operation forces (SOF), it is rare that standard blood pressure and pulse oximetry may be available. Although significant reductions in blood pressure (BP) and oxygen carrying capacity of the blood (PaO2), and elevations in heart rate (HR) can be measured in the civilian arena and are routinely used to assess progression toward circulatory collapse [3], compensatory mechanisms that buffer against changes in BP and PaO2 make these measurements poor predictors for *early* assessment of shock [4]. This notion was supported by preliminary data from our laboratory demonstrating that arterial O2 saturation and BP changed very little (Figure 1, Panels A & B) during a significant (as much as 2 liters) gradual reduction in central blood volume in humans that caused dramatic reductions in stroke volume and cardiac output (Figure 1, Panels D & E). In addition, elevated HR (Figure 1, Panel C) in a wounded soldier may be impossible to accurately interpret since “fight-or-flight” responses are a natural consequence of battle. Therefore, a definition based on the absence or presence of hypotension as measured by changes in mental status, pulse character, and/or HR can be misleading since it does not represent the underlying problem of or the solution to hemorrhagic shock.

![Figure 1: Hemodynamic and arterial O2 saturation responses to graded reductions in central blood volume. Lower body negative pressure (LBNP) was used to transiently redistribute blood away from the heart, thereby creating central hypovolemia in intact humans. Values are mean ± 1 standard error.](image)

Monitoring for the onset of circulatory shock in the civilian trauma patient has also focused on the clinical “gold standard” assessments of BP, arterial O2 saturation, or simple pulse palpation (rate and character). Unfortunately, these measurements in a wounded soldier on the austere battlefield environment probably will be even more imprecise, subjective, and inconsistent. More important, the appearance of hypotension and other signs and symptoms of shock do not mark the beginning of circulatory compromise, but rather represent the beginning of decomposition, i.e., a point in time when it may be too late to introduce effective LSI. This notion was reaffirmed from a preliminary study performed from the USAISR animal database. With the use of specific data mining and multivariate regression analysis, it was demonstrated that the mean arterial
pressure was a predictor of cardiovascular collapse but that the predictive power gave too little response time to be useful to a combat medic performing triage and resuscitation (J. Ward, unpublished data).

Since the appearance of hypotension and reduced PaO2 reflect late events in the process of hemorrhagic shock, it is critical to identify physiological signals that will be altered during the earliest time period of blood volume loss. A common denominator in development of shock is the inadequate oxygen delivery (DO2) to the tissue that is associated with reductions in blood flow (cardiac output) or metabolic alterations (reduced pH or base excess). Increased cardiac output and DO2 correlate well with survival while failure to stabilize cardiac output and DO2 is highly correlated with death [4-7]. Therefore, an algorithm that includes some indicator of oxygen delivery (e.g., stroke volume, cardiac output) may represent a better tool for the early prediction of circulatory shock than measurements currently in use for this purpose.

2.0 DESCRIBING TRIAGE CHALLENGES TO THE MEDIC

In the Future Force Warrior battlefield environment, soldiers will be widely dispersed, being separated by time and distance from medic and/or buddy aid. Independence of objective force operations places a requirement on far-forward treatment, stabilization, and maintenance of wounded soldiers with technology that provides moment-to-moment real-time monitoring of sensitive predictors for the onset of hemorrhagic shock and requirements for LSI. Large gains can be potentially achieved far-forward by simplifying and improving initial assessment of injury, appropriate intervention, and priorities for early evacuation.

Optimal management designed to prevent the onset of circulatory shock requires a recognition and integration of multiple complex physiological responses with varying time courses. The resulting challenge is that shock is easily diagnosed in late stages when therapy is ineffective while early diagnosis is difficult in the absence of measurements that represent physiological responses associated with the underlying mechanisms of shock. The solution to this dilemma is to identify the physiologic signal(s) that provides the best early indicators of blood volume loss and impending circulatory collapse. Such requirements for complicated information and decision-making can overwhelm a physician well-trained in critical care medicine much less a first level responder (medic). Human capabilities for making the most appropriate and timely decisions for application of an effective LSI can be augmented by new technologies that provide automated data mining, trending and decision support software. Previous efforts in this direction have centered upon developing hardware for casualty assessment. However, before developing hardware, an effective database of multiple physiologic signals associated with BP regulation must be constructed and evaluated to identify the best early predictors of impending cardiovascular collapse. Development of the optimal hardware (medical monitoring devices) will depend on validating an algorithm that identifies primary predictive physiological signals. This algorithm should provide the medic with essential, continuous information about the severity and clinical progression of the casualty and remote triage decision-making for prioritization of care and evacuation. Therefore, the result of the research in this Program Area should significantly enhance the decision making capability of the medic and subsequently improve casualty outcome on the battlefield.

The physiology of the injured soldier suffering from severe hemorrhage is very dynamic, yet pre-evacuation care and monitoring have traditionally been based on isolated measurements even under the best circumstances. The absence of frequent physiological measurements obtained from the wounded soldier forces battlefield medics to make rapid decisions about priority of care and application of interventions based upon isolated “snapshot” data points (e.g., BP, pulse character, respiratory rate, mental status) without the benefit of observing trends and the dynamic nature of the evolving trauma physiology. Thus, the current
process of combat casualty care can be greatly improved by providing appropriate continuous physiological observations. In support of this concept, data from civilian trauma literature shows that temporal patterns of physiological responses during hemorrhage are more informative than single measurements because they provide a history of physiologic events that lead to shock [3]. It is therefore clear that identification of the best early predictors of hemorrhagic shock can only be accomplished by simultaneous and continuous measurement of various physiological signals (responses) associated with BP regulation that have been proven to be accurate predictors of cardiovascular collapse.

3.0 RESEARCH ACTIVITIES IN ADVANCED CAPABILITIES FOR COMBAT MEDICS

Realizing the limits of current triage capabilities (i.e., absence of critical continuous measurements such as BP, cardiac output and DO₂), an algorithm that facilitates remote triage on the battlefield is one of the primary objectives of current Combat Casualty Care research. Such a reliably predictive algorithm does not currently exist but is critically needed. The focus of the research conducted in the Program Area on Advanced Capabilities for Combat Medics will therefore be placed on the development of extensive databases that include multiple physiological measures obtained from pre-hospital and in-hospital trauma patients and models of central hypovolemia in both humans and animals. By defining the outcome variable as time required to reach cardiovascular instability, the resulting database should provide the foundation for development of an algorithm capable of predicting the need for a LSI. In order to develop an accurate triage algorithm that predicts progression toward the onset of cardiovascular collapse, it will be necessary to collect numerous physiologic signals simultaneously in models of central hypovolemia (i.e., hemorrhage). The USAISR has three general models of cardiovascular collapse (hemorrhagic shock) in animals and humans from which extensive databases are being developed.

3.1 Animal Hemorrhage Models

A number of large and small animal models of controlled and uncontrolled hemorrhage have been developed and used at USAISR to investigate the physiology of hemorrhage. Our investigators have designed unique methodologies to understand more fully the relationship between blood loss and BP in uncontrolled vs controlled hemorrhage. A major advantage of the use of animal models is the ability to make invasive physiologic measurements that otherwise cannot be easily attained in human subjects. In addition, the introduction of injury with hemorrhage using animals provides a unique capability to investigate the contribution of tissue trauma to the prediction of survivability. With the use of animals that are extensively instrumented with both invasive and non-invasive physiological monitoring sensors, numerous and various hemodynamic and metabolic variables can be measured before, during and after recovery from moderate to severe hemorrhage. Most unique to the animal hemorrhage model is the ability to identify survival time as a clinical outcome for predicting the need for an LSI.

3.2 Trauma Patient Models

Although animal models offer numerous advantages to the study of mechanisms underlying hemorrhagic shock, the cardiovascular system and its regulatory components in animals do not necessarily function with responses identical to those observed in humans. Perhaps more importantly, most animal experiments require the use of anesthesia that can significantly alter autonomic reflex responses and eliminate the ability to assess significant human characteristics of mentation. It is therefore prudent that animal research be supplemented with a human clinical research arm that extends the applicability of experimental results.
The USAISR is continuing to develop a unique database that has been initiated in collaboration with Texas A&M University and trauma center at the University of Texas Health Science Center at Houston, with plans to extend collaborations with trauma centers at Massachusetts General Hospital, and Dartmouth Medical College, and in San Antonio. This part of the research plan will provide the unique opportunity to collect non-invasive, near continuous physiologic measurements on large numbers of injured patients during the initial phases of care by the first responder. This database will be a large storage reservoir for physiologic data, clinical interventions, and outcome results of pre-hospital trauma patients. The ultimate aim of the research on trauma patients will be to collect data from the point of injury through the ambulance phase, into the emergency center and ultimately through the operating room and intensive care unit. These data will be critical to the development of an accurate algorithm for remote triage on the battlefield because civilian trauma patients represent an operational human model for military casualties [8]. The USAISR is also in the unique position to expand the trauma patient database to include burn and trauma patients undergoing ‘elective’ hemorrhage during surgery and recovery in the USAISR Trauma Division.

3.3 Human Hypovolemic Model
Data from trauma patients will be instrumental in providing etiology and military relevance for the understanding of hemorrhagic shock in humans. However, the absence of physiological measurements at the time of injury until the moment that a medic arrives limits the ability to identify early predictors of clinical outcome. In an effort to extend the research capabilities to investigate mechanisms and early predictors of cardiovascular collapse during hemorrhage in humans, USAISR investigators have introduced a model designed to safely and noninvasively induce central hypovolemia in conscious human subjects, thereby eliciting hypotension and subsequent cardiovascular instability like that resulting from hemorrhage [9,10]. This technology is based on the ability to redistribute blood away from the central circulation to the lower extremities with the use of lower body negative pressure (LBNP, Fig. 2).

![Subject placed in the LBNP device.](image)
Application of LBNP provides the capability of inducing cardiovascular and autonomic responses similar to those resulting from hemorrhage [11,12]. For example, Figure 3 shows a comparison of relationships between average reduction in central venous pressure and increased sympathetic nerve activity during hemorrhage (450 ml) and −10 mmHg LBNP in 9 subjects [11]. It is clear that the relationships virtually mirror each other, suggesting that a blood loss to the central circulation of approximately one-half liter can be induced by each 10 to 15 mmHg LBNP. This assumption is based on well-established linear relationships between increasing LBNP and decreasing cardiac filling (central venous) pressure and stroke volume [13-15].

Figure 3: Comparison of relationships between central venous pressure (CVP) and sympathetic nerve activity (SNA) during -10 mmHg LBNP (open circles, broken lines) and 450 ml hemorrhage (closed circles, solid lines) in 9 human subjects. Circles and lines represent mean ± SE values. † P < 0.05 compared with baseline. Data modified from Rea et al. [11].

Figure 4 demonstrates the similarity in typical elevations in HR and reductions in cardiac output from a group of ten pigs during actual hemorrhage [16] compared with responses from a group of ten healthy test subjects to a graded LBNP protocol [13]. In the absence of effective resuscitative measures, compensatory reflex mechanisms fail to adequately compensate as levels of LBNP gradually increase, and a subsequent collapse of blood pressure regulation ensues [9,10] with frank onset of severe hypotension (i.e., shock) and bradycardia similar to that reported in humans during severe hemorrhage [17-19]. The comparison of hemorrhage and LBNP data presented in Figures 3 and 4 demonstrate the similarity and potential for duplicating hemodynamic responses to actual hemorrhage with application of LBNP. Therefore, application of LBNP will provide a noninvasive method of investigating continuous and simultaneous cardiovascular responses and underlying mechanisms associated with hemorrhage in human subjects under conditions of controlled, experimentally-induced hypovolemic hypotension.
Figure 4: Comparison of elevated heart rate and reduced cardiac output during 65 min of hemorrhage in ten pigs (left panels) and graded LBNP in ten human subjects (right panels). Circles and lines represent mean ± SE values. Data modified from Hannon [16] and Convertino [13].

4.0 DATA COLLECTION AND ANALYSIS

Figure 5 represents a diagrammatic summary of the cascade of activities that reflect the research plan for the Program Area for Advance Capabilities for Combat Medics in the Combat Casualty Care Research Program. First, integration of data from existing and ongoing animal and human experiments into a comprehensive trauma informatics database will significantly contribute to the development of new algorithms for automated remote triage of combat casualties. The primary strategy for development of a valid algorithm for early prediction of circulatory collapse will be focused on the simultaneous and continuous measurement of numerous physiological variables from human and animal subjects that comprise our hemorrhage trauma models. Measurements will be targeted to physiological responses associated with BP regulation. As specific physiologic measures are identified from our laboratory experimental models, new non-invasive devices that provide such measures can be added to current trauma patient monitoring for validation of algorithms. The resulting analog physiological signals will be collected and stored in a database that will allow for retrospective waveform analysis and data mining. Our analysis will focus on the hypothesis that it is possible to estimate mean time to circulatory collapse and the requirement for LSI from hemodynamic signals recorded in the USAISR database of hemorrhagic shock protocols. In addition to analysis of numerical responses, information content related to the morphology of specific physiological signals, their changes and variability over time, and their interrelationships will be analyzed.
5.0 ALGORITHM DEVELOPMENT

This research will apply the techniques of data mining to estimate mean time to circulatory collapse from hemodynamic, respiratory and metabolic signs recorded in the USAISR database of hemorrhagic shock protocols. Data mining is exploration and analysis, by automatic or semiautomatic means, of large quantities of data in order to discover meaningful patterns and rules. Selected files containing data on human and animal models of hemorrhage and subsequent cardiovascular collapse will be analyzed. The independent variables will be the hemodynamic, neural, and metabolic parameters and the dependent variable will be time to circulatory collapse. Circulatory collapse will be defined by a precipitous fall in mean arterial pressure that becomes too low to maintain an adequate supply of cerebral blood flow or mental function. Independent variables will include (but are not limited to) HR, BP, stroke volume, cardiac output, peripheral blood flow, cerebral artery blood flow, arterial O₂ saturation, blood gases and metabolites, autonomic nervous activities, sublingual CO₂, ECG waveform, peripheral pulse waveform, vascular volume status, mentation, and clinical outcome. Significant early predictor(s) of failure (cardiovascular collapse in human subjects and mortality in animals) will be identified using multiple logistic regression statistics. Methods will include correlation coefficients, multiple logistic regression, Reed-Muench analysis, Kaplan-Meier survival analysis, cluster analysis, and discriminate analysis.
6.0 DEVICE DEVELOPMENT AND TRANSITION FOR BATTLEFIELD USE

The resulting algorithm for early prediction of cardiovascular collapse that evolves from the Research Program Area will inherently identify the specificity and frequency of physiologic measures required in order to provide the most effective casualty care and remote triage. The resulting physiologic measures extracted from the remote triage algorithm can then be used to direct decisions regarding development or identification of medical monitoring devices or technologies that can be worn by the soldier. For example, a small computer that includes the algorithm could be part of the monitoring system worn by the soldier. A personal digital assistant (PDA) device carried by the battlefield medic would provide a simple visual code (green, yellow, red) of the soldier’s medical status that can be transmitted via global-positioning satellites. If integrated into the proposed Warrior Physiological Status Monitor (WPSM), the Advanced Medical Monitoring Device could reduce combat mortalities by enabling combat medics to: 1) commence triage within moments after a soldier is wounded; 2) receive more accurate information of wound severity and progression to shock; and, 3) optimize available treatment and evacuation. Finally, since the killed-in-action rate for battlefield medics has been as high as double that of infantryman, the advanced diagnosis system could be instrumental in reducing battlefield mortality of medics by providing early identification of dead soldiers.

7.0 SUMMARY

With the use of experimental protocols that utilize human and animal trauma models of central hypovolemia leading to cardiovascular collapse, the goal of the Program Area for Advanced Diagnosis for Combat Medics is to provide an automated capability for remote trauma triage on the battlefield. This goal will be accomplished through an extensive series of research projects designed to provide continuous data acquisition of numerous non-invasive physiological signals (measurements) associated with BP regulation. With the use of data mining, neural networks, multivariate logistic regression analysis, and decision tree analysis, an algorithm predictive of clinical outcome and the need for life saving procedures can be generated from the resulting physiologic database. Not only will these data be utilized to direct the future of combat casualty care monitoring systems, but they will facilitate rational decisions concerning bandwidth requirements and, perhaps more importantly, what physiologic measurements are truly important predictors of outcome and LSI. This research approach will provide the first data-driven answer for remote triage for the Future Force Warrior. The resulting algorithm will enhance the diagnosis and acute treatment of hemorrhagic wounds. It is in this manner that our approach will produce an enhanced human capability for advanced diagnosis and remote triage of combat casualties and will ultimately be able to improve survivability of combat casualties.

8.0 REFERENCES


Skin Allograft Acceptance with Anti-CD154 in a Non-Human Primate Model

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ABSTRACT

Background: Traditional burn wound management involves application of topical antimicrobial agents with frequent dressing changes for superficial partial thickness burns and early excision and grafting of deep partial or full thickness burns. Combat related injuries tend to affect large areas and treatment has been often limited to split-thickness skin autografts. This approach has been limited by its requirement for an autologous donor graft sites. Allogenic skin transplantation would alleviate many of these problems, but has remained impractical due to graft rejection. To date, no clinically available intervention has been reported to induce long-term primary skin graft survival. However, murine models utilizing either costimulation blockade or costimulatory blockade together with donor specific transfusions (DST) have met with limited success. Previously we have used a humanized monoclonal antibody (hu5C8) directed against CD154 to induce long-term graft survival in a primate renal allograft model without the use of DST. In this work, we have applied these regimens with the addition of DST to a primary skin transplant model.

Methods: Ten animals were transplanted with full thickness skin allografts mismatched at both class I and class II major histocompatibility loci. Of these, two were given no treatment, five were treated with anti-CD154 mAb alone, and three received anti-CD154 mAb combined with whole blood DST. All recipients also received autografts.

Results: Treatment with both hu5C8 alone and hu5C8 plus DST greatly prolonged allograft survival with mean survival time in the monotherapy group of > 226 days and mean survival time in the DST group of > 263 days.

Conclusion: These results suggest that costimulation blockade with anti-cd154 can attenuate acute rejection of skin allografts and may lead to long-term survival of these grafts without chronic immunosuppression. Further, we noted that dst provided no survival advantage over anti-cd154 monotherapy alone. The ability to improve and simplify burn wound management has significant impact upon the ability to return our operational forces to full duty. Further studies are ongoing to obtain durable graft survival and thereby transition these immunomodulatory strategies into clinical practice.

1.0 INTRODUCTION

Skin allografts remain an extremely rigid test of any intervention designed to prevent allograft rejection. Despite considerable success in solid organ transplantation, skin allografts have not been successfully applied clinically, and experimental success has generally required ablative therapy. Current treatment strategies for burns and similar injuries have been limited to skin autografts to provide wound coverage but only limited functional restoration. Significant burns account for roughly ten percent of all combat related casualties and a considerable portion of accidents to active duty service members. Injuries that require coverage for large wound surface areas are limited to split thickness skin grafts (STSG) which provide less than optimal cosmetic and functional outcomes when compared with full thickness grafts or myocutaneous flaps. The infectious risks present in such large wounds as in burn or multi-system trauma patients may preclude standard global immunosuppression strategies. Therefore, a treatment regimen facilitating long-term acceptance of full thickness skin allografts would be advantageous allowing patients to have improved function and cosmesis. Furthermore, immunoregulatory strategies applied to skin allografts would likely be successful for other peripheral injuries (digits, limbs, facial parts) providing for additional improved outcomes for injured patients.

In vivo, T lymphocytes require at least two signals for complete activation. These signals are comprised of an antigen specific signal received through the T cell receptor and a second costimulatory signal, delivered predominantly through CD28 (1) as well as CD154 (2) and their cognate receptors CD80/86 and CD40 on the APC. These signals allow CD4 T cells to produce sufficient IL-2 and other cytokines to allow autocrine-driven clonal expansion. In the absence of a co-signal anergy is often induced.

Long-term skin graft acceptance of allogeneic skin has been previously achieved in a mouse model when costimulation blockers were utilized (3,4). Some have used strategies involving both costimulation blockade and donor specific transfusion (DST) (5,6). Additionally, we and others have demonstrated that blockade of CD154 and CD40 interactions with a humanized anti-CD154 monoclonal antibody (hu5C8) has been shown in both small animal and primate models to induce graft survival in solid organ transplants (7,8). To date, the use of hu5C8 in human clinical trials has been put on hold as a result of reported thrombotic events in autoimmunity patients.

In rodent studies, donor specific transfusions clearly are superior to therapies that use costimulation blockers alone. Several groups have shown prolonged survival of islet, cardiac, and skin allografts with the combination of DST, anti-CD154, and thymectomy (5,9). Although the mechanism by which DST influences the immune response remains unclear, one hypothesis is that it induces activation of alloresponsive CD8+ cells which in turn lead to deletion or anergy of these cells after costimulation blockade(9). We have previously reported the effect of a hu5C8-based treatment regimen previously shown to be effective in renal transplantation to a non-human primate model with and without the use of DSTs and now update those results. (10).

2.0 METHODS

2.1 Skin Allografts. Animals were divided into three groups based on their treatment after transplant (see below). All received both mismatched allografts and autografts. Anesthesia was administered by IM ketamine (10 mg/kg) and xylazine (2 mg/kg) and animals redosed as necessary. Full thickness two by two centimeter abdominal skin grafts were procured under aseptic conditions and sharply defatted in normal saline. Wounds were closed with 4-0 nylon sutures and the animal then placed in the prone position. Skin
ellipses were discarded from the lower back at the level of the iliac crest and 2 mg hu5C8 was injected into the graft base (allografts only). Both allografts and autografts were then secured with simple interrupted 4-0 nylon sutures with hair follicles reversed for later identification. Dressings were placed, changed on postoperative day 3 and sutures removed on postoperative day 10. All experiments were approved by the Naval Medical Research Center IACUC under animal use protocol 97-17.

2.2 Treatment. Group one received no therapy to prevent rejection and served as allograft controls (n=2), group two received anti-CD154 monoclonal antibody alone (hu5C8, Biogen) (n=5), and group three received hu5C8 with DST (n=3). All experimental animals were treated with 2 mg of hu5C8 injected into graft beds prior to transplantation. Anti-CD154 (hu5C8) was given at 20mg/kg IV for the non-DST group. Recipients of DSTs were transfused with 20mg/kg hu5C8, 20 ml donor whole blood, and 100 units heparin given at the time of transplantation. All experimental animals where then placed on a six month dosing regimen of hu5C8 with intravenous injections of 20 mg/kg on days 1, 3, 10, 18, 28, and then monthly. Repeat challenge and third party grafts from MLR high responder donors were placed at day 300 in one matched DST and non-DST treated pair.

2.3 Postoperative monitoring. Skin biopsies were performed on postoperative day 120 from both treatment groups and at the onset of rejection (day 50) in one animal. Dermal elasticity was determined by palpation. Both pre and post-operatively blood was drawn for MLR analysis. Unidirectional MLRs were performed pre-operatively as previously described (7). Briefly, peripheral blood mononuclear cells (PMBC) were isolated by ficoll gradient. The PMBC were washed with PBS then resuspended in RPMI-1640 cell culture media (Life Technologies, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum, 2 mM L-glutamine, and penicillin/streptomycin. Gamma irradiated (50Gy) donor cells (105/well) served as stimulators and were co-cultured at a ratio of 1:1 with responder cells. The cultures were incubated at 37°C for 5 days then pulsed with 10 Ci of H3 thymidine and incubated for an additional 24 h before harvest.

2.4 Histology. Skin biopsies were performed under ketamine and xylazine chemical restraint. Tissues were obtained using full thickness biopsy and closed with interrupted sutures. Tissue samples were embedded in O.C.T. compound (Tissue Tek/Sakura Finetek, Torrance, CA), snap-frozen in a dry ice/ isopentane bath, and stored at -70°C. Six micrometer frozen sections were stained with hematoxylin and eosin using standard histological techniques.

3.0 RESULTS

3.1 Allograft controls. Animals (n=2) that received neither hu5C8 alone nor a combination of hu5C8 and DST demonstrated graft rejection at five and seven days respectively as evidenced by necrosis (table1). Histology obtained during rejection demonstrated epidermal necrosis and lymphocytic infiltration consistent with acute cellular rejection.

3.2 Anti-CD154 monotherapy. Five animals were transplanted using treatment with hu5C8 alone. Mean survival time in this group was > 262 days. Approximately seven days after transplantation, all grafts demonstrated erythema, which was not present in control autografts. These subsequently resolved without further treatment. All grafts demonstrated normal dermal elasticity and the presence of hair growth. Four animals rejected their allografts at 314, 310, 140, and 50 days. The animal that rejected its graft at day 314 underwent repeat challenge and third party grafting at day 300, and promptly rejected these grafts at day 14.
and 8, respectively. Rejection was evidenced by graft erythema, edema, ulceration and eventually loss. Histology at the onset of graft loss was consistent with acute rejection with a large lymphocytic infiltrate in the dermis and pykotic keratinocytes consistent with apoptosis (figure 2b). One animal remained without signs of rejection at >500 days post transplant and which point the animal was euthanized (table 1). Histology obtained by skin biopsy in the long surviving grafts on postoperative day 110 showed no epidermal necrosis and mild inflammation when compared with controls.

3.3 Anti-CD154 and DST. Three animals were transplanted using hu5C8 in combination with a single whole blood transfusion. Mean survival time for this group is currently > 247 days. One of these animals remained well with healthy allografts until >500 days following transplantation at which point the animal was euthanized (table 1). Interestingly, one died on postoperative day two from a febrile illness clinically consistent with sepsis. Blood cultures and a detailed necropsy did not reveal a cause of death. The third animal rejected its allograft at 239 days following transplantation (table 1). This animal underwent repeat challenge and third party grafting at day 300 and rejected both of these grafts at one week. As with the previous group, approximately seven days after transplantation all grafts demonstrated the onset of erythema, which subsequently resolved without treatment. All grafts exhibited normal dermal elasticity and the presence of hair growth. Histology from day 110 biopsy showed viable epithelium with low numbers of mixed inflammatory cells, similar to results with anti-CD154 mAb monotherapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival</th>
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<tr>
<td>Allograft Control</td>
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<tr>
<td>hu5C8 w/ DST</td>
<td>2**, 239*, &gt;500</td>
<td>*Reject, **POD2 death, others AW</td>
</tr>
<tr>
<td>hu5C8 w/o DST</td>
<td>314*, 310*, 140*, 50*, &gt;500</td>
<td>*REJECT, OTHERS AW</td>
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Table 1: Summary of all animals

4.0 DISCUSSION

These results demonstrate that costimulation blockade with anti-CD154 mAb greatly delays and, in some cases, prevents rejection of skin allografts. Skin grafts transplanted with this regimen demonstrate hair growth, no wound contracture and normal function. As the mean survival time of DST treated animals in this small series is significantly less than non-DST treated animals, DST seems to provide no survival advantage. However, additional studies will have to be conducted before this conclusion is verified. The ability of a pair of these animals to reject both repeat challenge and third party grafts placed two months after cessation of antibody therapy confirms that tolerance is not achieved in this model. Previously reported results in a murine model showed long term graft acceptance with the addition of thymectomy to treatment regimens combining DST and a two week course of anti-CD154 mAb (50 days vs. greater than 100 with thymectomy) (6). Our results involve the use of a six-month treatment protocol similar to that reported in our renal transplant models (7). The effects of anti-CD154 mAb therapy may be mediated through both the innate immune system by interrupting dendritic/T cell interactions and the acquired immune system directly. These interactions
possibly lead to either clonal deletion or apoptosis of alloreactive T cells which may lead to long lasting antigen specific anergy. (2)

The cause of the postoperative day two death in the DST treated animal remains unclear. Although the clinical picture resembled sepsis both necropsy and laboratory evaluation did not reveal a source. Other possibilities include systemic inflammatory response syndrome (SIRS) from cytokine release as seen with IL-2 treatment for renal cell carcinoma or possibly a thromboembolic phenomenon related to antibody crosslinking with platelets. However, we have in vitro data suggesting that neither cytokine release nor platelet crosslinking occurs as a result of anti-CD154 mAb treatment. The etiology of this death remains speculative, future use of DST will need to be closely monitored.

The erythema seen in all transplanted animals during the first week may represent the same process of early lymphocytic infiltration reported in renal allografts treated with anti-CD154 mAb, which we were unable to be associated with any functional abnormalities (7). The episodes of delayed rejection were preceded by a picture similar to the erythema seen initially and biopsy at that time showed a large diffuse infiltrate, comparable to that seen in rejecting renal allografts (7). This may represent reemergence of alloreactive CD8+ T cells which then participate in graft rejection.

REFERENCES


Evaluating an Ultrasound Algorithm for Patients with Blunt Abdominal Trauma

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ABSTRACT
The ideal assessment of the blunt abdominal trauma (BAT) patient would be sensitive, specific, economical, fast, and without complications. By combining ultrasound (US) with computed tomography (CT) and diagnostic peritoneal lavage (DPL), an effective algorithm can be derived to accurately evaluate BAT. We prospectively evaluated a series of patients with suspected blunt abdominal trauma using an algorithm with ultrasound as the initial screening modality to determine if it would be more sensitive, specific, and cost-effective than each diagnostic modality alone. 

Methods: One hundred ninety-one patients over the age of 18 with suspected BAT were evaluated according to an established algorithm. Ultrasound was the initial diagnostic technique.

Results: In this study, US had a sensitivity of 84.0%, a specificity of 98.7, and an accuracy of 96.7%. CT had a sensitivity of 100%, a specificity of 100% and an accuracy of 100%. The algorithm had a sensitivity of 100%, a specificity of 98.7% and an accuracy of 99.0%. Of the patients entered in the study, 9% received a laparotomy. The non-therapeutic laparotomy rate was 11%.

Conclusions: An algorithm for blunt abdominal trauma that utilizes ultrasound as the initial diagnostic technique can accurately assess intrabdominal hemorrhage in unstable patients and act as a screening tool for computed tomography in stable patients.

1.0 INTRODUCTION
Fifty-seven million people become trauma victims every year in the United States, making it a significant cause of morbidity and mortality. Trauma is the fourth leading cause of death in the United States overall and the most common cause of death in people under the age of 44. Approximately 20% of injured trauma victims will have residual long-term disabilities. Injuries related to blunt abdominal trauma (BAT) may follow direct impact, acceleration-deceleration, and shearing forces to the human body. These occur most commonly from automobile collisions, followed by falls and assaults. In motor vehicle collisions and falls, the process of rapid deceleration creates a situation where the body’s internal organs continue moving after the musculoskeletal system has been stopped.

The evaluation of patients with suspected blunt abdominal trauma presents a diagnostic challenge. Patients often do not present with the classic signs of intraabdominal injury such as abdominal pain or unexplained hypotension. In addition, trauma centers often have a high proportion of patients with an altered mental status due to chemical substances or head injury, making the clinical history and physical exam less reliable.
In the past, all patients with suspected intraabdominal injury were surgically explored. With this strategy, negative laparotomies, in which no injury is found, resulted in morbidity 18% of the time and non-therapeutic laparotomies, in which there is no surgical intervention for intraabdominal injuries, resulted in a morbidity rate of 45%. Exploratory laparotomy following BAT still remains mandatory for certain patients (peritonitis, free air, progressive abdominal distention with unexplained hypotension), but in the absence of these more overt clinical findings several modalities are typically utilized to increase diagnostic accuracy.

In 1965 Root introduced diagnostic peritoneal lavage (DPL). DPL is a relatively fast procedure, being performed in 3-26 minutes. It can be performed during trauma resuscitations with a sensitivity of 87-99% and a specificity of 97-98%. The procedure requires peritoneal puncture and is associated with only a 1% incidence of significant complications but it is unable to adequately detect retroperitoneal and diaphragmatic injury. DPL is relatively contraindicated in pregnant patients, those with multiple previous operations, pelvic fractures, or clotting disorders.

Computed tomography is increasingly utilized in BAT patients since it is a non-invasive test and has a sensitivity of up to 97% and a specificity 98-99%. CT permits localization and grading of injuries, but cannot be safely performed on unstable or uncooperative patients due to the requirement for transport to the radiology suite and time constraints. CT can take 60 – 90 minutes to obtain when transport and setup times are included with a cost that is considerably higher than other diagnostic modalities. CT also carries the risk of complications from intravenous contrast injection and allergy.

The use of ultrasound in the United States has become increasingly popular among trauma surgeons for providing a quick, reliable assessment of the thorax and abdomen in BAT. Numerous publications, including one with 1000 patients examined prospectively, have described its speed, portability, and low-cost in this patient population. It has also been shown repeatedly that housestaff can interpret US results as reliably as radiology personnel.

The ideal assessment of the BAT patient would be sensitive, specific, economical, fast, and without complications. By combining ultrasound with computed tomography (CT) and diagnostic peritoneal lavage (DPL), a potentially cost effective algorithm can be derived to accurately evaluate blunt abdominal trauma patients. We prospectively evaluated a series of patients with suspected blunt abdominal trauma using an algorithm with ultrasound as the initial screening modality to determine if it would be more sensitive, specific, and cost-effective than each diagnostic modality alone.

2.0 PATIENTS AND METHODS

This study was conducted at the Ryder Trauma Center, Jackson Memorial Hospital. This is the only Level I trauma facility for all of Dade County, Florida. The study was approved by the Institutional Review Board at Jackson Memorial Hospital. Five hundred eighty-five patients were seen over a two-month period ending in August 1996. One hundred ninety-one patients over the age of 18 with suspected BAT, in which physical examination alone would not be sufficient to completely assess intraabdominal injuries, were evaluated according to an established algorithm (figure 1). The remaining 398 patients were evaluated with physical examination alone and were not included in the study.

Ultrasound was the initial diagnostic technique in all 191 patients. If the US examination was negative, the patients were initially observed. If the patient showed no further BAT related symptoms (hypotension, abdominal pain, hematuria), the assessment for BAT was terminated. Hemodynamically stable patients that
developed hematuria, transient hypotension, or abdominal pain during observation received a CT scan. If a patient under observation developed persistent hypotension, a repeat ultrasound was performed. If the initial US was positive, stable patients received a CT examination to further evaluate and grade the injury, while unstable patients were taken for exploratory laparotomy. If the US was deemed indeterminate and the patient was stable, a CT scan was performed. In unstable patients with an indeterminate US, DPL was performed. At any time during the BAT work-up, a patient could be taken to the operating room if they developed peritoneal signs on physical examination.

The following definitions were used for the algorithm: 1) true positive- an injury was detected and confirmed by another diagnostic technique or laparotomy, 2) true negative- if no injury was detected by the techniques and none developed later, 3) false positive- a non-therapeutic exploratory laparotomy, 4) false negative- passage through the algorithm without a detected injury and a subsequent laparotomy revealing intraabdominal injury. At anytime during the diagnostic workup, the surgeon could interrupt the algorithm for laparotomy based on clinical judgment. This was considered as a positive diagnosis of intraabdominal injury according for the algorithm and resulted in a false positive for the algorithm if the laparotomy was negative.

US was performed by dedicated technologists using an Accuson 128x P/10 (Mountain View, California) with a 3.5-MHz sector or curvilinear transducer to identify free fluid in six areas as previously described: the pericardium, the subphrenic space bilaterally, splenic tip, subhepatic space, and the pelvis. The liver and spleen were also evaluated for parenchymal injury. An attending radiologist or senior radiology resident interpreted all scans. The US or CT was deemed positive if free fluid or a parenchymal injury was clearly identified. A negative US or CT was recorded when no visceral injury and no free intraperitoneal fluid was found. The US or CT examination was deemed indeterminate if there was questionable free fluid, questionable visceral injury, or if the examination was technically limited. A DPL was ruled positive if there was one or more of the following 1) initial aspirate yielded > 5cc of blood, 2) RBC > 100,000 RBCs/mm³ or, 3) WBC > 500 WBC/mm³.
3.0 RESULTS

A total of 191 patients with BAT were evaluated with the algorithm over a two-month period (figure 2). One hundred and fifty-nine of these patients had a negative US, 23 patients had a positive US, and 9 patients had an indeterminate result.

All 159 patients with a negative US were admitted for observation. One hundred and forty-three of these patients received no further tests. One patient developed persistent hypotension and received a second US with a negative result. Further evaluation revealed major thoracic injuries as the cause of hypotension, with no intraabdominal injury. Fifteen patients with a negative US had persistent abdominal pain or transient hypotension. One patient was taken to the operating room after abdominal pain progressed to peritoneal signs (table 1). Operative findings revealed a jejunal injury. Fourteen negative US patients received a CT scan. Eleven had a negative CT. Three patients had a positive CT scan with missed injuries that were managed non-operatively. In this group, one patient had a subcapsular hematoma of the liver with no free fluid present, the second patient had a splenic laceration with a small amount of perisplenic fluid, and the third patient had a liver laceration with a small amount of perihepatic fluid. The overall negative predictive value of US was 97% (155 TN / 155 TN + 4FN).
Twenty-three of the total 191 patients had a positive US result. Twelve initially had stable vital signs, and received a follow up CT. In 2 patients, the US was deemed positive and the CT negative. One patient had stable vital signs but persistent abdominal pain. The patient subsequently developed peritoneal signs and was taken for exploratory laparotomy in which no free fluid or organ injury was identified. The abdominal pain was determined to be referred pain from lower rib fractures and the patient was counted as a false positive in the algorithm. The other patient with a positive US and negative CT was observed with no subsequent evidence of intraabdominal injury. Ten patients had positive CT scans after positive US. Four of these patients became hypotensive later and were taken to the operating room and found to have intraabdominal injuries, while the other six were managed non-operatively.

Eleven patients had a positive US and unstable vital signs, and were taken directly to the operating room. All eleven of these patients were found to have intraabdominal injuries. The overall positive predictive value of US was 91% (21 TP / 21 TP + 2 FP).

Nine patients had an indeterminate or questionably positive US result. All of these patients were hemodynamically stable and were evaluated with CT. Three out of the 9 indeterminate patients had a negative CT with no subsequent injury while three patients had a positive CT with non-operative injuries. These injuries were a grade 3 liver laceration, a grade 2 liver laceration, and a large subcapsular hematoma. Three patients had an indeterminate CT. Two were observed and one had an exploratory laparotomy that showed a hematoma on the sub-diaphragmatic aorta.

In this study, US had a sensitivity of 84.0% (21 TP/ 21 TP + 4 FN), a specificity of 98.7% (155 TN/ 155 TN + 2 FP), and an accuracy of 96.7% (155 TN + 21 TP / 21 TP +155 TN + 2 FP + 4 FN). CT had a sensitivity of 100% (13 TP/ 13 TP + 0 FN), a specificity of 100% (17 TN/ 17 TN + 0 FP) and an accuracy of 100% (17 TN + 13 TP / 17 TN +13 TP + 0 FN + 0 FP). The algorithm had a sensitivity of 100% (28 TP/ 28 TP + 0 FN), a specificity of 98.7% (161 TN/ 161 TN + 2 FP) and an accuracy of 99.0% (161 TN + 28 TP / 28 TP +161 TN + 0 FN + 2 FP). Of the patients entered in the study, 9% received a laparotomy. The non-therapeutic laparotomy rate was 11%.
Figure 2: Algorithm Results

Key: US = ultrasound, CT = computed tomography, Lap = laparotomy, (+) = positive, (-) = negative, (?) = indeterminant, AP= abdominal pain, H = hematuria, BH = brief hypotension
* patient taken for laparotomy based on clinical findings
### Table 1: Patients undergoing laparotomy

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>US results</th>
<th>CT</th>
<th>CT result</th>
<th>OR</th>
<th>OR result (procedure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td></td>
<td>X</td>
<td></td>
<td>+</td>
<td>Small bowel injury (resection)</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>Fluid in Morrison’s pouch; liver laceration</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Bladder injury (repair); liver laceration (hepatorraphy)</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>Abundant free fluid in abdomen</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic hilar injury (splenectomy); Liver subcapsular hematoma</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>Abundant free fluid in abdomen; liver laceration</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic laceration (splenorrhaphy); Liver laceration (hepatorraphy)</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>Pelvic, perisplenic, subhepatic fluid</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Liver laceration (hepatorraphy)</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>Fluid in abdomen and pelvis</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Liver laceration (hepatorraphy); vena cava injury (repair)</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>Perihepatic, perisplenic, pelvic fluid</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Mesenteric injury (ligation of bleeding vessel)</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>Abundant free fluid in abdomen</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic injury (splenectomy)</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>Free fluid in abdomen; splenic laceration</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic injury (splenectomy); Liver injury (hepatorraphy)</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>Abundant free fluid in abdomen</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic injury (splenectomy)</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>Perisplenic fluid</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Splenic injury (splenectomy)</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>Fluid in Morrison’s pouch, splenic parenchymal injury</td>
<td>X</td>
<td></td>
<td>+</td>
<td>Liver laceration (hepatorraphy); splenic laceration (splenorrhaphy)</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>Liver laceration, fluid in Morrison’s pouch</td>
<td>+</td>
<td>Liver laceration; paracolic fluid</td>
<td>+</td>
<td>Liver laceration (hepatorraphy)</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>Fluid in Morrison’s pouch</td>
<td>+</td>
<td>Subhepatic fluid</td>
<td>+</td>
<td>Splenic subcapsular hematoma (splenorrhaphy)</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>Perisplenic fluid</td>
<td>+</td>
<td>Free fluid in abdomen, pelvis; fractured spleen</td>
<td>+</td>
<td>Splenic injury (splenectomy)</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>Perisplenic, subhepatic fluid; splenic parenchymal injury</td>
<td>+</td>
<td>Abundant free fluid; shattered spleen</td>
<td>+</td>
<td>Splenic injury (splenectomy)</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>Fluid in Morrison’s pouch; perisplenic fluid</td>
<td>-</td>
<td>Rib fractures</td>
<td>-</td>
<td>No intraabdominal injury</td>
</tr>
<tr>
<td>18</td>
<td>?</td>
<td>Liver laceration</td>
<td>?</td>
<td>Diaphragm rupture; aortic aneurysm</td>
<td>-</td>
<td>Hematoma superficial to aorta</td>
</tr>
</tbody>
</table>

(+) = positive test, (-) = negative test, (?) = indeterminate test (X) = not performed
4.0 DISCUSSION

The principle finding of this study is that an algorithm utilizing ultrasound is 100% sensitive and 99% specific for detecting injury in blunt abdominal trauma. It is also cost-effective and results in a low rate of non-therapeutic laparotomy.

These results compare favorably with other algorithms used to assess BAT patients. Mele et al. examined an algorithm with CT and DPL with no missed injuries and no non-therapeutic laparotomies. Bode et al. utilized a protocol with US and CT in 1,671 BAT patients with no non-therapeutic laparotomies, but two missed injuries were discharged home. Shih et al. used a diagnostic algorithm with CT and US and had two non-therapeutic laparotomies and no missed injuries. Boulanger et al. compared an algorithm similar to the one described here to an algorithm utilizing only CT and DPL and showed no difference in diagnostic accuracy between the two groups. The algorithm utilizing US however, had a significantly faster work-up time and lower cost.

We have found that US is best used in two situations. In stable patients, US potentially serves as an extension of the physical examination and therefore may act as a triage for CT. Because CT is highly sensitive, many would argue that it be used as the primary diagnostic technique for the assessment of BAT patients. This is problematic because CT is expensive with a hospital cost of around $500 per scan. CT is also time consuming and necessitates approximately 60-90 minutes of direct patient care if transport and setup time are considered. Time can be an important factor in centers where demand for CT exceeds the available scanning time. By using CT based on our algorithm, 145 scans were avoided during the study period. Over two-months, this resulted in a cost savings of approximately $72,000.

Ultrasound missed 4 injuries in stable patients during the study. Three were non-operative solid organ injuries discovered by CT. Solid clots can have similar echogenicity to surrounding tissue, and this contributes significantly to the greater sensitivity of CT over ultrasound. The remaining patient had a small bowel injury, and this injury was detected with physical examination. The ability of US to detect isolated hollow viscous injury is poor, since often no free fluid is present initially. Similarly, CT and DPL have a relatively low rate of detection in this type of injury and physical examination findings continue to be an important indicator for bowel injury in the BAT patient. Ultrasound findings were falsely positive in two stable patients, but they did not undergo laparotomy because they were hemodynamically stable and their follow-up CT was negative.

The second indication for US in BAT is for unstable patients. Ultrasound can quickly assess if the injury is intraabdominal or if other body compartments such as the thorax, pelvis, or head should be the focus of diagnostic work-up. These patients cannot safely undergo CT scanning because access to the patient by healthcare providers for continued resuscitation becomes problematic. These types of patients previously received a DPL during their initial trauma evaluation and resuscitation.

All positive ultrasounds in unstable patients were confirmed by laparotomy. There was one negative US in an unstable patient who was shown to have a thoracic injury. This highlights the utility of US in the unstable patient. Before proceeding directly to the laparotomy, the surgical team needs to be aware of whether an intraabdominal injury is present.

DPL was not used in the study, although it was included in the algorithm. No patients met criteria for DPL, but the option should be maintained for unstable patients with indeterminate US. CT proved to be 100%
Evaluating an Ultrasound Algorithm for Patients with Blunt Abdominal Trauma

sensitive and specific when used after US screening. There was one negative laparotomy in the study. This patient had all tests negative but was taken to the OR on the basis of physical examination findings and was shown to have no intraabdominal injuries.

A potential weakness of this study is that patients that were not considered to have an injury were observed only until discharge. Although no patient that was discharged presented with subsequent injuries to our center or clinic, it is possible that they may have sought care elsewhere. In addition, the study was conducted prospectively but without randomization of patients to a comparative clinical pathway or a control group. This needs to be addressed in future studies.

This study demonstrates that US offers the clinician a number of advantages in the assessment of BAT patients. All BAT patients, including those that are hemodynamically unstable and those with an altered mental status can be quickly scanned during the resuscitation to give the physician an immediate indicator if intraabdominal hemorrhage is present. Those with a negative US and no other symptoms or abnormal findings can be safely discharged after an observation period. Those with positive US results can be taken immediately for laparotomy if they are hemodynamically unstable. The CT scan can then be reserved for stable patients with a positive US in order to better characterize the injury or for symptomatic patients with a negative US. This is financially and clinically sound because it greatly reduces the number of CT scans while identifying injuries US is more likely to miss such as confined solid organ hematomas and bowel injuries.

We conclude that an algorithm for blunt abdominal trauma that utilizes ultrasound for unstable patients and as a screening tool for CT in stable patients is highly economical and is an accurate method for detecting intraabdominal injury.

5.0 REFERENCES

Evaluating an Ultrasound Algorithm for Patients with Blunt Abdominal Trauma


Six-Hours-Rule – A Dogma for Military Surgery?

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Today, the six-hours-rule is a delicate item for military logistics and it is a great challenge for medical services to provide an adequate treatment during the first hours after wounding.

Up to now “Six-hour-rule has never been validated…” (R. Coupland, 1989)

DEFINITION

Six-hour-rule (NATO-ACE-Directive Number 85-8):

A principle of support given by the medical service. Surgical treatment should take place as soon as possible, but at last six hours after wounding. This principle directs the location of the first line surgical unit, which can provide life-saving and limb-saving surgical procedures. The unit must be reachable within 4 hours after wounding.

HISTORY

The rule is the result of traditional surgical experience. Early wound debridement and open wound treatment led to postprimary, secondary healing without infection. Sepsis and death were mostly caused by un-done, late or wrong wound treatment. The rule respects the intolerance of ischemia of traumatised tissue, especially of the skeletal muscle. In addition, the six-hours-rule considers the physiological pathway of contaminated wounds. Germs penetrate healthy tissue with 1mm per hour. Many studies show that the spreading of infection can be reduced by early infusions of potent antibiotics. In this case the virulence of most the common germs will be three to five times less.

TREATMENT STANDARDS IN CIVILIAN CASES OF POLYTRAUMA

Investigations in civilian polytrauma cases show death in 50 % during trauma, 30 % within 2 hours and 20 % caused by multi-organ-failure after 10 to 21 days. Mean rescue times of 20 to 40 minutes are usual.

Reduction of rescue time improves the chances to survive.

CONSEQUENCE

Depending on priority (P) of the injury:

P 1 –severe:
life-threatening haemorrhage, occlusion of airways
- 6-hours-rule not useful

Six-Hours-Rule – A Dogma for Military Surgery?

P 2 - less severe-
fractures of long bones (bullet, shell), severe damage of soft tissue, thoracic/abdominal trauma without
life-threat
- 6-hours-rule is restrictedly useful
P 3 -moderate-
Wounding of soft tissue, not-penetrating wounds
- 6-hours-rule is crucial, with a prolongation of the time factor by the additional use of antibiotics
P 4 -lethal-
Deleting, complex injury pattern
- 6-hours-rule is senseless

SITUATION OF THE GERMAN MEDICAL SERVICE

Adequate and best medical treatment of the victims is the political decision: immediately - professional -
complete.

“.. In case of wounding all German soldiers must be provided with medical procedures which must be
comparable with a result provided at home...”(GE surgeon general,1993)

By consequence, treatment according the six-hour-rule is mostly not appropriate - polytraumatised
soldiers should reach clinical treatment within ”the golden hour”.

The logistical challenge is to guarantee a modern, up-to-date medical care in field conditions.

LITERATURE

[1] Bunn F., I. Kwan et al., Effectiveness of Pre-Hospital Trauma Care, Cochrane Injuries Group, 2001,http://www.cochrane-injuries.lshtm.ac.uk


Unfallchirurg 2002 ,105: 974 – 985


ILLUSTRATIONS

Penetrating abdominal trauma – Open surgical treatment

Penetrating lung trauma

Mine Injury

Penetrating extremity trauma
Six-Hours-Rule – A Dogma for Military Surgery?

External fixation – Mine-trauma

Wounded transported to the Kabul Field Hospital

CT-scan - Kabul Field Hospital
ICU - Kabul Field Hospital

Air transport
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**Treatment standards in civilian cases of polytrauma:**

Investigations in civilian polytrauma cases show death in 50 % during trauma, 30 % within 2 hours and 20 % caused by multi-organ-failure after 10 to 21 days. Mean rescue times of 20 to 40 minutes are usual. Reduction of rescue time improves the chances to survive.

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  - Wounding of soft tissue, not-penetrating wounds
  - 6-hours-rule is crucial, with a prolongation of the time factor by the additional use of antibiotics

- **P 4 – lethal:**
  - Deleting, complex injury pattern
  - 6-hours-rule is senseless

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By consequence, treatment according the six-hour-rule is mostly not appropriate - polytraumatised soldiers should reach clinical treatment within "the golden hour". The logistical challenge is to secure a modern, up-to-date medical care in field conditions.

**Literature**

1. Bunn F., I. Kwan et al., Effectiveness of Pre-Hospital Trauma Care, Cochrane Injuries Group, 2001, http://www.cochrane-injuries.lshtm.ac.uk
Sequestration of Blood Plasma Iron as a Marker of Systemic Response to the Blast Lung Injury

Assessment with Electron Paramagnetic Resonance (EPR) Spectroscopy

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SUMMARY

Impact of blast shock waves (SW) with the body wall produces blast lung injuries characterized by bilateral traumatic hemorrhages. Such injuries often have no external signs, are difficult to diagnose, and therefore, are frequently underestimated. Predictive assessment of acute respiratory distress syndrome outcome in SW-related accidents should be based on experimental data from appropriate animal models. Blood plasma transferrin is a major carrier of blood iron essential for proliferative “emergency” response of hematopoietic and immune systems as well as injured tissue in major trauma. Iron-transferrin complexes ([Fe3+]TRF) can be quantitatively analyzed in blood and tissue samples with low-temperature EPR techniques. We hypothesized that use of EPR techniques in combination with assays for pro-inflammatory cytokines and granulocytes in the peripheral blood and BAL would reveal a pattern of systemic sequestration of [Fe3+]TRF that could be useful for development of biomarkers of the systemic inflammatory response to lung injury. With this goal we (i) analyzed time-dependent dynamics of [Fe3+]TRF in the peripheral blood of rats after impacts of SW generated in a laboratory shock-tube and (ii) assayed the fluctuation of granulocyte (PMN) counts and expression of CD11b adhesion molecules on the surface of PMNs during the first 24 h after SW-induced injury. Sham-treated

animals were used as control. Exposure to led to a significant decrease in the amount of blood \([Fe^{3+}]\)TRF that correlated with the extent of lung injury and developed gradually during the first 24 h. Thus, sequestration of \([Fe^{3+}]\)TRF occurred as early as 3 h post-exposure. At that time, the steady state concentration of \([Fe^{3+}]\)TRF in blood samples decreased from 19.7±0.6 µM in controls to 7.5±1.3 µM in exposed animals. The levels of \([Fe^{3+}]\)TRF remained decreased throughout the entire study period. PMN counts increased 5-fold and 3.5-fold over controls respectively, at 3 and 6 h postexposure. These effects were accompanied by an increase in expression of CD11b on the surface membrane of PMNs. Extensive release of cytokines IL-1, IL-6, MCP-1, and MIP-2 was observed in BAL fluid and blood plasma during 24 h postexposure. We conclude that EPR monitoring of blood \([Fe^{3+}]\)TRF can be a useful approach for assessment of systemic pro-inflammatory alterations due to SW-induced lung injury.

1.0 INTRODUCTION

Detonation of explosives generates blast shock waves (SW) which are usually characterized by of (i) amplitude of blast overpressure, (ii) frequency of sound, and (iii) kinetic energy of striking force, and are considered to be major factors that produce the blast-injured casualty [1 - 4]. A pattern of striking injuries ranges from displacement and rupture of bone and organs to amputations of limbs and can be assessed by the existing biomechanical models for high-velocity, low-mass impacts [1, 5, 6]. The effects produced by SW-generated sound and SW-generated overpressure are more complex and remain beyond the framework of predictive biomechanical models. These damaging effects often have no external signs, can be painless, difficult to diagnose and prediction of outcomes, are frequently underestimated, and therefore, need to be assessed at cellular and molecular levels [7-9].

Interaction of SW with the body wall or body armor produces two types of energy waves, high frequency \{0.5 to 1.5 kiloHertz (kHz)\} low-amplitude stress waves, and long duration \{(2 – 3 msec)\} low frequency (below 0.5 kHz) share waves; both types of energy waves can be transmitted directly though tissues [3, 4, 10]. Stress waves move faster than the velocity of sound in tissue. Like ultrasound, they deposit energy wherever they are reflected or change their frequency, resulting in biomembrane fragmentations, edema, and hemorrhage [3, 4]. Therefore, lung, which contains many air/fluid interfaces where density changes abruptly, is particularly susceptible to stress wave injury [3, 4].

Share waves produce gross thoracic deformation in association with compression, distortion and stretching of alveolar walls. In this case, internal hemorrhage and pulmonary contusion are due to hyperbaric effects when stretching of alveolar capillaries overcomes their natural elasticity and barotrauma-like injury takes place [3, 4, 11]. Thus, it has been suggested that the criterion for injury due to blast-induced waves of high frequency is overpressure impulse, while overpressure per se is the determining factor if the wave frequency is low [12].

Multiple patho-physiological observations in animal models and clinics suggest that the major primary features of the SW-induced lung blunt trauma are pulmonary contusion, hemorrhagic lesions, edema, and circulatory depression [4, 13, 14]. The extravasated blood may be responsible for initiating a cascade of systemic reactions that involve expression and release of various vasoactive and pro-inflammatory humoral factors and vascular components. These presumably can activate inflammatory leukocytes for migration from the peripheral blood into the injured areas, where release of proteolytic enzymes and oxygen-derived free radicals aggravate parenchymal injury. Indeed, it has been documented recently that blast-induced blunt trauma is often complicated by secondary (indirect) acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), apparently due to infiltration of neutrophils (PMNs) [15].

Among the essential biochemical changes related to the inflammatory response to trauma is the increased turnover of serum concentrations of transferrin-chelated iron \((Fe^{3+})TRF\), which represents the main pool of blood plasma iron [16 – 18]. The levels of plasma iron \((Fe^{3+})\) fall as a result of iron redistribution from plasma to tissues [16]. The trauma-induced iron sequestration (hypoferremia) has been observed in the
pathogenesis of trauma of different etiology and has been documented in blast lung injuries of different severity [19, 20].

The objective of the present research was to evaluate [Fe\textsuperscript{3+}]TRF status in the peripheral blood of rats during an inflammatory response following exposure to medium amplitude \{($\sim$90 or $\sim$120 kiloPascals (kPa))\} low-frequency 260±5 Hertz (Hz) SW, as a biomarker of severity of lung injury.

Each experiment included a pathology assessment using an injury scoring system developed for blunt trauma injuries to derive a severity score for lung damage [21]. Inflammatory response was estimated from immunoassays for pro-inflammatory cytokines and assessments of alteration in inflammatory leukocytes in the peripheral blood and injured lung. Amounts of [Fe\textsuperscript{3+}]TRF in whole blood and blood plasma samples were measured by quantitative electron paramagnetic resonance (EPR) spectroscopy. The observed alterations in the amounts of blood [Fe\textsuperscript{3+}]TRF were correlated with estimated injury score (IS) ratios in each animal. The gathered data suggested that stress-induced tissue sequestration of [Fe\textsuperscript{3+}]TRF from the peripheral blood can be considered as a potential biomarker of the systemic inflammatory alterations due to blast lung trauma.

2.0 MATERIALS AND METHODS

2.1 Treatment of Animals

CVF Sprague-Dawley rats, weight 280-320 g, were purchased from Charles Rivers Laboratories Inc., Wilmington, MA. The rats were acclimatized for 7 days during which they were maintained in a unidirectional filtered-air room with 12/12 h light/dark cycle and allowed food and water \textit{ad libitum}. Following acclimatization, rats were randomly assigned to the “SW exposure” and “control” groups. Then, all animals were anesthetized with ketamine + xylazine (60 mg + 5 mg per kg of body weight, IP), and animals from the “exposure” group were subjected to lung trauma by impact of SW. The compressed air-driven shock tube generated a single SW with main harmonic frequency at 260±5 Hz and peak overpressure at 90±5 kPa or 122 ± 8 kPa (at peak overpressure, see Fig. 1). The pressure-time history of SW was recorded at each exposure. This study focused on the effects at different time periods and used a sequential approach to post-exposure events. Phase I of the study design called for analysis of pro-inflammatory alterations and alterations in [Fe\textsuperscript{3+}]TRF at 1, 3, 6, 12, and 24 h following exposure to SW with main harmonic frequency at 260±5 Hz and peak overpressure at 122 ± 8 kPa. Previous experiments demonstrated that at this level of overpressure produced the most consistent lung injury [22]. Accidents of lethal outcome (mortality rate was ~25%) usually developed during the first 15 min of post-exposure and were excluded from further analyses. Thus, the 32 rats that survived were subjected to blood and lung tissue sampling at 1, 3, 6, 12, and 24 h following exposure. Eighteen control animals underwent all procedures (i.e. anesthesia, suspension, time delays, etc.), except exposure.

Phase II was implemented in order to assess alterations in the amounts of TRF-[Fe3+] in the peripheral blood at 24 h following exposure to SW of either 90±5 kPa (7 animals) or 122±8 kPa (7 animals) to determine overpressure-dependent responses of the above parameter. Five sham-treated animals underwent all procedures (i.e. anesthesia, suspension, time delays, etc.), except exposure to SW.

Blood was drawn by cardiac puncture. Each individual animal was assigned only one sampling procedure. Blood samples for EPR, flow cytometry, and ELISA analyses were collected separately in 1 cc and 3 cc syringes. Gross lesions were recorded and photographed. To avoid possible re-distribution of the infiltrated cells, the histology assessment was conducted in non-perfused lung.
Animal handling and treatments were conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals and experiments involving animals adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council. The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Figure 1. A graph illustrating the pressure-time history of a real SW with arrowed peak overpressure. Positive and negative phases of SW are arrowed. Peak overpressure has been defined as maximum pressure at the positive phase of SW.
2.2 EPR Analysis

Assessment of blood [Fe^{3+}]TRF was conducted with low-temperature EPR spectroscopy as described previously [20]. Briefly, 0.6 ml of blood was collected in 1 cc syringes free from latex and silicone oil (Henke-Sass, Wolf; Gmbh, Germany) to make blood samples of constant volume and geometrical profile. The drawn blood samples were frozen immediately in liquid nitrogen. The frozen samples were kept at -196 °C until EPR analysis was completed. Concentrations of blood [Fe^{3+}]TRF were calculated using standard solutions of apo-transferrin loaded with known amounts of [Fe^{3+}]. To prepare standard solutions of iron ([Fe^{3+}]) complexes with apo-TRF, i.e. [Fe^{3+}]TRF, a 0.1 mM solution of commercial human apo-TRF (M.W. ~80 kDa) (Sigma-Aldrich Chemical Co., St. Louis, MO) in Hanks’ balanced salt solution (HBSS) (Life Technologies, Gaithersburg, MD) was titrated with 1.0 mM complex of [Fe^{3+}] with nitrilotriacetic acid ([Fe^{3+}]NTA) in 0.5 M sodium bicarbonate buffered solution, pH 7.2, for 10 min. [Fe^{3+}]NTA was prepared by incubation of nitrilotriacetic acid (N,N-bis[carboxymethyl]glycine) trisodium salt (Sigma-Aldrich Chemical Co., St. Louis, MO) with ferric chloride (Fisher Scientific, Fair Lawn, NJ) in 0.5 M sodium bicarbonate (Fisher Scientific, Fair Lawn, NJ) buffered solution, pH 7.2, for 10 min at room temperature. The frozen samples of [Fe^{3+}]TRF solutions were kept at -196 °C during the EPR experiments. The characteristic profile of the recorded low temperature EPR spectra of [Fe^{3+}]TRF standard solutions were compared to the spectrum of 0.1 mM solution of holo-transferrin (Sigma-Aldrich Chemical Co., St. Louis, MO) in Hanks’ Balanced Salt Solution (pH 7.4). The recorded EPR spectra were integrated using the WINEPR program package (Bruker, Co). The second integral values were used to calculate concentrations of [Fe^{3+}]TRF in blood samples.

2.3 Flow Cytometry

For flow cytometry analysis of CD11b in PMNs, blood was drawn into syringes that contained 50 USP U heparin/ml (SoloPark Laboratories, Franklin Park, IL, 60131). The samples on a given day from the exposed and control animals were processed in parallel. The 1cc syringes that contained heparinized blood samples were kept on ice at 0°C until the cells were labeled with monoclonal antibodies. Cell labeling for flow cytometry was conducted as follows: aliquots (100 µl) of the heparinized blood were transferred into sterile polypolyene tubes and labeled either with 5 µg/ml of mouse anti-rat CD11b-biotin antibody, (IgA), (BD PharMingen, San Diego, CA) or 5 µg/ml of mouse IgA isotype-biotin antibody, (BD PharMingen, San Diego, CA) for 20 min at room temperature. Then, 2 ml of 1x BD Facs Lyse Solution (BD Bioscience, San Diego, CA) were added to each sample to lyse the erythocytes. After 10 min, the leukocytes were centrifuged at 2,000 x g for 5 min, washed twice with Tyrode’s buffer, resuspended in 0.1 ml of Tyrode’s buffer, and incubated with 20 µl of streptavidin-PE (17 µg/ml) (BD PharMingen, San Diego, CA) for 20 min at room temperature. Then, 500 µl of Tyrode’s buffer were added and the cells were assessed by flow cytometry. Unlabeled control cells were treated as above, except, they were not labeled with CD11b-biotin antibody nor incubated with streptavidin-PE.

A Becton-Dickinson FACSort flow cytometer and CELLQuest Pro software (Becton Dickinson) were used for acquisition and analysis of the data. PMNs were identified by their side- and forward light scatter coupled with fluorescence in the FL2 channel due to CD11b-specific fluorescence labeling with antibody conjugated to phycoerythrin (PE). The CD11b expression on PMNs was evaluated by creating FL2 (CD11b) histograms and gating on FL2 positive cells in the FL2 vs. SSC dot plot. Positive CD11b specific immunofluorescence of PMNs was distinguished from the isotype immunofluorescence (negative control for nonspecific immunoadherence) in all samples. CD11b expression was proportional to the mean fluorescence intensity (MFI) in the FL2 channel and is presented in relative fluorescence units. The PMN numbers in blood samples are presented as PMN counts per 10000 events.
2.4 Analysis of MIP-2 in Blood Plasma Samples

For enzyme-linked immunosorbent assay (ELISA) analysis of rat MIP-2, 3 ml of blood were drawn into a solution of ethylenediaminetetracetic acid disodium salt (EDTA) (1mg/ml in 0.1 M PBS pH 7.4). Cells were removed by centrifugation (2,000 x g for 5 min). The blood plasma samples that exhibited signs of hemolysis were excluded from further analysis. Blood plasma was aliquoted into 1.5 ml polypropylene vials and stored at –80 °C until analyzed. Concentrations of blood plasma MIP-2 were quantified with an immunoassay kit from BioSource International Inc. (Camarillo, CA) according to the manufacturer’s protocol.

2.5 Cytokine Analysis in BAL Fluid

BAL fluid samples were obtained though the trachea after incision in the neck following euthanasia. The lungs were lavaged with 3 ml volume of Dulbecco’s Phosphate Buffered Saline (pH 7.4) though a cannula inserted into the trachea. Cell pellets were pooled from the lavages and centrifuged at 1,200 g for 10 min. The supernatants were collected and frozen immediately in liquid nitrogen, and stored at –80º C until analyzed for IL-1b, IL-6, INF-g, and MCP-1 cytokines using LINCOplex Rat Cytokine/chemokine Kit and the Luminex® 100 IS System (LINCO Research, Inc., St. Charles, MO, www.lincoresearch.com).

2.6 Evaluation of Blunt Trauma Injury

Injury score (IS) for blast-induced pulmonary trauma in rats was assessed using an injury scoring system developed recently for air blast-induced blunt trauma [21]. Briefly, the scoring system utilized a packet of scoring sheets to aid in assessment of lesions [21]. Each sheet was designed to provide a quantitative assessment of the severity of the lesions in lung as defined by the equation:
\[ IS = (E+G+ST)(SD) \]

where E, G, ST, and SD were defined as:
E, extent of injury in terms of lung lobes, (0-5 range); G, injury grade, which included the extent of surface area of the lesions, (0-4 range); ST, severity type element that classifies the type of the worst-case lesions (i.e. petechiae, punctures, ruptures), (0-5 range); SD, severity depth element which indicates the depth of disruption of the worst-case lesion, (1-4 range). The Severity of Injury Index (SII) was then calculated as:

\[ IS/Maximum \ Possible \ IS \text{ for rat lung, i.e.} \ “56” \text{. Thus, the assigned lung SII was 0.0 for “negative injury level,” 0.03 though 0.04 for “trace injury level,” 0.05 though 0.21 for “slight injury level,” 0.22 though 0.36 for “moderate injury level” and 0.37 though 0.64 for “extensive injury level.” At the selected experimental conditions the estimated SII levels ranged from 0.22 though 0.36 and, therefore, were characterized as “moderate injury levels” [21].

2.7 Lung Tissue Preparation for Histology and Immunofluorescence Microscopy

Lung tissue samples were collected at necropsy, fixed in 4% buffered paraformaldehyde (pH 7.4), embedded and frozen in O.C.T. compound, and subjected to cryosectioning. The obtained specimens (10 µm sections) were stained with hematoxylin and eosin for histological examinations or processed for immunofluorescence analysis with light, or fluorescence confocal microscopy respectively.
2.8 Immunofluorescence Techniques and Image Analysis

The obtained lung specimens (see above) were processed for the immunofluorescence imaging as described previously (23). Briefly, the tissue sections were washed with phosphate buffered saline (PBS), incubated in PBS containing 2% paraformaldehyde and 0.1 % Triton X-100 for 20 min, washed three times with PBS, then once with PBS containing 0.5% BSA and 0.15% glycine (buffer A). Any non-specific binding was blocked by incubating the samples with donkey normal serum (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, www.scbt.com) diluted 1:20 in buffer A. The primary antibody against (I) VE-cadherin (goat polyclonal IgG from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, www.scbt.com) and (II) MPO (rabbit polyclonal IgG from Calbiochem, San Diego, CA, www.calbiochem.com) were used in 1:250 dilution in buffer A. This was followed by three additional washes with buffer A and incubation with secondary fluorochrome-conjugated antibody, and with Hoechst 33342 (Molecular Probes, Inc., Eugene OR, www.probes.com) diluted 1:5000. The labeled specimens were rinsed, mounted in Gelvatol (Monsanto Corp., St. Louis MO), and coverslipped for fluorescence microscopy. The specimens were analyzed with Nikon Eclipse E800 microscope (Nikon Plan Apo 60xA/1.4 lens) equipped with Bio-Rad 2100 confocal system. Image processing and analysis were conducted using C-Imaging software (Compix Inc., Cranberry Township PA).

2.9 Statistical Analysis

Tables of summary statistics and graphical displays were constructed to contrast the effects of primary outcomes variables. Analysis of variance procedures with Tukey post hoc correction examined the existence and nature of temporal trends among the treatment (viz., sampling time periods post exposure) level means. Significance is reported as p<0.05

3.0 RESULTS

3.1 Lung Injury Analyses: Pathology Scoring and Histology

It is well documented that impact of SW is associated with so called “primary injuries” that are largely restricted to the air-filled organs (e.g. lung, ears) [4, 14, 15]. The most consistent lung lesions that occurred after exposure to at peak overpressure of 122 ± 8 kPa, were bilateral diffuse parenchymal hemorrhages that involved the entire thickness of lobes as shown in Fig. 2B (compare to control, Fig. 2A).
Sequestration of Blood Plasma Iron as a Marker of Systemic Response to the Blast Lung Injury

Figure 2. Representative macroscopic views of lung harvested from a control rat (A) and rats subjected to “moderate” (B) injury at 24 h after exposure to SW at peak overpressure of 118 kPa. Arrows in panel “B” show hemorrhagic lesions.

Pulmonary contusions were often accompanied by apnea that lasted up to 10 min following exposure. Histopathologic examination revealed significant hemorrhage of alveolar septal capillaries (Fig. 3). Pulmonary infiltration of PMNs and attachment of PMNs to erythrocyte conglomerates was readily observed at 3, 6, 12, and 24 h (Figs. 3 C, D, E, F) but not at 1 h, (Fig. 3 B).

Figure 3. Representative histological sections of lung harvested from a sham-treated rat (A) and rats subjected to “moderate” injury at 1 h (B), 3 h (C), 6 h (D), 12 h (E), and 24 h (F) following SW exposure. Note, infiltrated PMNs attached to erythrocyte conglomerates, observed at 12 h and 24 h.
All sections were stained with hematoxylin and eosin as described under “Materials and Methods”. Images were digitally captured at 40x magnification (for “A”, “B”, “C”, and “E”), and at 20x and 10x magnification (respectively for “D” and “F”) to demonstrate the extent of PMN infiltration. Inset was captured at 100x magnification to demonstrate interaction of PMNs with extravascular erythocytes (in “E”).

Immunofluorescence imaging of hemorrhagic lesions revealed that endothelial sequestration and transmigration of granulocytes observed at 3 though 24 h postexposure was accompanied by an appearance of immunoreactivity of myeloperoxidase in alveolar wall (Fig. 4).

Several lines of evidence suggest that early effector cells in the pathogenesis of trauma-induced ARDS are the PMNs that have demarginated from vascular endothelium and/or have been released from bone marrow, and, then infiltrated and deposited in lung [24, 25]. PMN-mediated tissue injury does not occur unless PMNs, primed and attracted by chemokines, migrate into surrounding parenchyma [24, 25, 26]. Therefore, in the next set of experiments we analyzed levels of chemokine MIP-2 in blood and induction of CD11b in PMNs at different post-exposure time-points using ELISA and immunofluorescence techniques, respectively.
Figure. 4. Representative immunofluorescence images of lung specimens from sham-treated rat and rat subjected to “moderate” injury at 3 h following SW exposure. Assessment of infiltration of inflammatory leukocytes into alveolar septa was conducted with immunostaining for myeloperoxidase (MPO) and vascular endothelial marker - cadherin (VE-Cadherin).

Panels “A” and “B” bright field images of specimens from sham-treated and SW-exposed animals respectively. Panels “C” and “D” immunofluorescence images of mapping of VE-cadherin (green, Alexa 488) and MPO-abundant leukocytes (red, Cy3) in sham-treated and SW-exposed animals respectively. An appearance of the MPO immunoreactivity in alveolar septa in hemorrhagic lesions due to degranulation of leukocytes is arrowed in the panel “D”.

Note: sections of hemorrhagic lesions of SW-exposed lung were used for preparation of the specimens as described in Materials and Methods. Confocal digital images were taken using Nikon Plan Apo 60x/A/1.40 lens.

3.2 Cytokine/Chemokine Analysis of Blood Plasma and BAL Fluid

The rat chemokine MIP-2 (equivalent to human IL-8) is highly PMN-selective and, therefore, can be useful to predict early inflammatory phases of ARDS. Alteration in MIP-2 status was monitored in the peripheral blood of injured animals. Levels of MIP-2 in plasma were elevated at 1, 3, and 6 h following injury. Plasma MIP-2 increased from 2.9±2.6 pg/mL in control rats, to 33.0±7.9 pg/mL, 35.0±7.3 pg/mL and 78.9±20.5 pg/mL at 1 h, 3 h, and 6 h respectively, in injured rats.

Alterations in the chemokine/cytokines in BAL are shown in Fig. 5. A substantial increase in the levels at 24 h post-exposure occurred within MCP-1 that apparently associated with the extensive phagocyte activity at that time.

Figure. 5. Relative increase in the levels of inflammatory cytokines/chemokines in BAL fluid at 24 h following “moderate” lung injury. Collection of BAL fluid and the cytokines/chemokines assay were conducted as described in Materials and Methods.
3.3 Flow Cytometric Analysis of Blood PMNs

Fig. 6 shows that shock wave-induced moderate trauma was accompanied by rapid PMN recruitment and development of a neutrophilia at the first hour following exposure to shock wave as evidenced by increased PMN counts in blood samples. The number of PMNs in peripheral blood at this time increased from 1090±66 PMNs per 10000 events in the “control” group to 2948±359 PMNs per 10000 events in the “injury” group. PMNs in the blood of injured animals continued to increase at 3 h post-exposure to 5458±92 PMNs per 10000 events. A significant relative decrease of the above effect occurred during the later time-points of observation (i.e. 6, 12, and 24 h) (Fig. 6), which probably reflected PMN sequestration by target tissues. Still, the steady state counts of blood PMNs at 6, 12, and 24 h following exposure remained significantly above control counts, i.e. 3850±315 PMNs, 3351±293 PMNs, and 2400±442 PMNs per 10000 events.

The early (1 h) increase in the numbers of PMNs in the peripheral blood was not accompanied by a significant increase in expression of CD11b (Fig. 7). The observed increase in mean fluorescence intensity (MFI) for CD11b expression from 1928±51 A.U. to 2314±112 A.U. was not statistically significant. A significant augmentation of MFI was observed, however, at all time points from 3 h to 24 h following exposure (Fig. 7).

Figure 6. Effect of SW exposure on PMN counts in rat peripheral blood at different time periods of blood sampling.
Values are least squares means for PMN counts in box plots ranging from the 25th to 75th percentile. Bars indicate the 5th and 95th percentiles.
Figure 7. Effect of SW exposure on CD11b expression in PMNs of rat peripheral blood at different time periods of blood sampling. Values are least squares means for mean fluorescence intensity (MIF) in box plots ranging from the 25th to 75th percentile. Bars indicate the 5th and 95th percentiles.
Figure 8. Representative low-temperature EPR spectra of transferrin-bound iron (TRF-[Fe$^{3+}$]) in rat blood at 24 h following SW exposure.

(A) - EPR spectra of blood TRF-[Fe$^{3+}$] and respective second integral curves (B) from “1” – sham-treated rat (“negative injury”), “2” - rat experienced “slight injury”, “3” – rat after “moderate injury”. Injury Levels were estimated as described in Materials and Methods.

3.4 Low Temperature EPR Analysis of Transferrin-Bound Iron in Rat Blood Samples

The quantitative conditions for the effect of resonance microwave energy absorption by electrons (i.e. EPR) can be given by:

$$H = \frac{h \nu}{\beta g}$$

where $H$ = the resonance magnetic field, in Gauss (G),

(see spectra in Results)

$h$ = Planck’s constant ($6.625 \times 10^{-27}$ erg-sec)

$\nu$= resonance microwave frequency (9.463 GHz),

from EPR spectrometer settings

$\beta$ = the Boh magneton (constant)

$g$ = factor of spectral splitting

Since the $g$ factor values are constant for each type of paramagnetic center, the $g$ factor can be considered as a quantity characteristic of the molecule in which the unpaired electrons are located. Low temperature EPR signals of paramagnetic [Fe$^{3+}$] (i.e. high spin d$^5$Fe$^{3+}$, $S = 5/2$) coordinated in the interdomain cleft of each of the lobes (C and N) of the transferrin molecule are identical due to the nature of the ligands in the [Fe$^{3+}$] coordination sites. Note each of the two EPR signals from [Fe$^{3+}$] chelated in the C-lobe and N-lobe additively contributes to the resulting EPR spectrum of the diferric transferrin molecule. The main component of this spectrum (at 77o K) has a characteristic doublet maximum with major features at $g = 4.30$ and 4.40 (trough at $g = 4.03$) that belong to high spin d$^5$Fe$^{3+}$ in a rhombic environment (symmetry) (Fig. 8 A). During computation of the concentration of [Fe$^{3+}$] chelated by blood transferrin, diferric transferrin was considered to contain twice the amount of spins than each of C- and N-monoferic transferrins.

In heterogeneous, non-light transparent systems such as blood, spin concentration values may be the only possible quantitative measurement, and may be useful when the molecular species are unidentified or chelated within labile complexes. For quantitative estimates of spin concentration, usually the computed second integral of the derivative curve (Fig. 8 B) is compared with that of a standard sample. The spin concentration in an unknown sample ($N_x$) is related to the number of spins in a standard sample ($N_s$) by the equation:

$$N_x = k \frac{\Sigma_x}{\Sigma_s} N_s$$

where sub s and x designate the standard and unknown samples, respectively; $\Sigma$, the integral of the EPR absorption over the entire EPR signal; and $k$, a constant that includes EPR spectrometer settings and sample
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dimensional profile (geometry). Note sample geometry and EPR spectrometer settings were constant in the conducted experiments. The parameters $\Sigma_x$ and $\Sigma_y$ were calculated from EPR spectra of blood, blood plasma samples, and standard solutions of [Fe$^{3+}$]TRF.

Representative low temperature EPR spectra of [Fe$^{3+}$]TRF in blood samples from rats with “negative,” “slight” and “moderate” injuries are shown in Fig. 8 A, spectra 1, 2, and 3 respectively. The respective double integration values are shown in Fig. 8 B. As follows from the presented results, systemic response to SW-induced trauma in rat subjected to a “moderate” injury level was accompanied by ~60% decrease in the amount of blood [Fe$^{3+}$]TRF, while “slight” injury caused ~30% decrease of the same parameter in comparison with “negative” injury in “sham-treated” rat.

Based on the above observations we expected most consistent inflammatory alterations due to exposure to at peak overpressure of $122 \pm 8$ kPa, which produced “moderate” injury in rats. Fig. 9 shows that there were no significant alterations in the amounts of blood [Fe$^{3+}$]TRF at 1 h post-exposure, but significant [Fe$^{3+}$]TRF sequestration occurred at 3 h post-exposure. At that time, the steady state concentration of [Fe$^{3+}$]TRF in blood samples decreased from 19.9±2.3 μM in controls to 7.6±2.5 μM in exposed animals. The levels of [Fe$^{3+}$]TRF remained decreased thoughout the entire period of observations up to 24 h (Fig. 9).

![Figure 9](image)

**Figure 9.** Effect of SW exposure on the level of [Fe$^{3+}$]TRF in rat peripheral blood at different time periods of blood sampling. Values are least squares means for concentration of [Fe$^{3+}$]TRF in box plots ranging from the 25$^{th}$ to 75$^{th}$ percentile. Bars indicate the 5$^{th}$ and 95$^{th}$ percentiles.
Regression analysis of the amounts of blood [Fe$^{3+}$]TRF on injury levels (i.e. “negative”- 0, “slight”- 2, and “moderate”- 3) showed significant inverse correlation between these two events (Pearson correlation of injury level and [Fe$^{3+}$]TRF r = -0.90) (Fig. 10). Indeed, the amount of [Fe$^{3+}$]TRF in blood dropped from 22.7±3.7 µM for the “negative” group to 15.2±1.4 µM in the “slight” injury group (p<0.009, n=7 Tukey test), and to 7.2±2.8 µM in the “moderate” injury group (p<0.006, n=7, Tukey test). Similar effect was observed in blood plasma. Thus, while the amount of [Fe$^{3+}$]TRF in blood plasma in the “negative” group was 31.0±1.8 µM, the same parameter in the “moderate” injury group was only 12.3±2.2 µM (p<0.005, n=7 Tukey test).

Figure 10. Regression plot of the amounts of transferrin-bound iron in blood samples on injury levels at 24 h following exposure to SW. Note, “negative injury” (“0”) assigned to “sham-treatment”. Values are least squares means for concentration of [Fe$^{3+}$]TRF in box plots ranging from the 25th to 75th percentile. Bars indicate the 5th and 95th percentiles.

4.0 DISCUSSION

Detonation of explosive materials or firing of large caliber guns generates a wide spectrum of shock waves in the ambient environment. Exposure to shock waves can cause serious internal injury mostly to air-filled organs like lung and bowel, without external indications of trauma [1-4].

Recent observations suggest that several different mechanisms are probably responsible for the various manifestations of blast-induced pulmonary insufficiency. Thus, while the immediate pulmonary injury are mostly characterized by apnea, barotraumatic air embolism, and internal bleeding, the delayed respiratory malfunctions that occur 24-48 h after blast accidents, are likely due to response to the damage to the tissue barriers and extravasation of blood components [4, 26, 27]. Indeed, respiratory failure that occurs 24-48 h after blast accidents was mostly associated with subsequent systemic inflammation [4, 28]. To date, most
human studies of systemic inflammation have focused on patients with clinical manifestation of pulmonary insufficiency and ARDS, and the sequence of inflammatory events that lead to these pathologies has not yet been clearly delineated [4, 28]. Furthermore, predictive diagnostics for the outcome of blast-induced trauma must be based on the assessment of specific biomarkers of the systemic response to impacts of shock waves of different frequency ranges. However, currently there is only limited information on early post-exposure changes in pro-inflammatory indices. The present study mostly focused on the temporal pattern of the systemic pro-inflammatory responses in an animal model of lung blunt trauma produced by exposure to low-frequency (260±5 Hz), long duration (~4 ms) shock wave with peak overpressure of 122 ± 8 kPa. The shock wave with these characteristics produced “moderate” lung injury that resulted in a consistent inflammatory response during the 24 h post-exposure period. In particular, we assessed the dynamics of PMN counts, surface expression of the CD11b adhesion molecule, and sequestration of [Fe³⁺]TRF, the major carrier of blood plasma iron, in peripheral blood during post-trauma alterations.

We observed that the “moderate” lung injury from exposure to shock wave was accompanied by an increase in the number of circulatory PMNs as early as 1 h after BOP-exposure, which is indicative of mobilization of the pool of margined PMNs into the free circulation, as has been postulated recently [25]. A further increase in PMN counts observed at 3 h post-exposure period was probably due to the rapid release of mature PMNs from bone marrow reserve [25]. Mobilization of large numbers of PMNs into the circulation enables the injured host to shuttle the granulocytes to injured tissues, where they mediate the healing process. Indeed, in our experiments infiltration of PMNs in hemorrhagic edema areas occurred as early as 3 h post injury and continued though the following 24 h. In the same time sequence as the PMN infiltration occurred the increase in the immunofluorescence of myeloperoxidase (a marker of PMN degranulation) was detected in alveolar septa of injured lung. After the increase in the number of circulatory PMNs at 1 h and 3 h, the numbers of circulatory PMNs began to decline at 6 h presumably due to tissue sequestration [25]. This is consistent with the pronounced induction of CD11b expression observed at the 3 h post-exposure period, which continued throughout the twenty-four hour observation period. The expression of CD11b adhesion molecules on PMNs is an essential attribute specific for PMN interaction with the vascular endothelium [25].

It has been suggested that CD11b surface expression on PMNs is driven by pro-inflammatory cytokines (e.g. MIP-2 in rodents) and occurs before the PMNs reach the site of injury [25]. Our observation that MIP-2 levels increased 11-fold at 1 h post exposure to SW is consistent with that hypothesis, and suggests that MIP-2 plays a role in the increased expression of CD11b in circulatory PMNs prior to their migration into the lungs.

The systemic response to SW-induced trauma was associated with a decay in blood [Fe³⁺]TRF complexes, as determined by EPR spectroscopy. TRF is the major carrier of [Fe³⁺] in the extracellular blood liquid, and [Fe³⁺]TRF can be detected in whole blood as well as in blood plasma, or serum. The TRF molecule containing two iron-binding sites in the C- and N-terminals can exist in both blood plasma and buffered solutions in four forms, iron-free or apo-, monoferric (C-terminal iron), monoferric (N-terminal iron), and diferric. At normal extracellular [Fe³⁺] concentrations random distribution of [Fe³⁺] on available TRF binding sites usually gives rise to a population of molecules of which ~50% are apo-TRF, ~40% monoferric, and ~10% diferric TRF [29].

EPR spectroscopy, like all other forms of spectroscopy, monitors the net absorption of energy from a radiation field when molecules change their energy state. The main feature of EPR spectroscopy is that it deals primarily with resonance microwave energy absorption by electron magnetic dipoles (spins) of paramagnetic centers (i.e. free radicals, metal ions) that is usually recorded as first derivative of absorption spectrum. [Fe³⁺] ions distributed among the different [Fe³⁺]TRF complexes have indistinguishable EPR parameters and, therefore, contribute equally to the low temperature EPR signals detected in solutions and biological samples. The EPR absorption of [Fe³⁺]TRF complexes is proportional to the amount of the loaded iron, and the contribution of C- and N-monoferric, and diferric TRF to the EPR absorbance is additive, based on the amount of the chelated [Fe³⁺].
As follows from our results, at 24 h postexposure, the decrease in the level of blood plasma [Fe\(^{3+}\)]TRF correlated with injury levels. The observed tissue sequestration of [Fe\(^{3+}\)]TRF from blood was most likely essential for appropriate immune and/or hematopoietic responses to occur following damage [30, 31]. It is well documented that a fall in plasma iron concentration and alteration in the tissue distribution of iron are essential parts of the trauma-associated systemic response. In particular, acute conditions such as infectious diseases, myocardial infarction, fractures, wounds, and surgery are characterized by a profound and prolonged iron sequestration that lasts beyond the recuperation period. The mechanism of these phenomena remains unknown, however, it is considered to be a part of the acute phase non-immune inflammatory response which leads to turnover of iron though the TRF transport system.

In conclusion, the characteristics of a useful biomarker should include biomedical plausibility, sensitivity, specificity, reproducibility, ease of performance and low cost. One of the most important requirements is that the biomarker efficiently and effectively provides information pertained to the question being asked. The data presented suggest that assessment of [Fe\(^{3+}\)]TRF in blunt trauma can provide a good deal of information on severity of injury and satisfy most of the above requirements for an effective biomarker.

5.0 ACKNOWLEDGMENTS

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6.0 REFERENCES

Sequestration of Blood Plasma Iron as a Marker of Systemic Response to the Blast Lung Injury


Optical Coherence Tomography Evaluation of Tracheal Inflammation

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ABSTRACT

Background: Methods for obtaining real-time in-vivo histologic resolution non-invasive endoscopic optical imaging would be a major advance for pulmonary diagnostics and treatment in civilian and military medical applications. Optical coherence tomography (OCT) is a rapidly evolving technology based on near infrared interferometry that may provide these capabilities. Purpose: The purpose of this study is to evaluate the feasibility of using OCT for detecting airway pathology in a septic animal model. Methods: Tracheas of New Zealand white rabbits were inoculated endobronchially with varying concentrations of live Streptococcus pneumoniae bacteria. After development of pneumonia/sepsis, the animals were sacrificed. OCT tracheal images and subsequent histological preparations of these experimental animals were compared to control rabbit tracheas for morphological features and quantitative tracheal mucosal thicknesses measurements. Results: Results revealed significant airway mucosal thickening in the experimental group consistent with tracheal edema. Morphological changes, including epithelial sloughing and glandular proliferation, were evident in regions of the experimental tracheas. Conclusions: This study suggests that OCT is a potentially valuable imaging modality capable of evaluating superficial airway pathology with high resolution in-vivo. Numerous applications of OCT can be envisioned in the realm of combat casualty medicine and may substantially increase the precision and accuracy of current bronchoscopic diagnostic techniques.

KEYWORDS

Optical Coherence Tomography, imaging, airway, pneumonia, inflammation, edema.

INTRODUCTION

Rapid and accurate evaluation of airway pathologic changes is paramount for minimizing morbidity and mortality in conditions such as acute burn or inhalation injury. Until now, assessment for proper clinical management relied primarily on visualization of abnormalities, during endoscopic biopsy or on frozen sections sent to pathology, and gross inspection at surgery. A means to obtain real-time non-invasive histologic imaging would aid in diagnosis, prognosis, and help to ensure higher yield biopsy samples, save operating time, and may also help avoid unnecessary interventions or repeated procedures.

Optical Coherence Tomography (OCT) is emerging as a new rapid acquisition high-resolution imaging modality that provides capabilities for real-time near histologic level evaluation. In attempting to approach the concept of “optical biopsy,” OCT offers the potential for surface and subsurface optical imaging (up to a depth of 1-3 mm) with high spatial resolution of tissue microstructure, without requiring contact between the optical probe and the tissue sample. Tissue layers, glands, small blood vessels, and cartilage can be visualized with resolutions approaching 10 micrometers with the use of superluminescent diode (SLD) laser prototype systems. These technologies, in combination with minimally invasive techniques, can be applied in combat casualty care to evaluate and examine tissue depth and level of injury, provide prognostic information, as well as a means to assess response to therapy, and potentially minimize the need for frozen sections or the uncertainties associated with gross examination.

The purpose of this study was to investigate the potential utility of OCT in detecting airway pathology in acute injury. In order to demonstrate this concept, an animal model of airway disease was employed in which the airways of rabbits were inoculated with live Streptococcus pneumoniae bacteria. Previous studies with animals have found that bacterial lung infection—and specifically infection with pneumococcus—induces an inflammatory process within the airways. Thus, it was hypothesized that minimally invasive OCT imaging of tracheas of S Pneumonia infected rabbits, would reveal morphological changes indicative of airway inflammation when compared to those of controls.

MATERIALS AND METHODS

SLD OCT Prototype

Optical coherence tomography theory has been discussed in detail in previous studies. Briefly, OCT uses a broadband near-infrared laser light source in which the emitted light is split into sample and reference beams. The sample beam is directed to the tissue being examined, which is reflected back and combined with the reflected reference beam that has travelled an equal distance from a reference mirror to create an interference pattern (Fig 1). The interference signal of the resultant light wave (a combination of the reflected sample and reference beam) is processed and translated digitally as a gray-scale or false-color map. The reflected signals at different axial tissue depths are determined by the comparison of the internal tissue surface reflected distances and the reference mirror as the mirror distance (delay line) is increased and decreased. The tissue sampling arm probe is then moved laterally, thereby creating a two dimensional image.
A simplified diagram of the OCT system we constructed in our laboratory is shown in figure 2. A low temporal coherence superluminescent diode light source (central wavelength $\lambda_0 = 1300$nm, FWHM $\Delta = 80$nm; AFC Technologies Hull, Quebec) was connected to a Michelson interferometer which split the light source into the sample and reference beams. These reflected beams were then recombined at the fiber coupler in the interferometer, producing an interference pattern that was detected by a photodiode. Signal processing and data acquisition was accomplished using a computer. Cross sectional images were constructed by repeating the measurements at adjacent lateral points along a sampling line. Axial line scan frequency was 500 Hz. Imaging depth was approximately 1~2 mm. Since the OCT light source is not visible, an aiming beam (laser diode with $\lambda = 650$nm) was coupled to the system to elucidate the exact location of the sampling site.
Figure 2: OCT Schematic. A schematic depicting the design of the superluminescent diode OCT prototype system used for these studies. Returning light is recombined at the partially reflecting mirror and read the photodetector. Results are processed and displayed. The light source of 1310nm broad band super-luminescent diode with 50 nm bandwidth (FWHM), and theoretical resolution of 10~15µm.

Animal Models

*Airway Inhalation Injury Induction.* 23 New Zealand white rabbits were inoculated with various quantities of S. pneumoniae $1.9 \times 10^3 – 2.4 \times 10^5$ cells using a sterile pediatric suction catheter on an approved protocol from the Institute of Surgical Research in San Antonio, TX. Animals were monitored at the time of exposure and 24, 48, 72 and 96 hours post exposure via blood work, pulmonary function tests, vital sign data, computerized tomography scans, and flow cytometry and cultures of bronchoalveolar lavage fluid to confirm diagnosis of pneumonia. On the fourth day following inoculation, surviving rabbits were sacrificed and their tracheas excised, placed in isotonic saline packed on ice and sent overnight to the Beckman Laser Institute. Effort was made to image all specimens as soon as possible, within two days of excision.

The normal control group consisted of 9 total tracheal specimens. Six tracheal specimens that underwent the same process as the septic tracheas without the inoculation with the S Pneumoniae. Another 3 specimens were obtained as part of an unrelated study to evaluate acute hemorrhagic shock hemodynamics. The hemorrhagic shock specimens were used to evaluate whether time lapsed when the tracheas are stored in saline affected the structure of the trachea. Successive amounts of blood were removed and replaced with saline within a three-hour period. At the completion of these experiments, the rabbits were sacrificed and their tracheas removed and maintained in isotonic saline and imaged on site at the Beckman Laser Institute on the campus of the University of California at Irvine. In the saline stored control group, the tracheas were harvested. The tracheas were imaged immediately after resection, then stored in saline. Serial OCT images were obtained daily up to 4-5 days post resection to note the effects of saline storage on tracheas mucosal, submucosa.
Optical Coherence Tomography Imaging

*Ex-Vivo Tracheal OCT.* Excised tracheas were cut open longitudinally along their musculofibrous membranes and divided into approximately 2 cm-long sections, each tracheal specimen yielded two samples, representing upper and lower trachea. Triangular notches were cut into opposite ends of each specimen to delineate the line of image acquisition, perpendicular to the cartilage rings. The tracheas were secured to pieces of cork using metal pins placed along their perimeter and covered by a layer of KY jelly to prevent desiccation during imaging (Figure 3). The tracheas were then placed on a moveable sample platform and a visible-light guiding beam was used to match the line of image acquisition with the triangular marking notches. The images constructed were displayed using a logarithmic intensity scale with the most backscattering areas represented in white and the least backscattering areas in black. The trachea was then prepared for standard H&E slides for comparison to the OCT images.

![Figure 3: Excised tissue set up for OCT imaging.](image)

**Histology**

Histology of excised tissue was prepared according to standard hematoxylin and eosin (H&E) histological staining method. OCT images and those of the histological sections were compared. Tissue slide examination and micrographs were performed with an Olympus BH2 light microscope (Olympus American, Melville, NY), and recorded using an Olympus DP10 camera (Olympus American, Melville, NY) for a light microscope and Olympus Digital Microfire 1.0 (Olympus American, Melville, NY).

**RESULTS**

We constructed a compact prototype OCT unit, which was capable of performing cross-sectional imaging with an axial and transverse resolution of 10-20 µm in complex airway tissues. 14 mm x 1.3 mm airway images were acquired and displayed in near “real-time” on a computer screen. High-resolution images of normal and diseased trachea were obtained using OCT. These images revealed levels of resolution capable of clearly identifying airway structures.
OCT images and the corresponding histology sections are shown in figures 4 for both normal and inflamed trachea. Trachea cartilage (C) was an identifiable landmark seen in OCT imagining which was confirmed with histology. OCT images were able to distinguish the submucosa (SM) from the lamina propia (LP). Also observed in both OCT mapping and histology sections were submucosal glands between cartilage rings (B). A slight variation in tissue structure occurred, with decreased submucosal layer thickness in the histologic specimens, which was believed to have resulted from tissue desiccation changes after excision and fixation. Injured trachea obtained from animals inoculated with S. pneumoniae demonstrated a thickened submucosa that was seen in both OCT imagining and histology.

### Normal vs. Septic Inhalation Injury Trachea

#### Normal trachea

![Image](image)

#### ISR trachea

![Image](image)

Figure 4: Top - Normal control trachea OCT (left) and corresponding standard H&E histology (right). Bottom - Inhalational injury (ISR) trachea OCT (left) and its corresponding standard H&E histology (right). Note the thickness of the submucosa as compared to control. Structural elements are clearly seen in corresponding images. (C – tracheal cartilage; SM – submucosa; LP – lamina propia B – cartilage rings; G – submucosal glands).
The average tracheal mucosal layer thickness above the cartilage rings for the control group was 150 +/- 2.5 um micrometers whereas that for the pneumonia group was 228 micrometers +/- 1.7 um). There was a significant difference (p < 0.005) between the infected mucosal thicknesses and the thicknesses from the other two control groups. There was no difference between the values obtained from the control hemorrhage vs intubated control animals (p = 0.50). A dose-response curve shows a weak correlation between the increase in mucosal thickness with dosage of S Pneumoniae inoculated (Figure 5). There was no significant difference in upper versus lower tracheal specimens, and storing the tracheal sample in saline for several days had no impact on image appearance or the thickness of the mucosal and submucosal layers.

**DISCUSSION**

These studies confirm the feasibility of high resolution OCT imaging of airway to obtain optical images at near histologic level in-vivo. Differences in tissue layers of the airway were clearly distinguishable and corresponded closely to standard H&E images subsequently obtained from the excised tissues. Studies in our lab have also demonstrated OCT capabilities in upper airway in ex-vivo specimens. There are many areas of combat casualty airway and thoracic clinical diagnostics and research where OCT may become useful as resolution improves. With availability of in-vivo high-resolution optical capabilities, responses to therapy in thoracic injuries and diseases might be better assessed in addition to more rapid and improved diagnostics. At the current level of resolution (10-20um), tissue layers, mucosa, and airway epithelium can be readily visualized. Architectural disruptions in diseased tracheal specimens (Fig 4) clearly demonstrated an
increase in mucosal thickness that suggested tissue damage. Structural changes can also be observed in acute airway injury, which could make the use of the current OCT technology with bronchoscopy valuable in the acute trauma setting. In airway burn and toxic inhalation injuries, delineation of submucosal edema, hyperemia, and blood flow changes by OCT (including use of Optical Doppler Tomography (ODT) techniques) \(^{34-44}\) could be a significant adjunct to bronchoscopy for the management of tracheal and bronchial injury and assessment of response to therapy.

The ability to visualize tissue structures in real-time at a near histologic level resolution opens up a wide range of potential areas for clinical and research applications for injured soldiers. In the future, when cellular level OCT resolutions are obtained, even greater uses for OCT in thoracic diagnostic can be envisioned. However, the depth of penetration of OCT is relatively shallow (1-3 millimetres) and is likely to remain limited by the degree of scattering inherent in complex biological tissues of the lung and thorax (thus, major advances in depth of penetration are unlikely). Nevertheless, there are many scenarios in combat casualty medicine in which surface and near surface high-resolution imaging are of great potential value.

The development of three-dimensional high-resolution OCT probes, endoscopes, and image processing and display technologies may allow for improved assessment of tissue injury. Further into the future, very small OCT probes could be designed to fit within needles to allow imaging at depths within tissues and organs with near real-time histologic resolution capabilities.

**SUMMARY**

This study has demonstrated the feasibility of high-resolution OCT for examination of airway injury. The current technology is limited to 10-20\(\mu\)m resolutions, and maximum depth of penetration is generally 2-3 mm. With further improvement in resolution, contrast, acquisition, display and processing, and development of specific thoracic probes, OCT may offer a significant advance for the diagnosis and treatment of patients with burn and toxic inhalational injury as well as other airway pulmonary diseases.

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Life Support for Trauma and Transport (LSTAT™) Patient Care Platform: Expanding Global Applications and Impact

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ABSTRACT

The Life Support for Trauma and Transport (LSTAT™) patient care platform looks like a stretcher, but is actually a portable intensive care unit and surgical platform only 15 cm thick. The LSTAT platform has recently been employed in ground based tactical situations ranging from Operation Iraqi Freedom to a humanitarian mission in Cambodia. There is a growing body of evidence to suggest that, compared to conventional methods, the LSTAT platform improves the efficiency and effectiveness of care from the site of injury, through transport and definitive surgical care. In addition to use by the U.S. military, other nation’s military medical communities have begun acquiring LSTAT platforms, as well. The platform is cleared by the U.S. Food and Drug Administration, as well as CE Mark authorities for the purchase and use of the platform by EU nations. The LSTAT also has received limited fleetwide airworthiness clearance by the U.S. Army and U.S. Air Force.

By integrating multiple medical, data and utility capabilities into a single compact and light weight system, continuous treatment and monitoring can be provided across the continuum of care on land, air or sea. Therefore, there is little need to disconnect the patient from therapy and monitoring, resulting in the consumption of less time and resources, simplified logistics, and the prospect of improved clinical outcomes. The integrated nature of the platform also accommodates more rapid technology insertion, such as closed loop control of ventilation, infusion and other therapies.

The LSTAT platform has been deployed operationally to a number of austere military environments around the globe by medical components of the U.S. Army, Navy, Air Force, Special Operations Forces, and National Guard. Preliminary qualitative and quantitative data is being collected to validate the benefits of the platform in both pre-hospital and in-hospital applications.

Preliminary data indicates the LSTAT platform is preferred by some medical personnel over conventional methods for the treatment and transport of critically injured patients in certain applications.

1.0 INTRODUCTION

After Desert Storm I, the U.S. military medical leadership agreed upon the need for a ‘trauma pod’: an evacuation platform that would provide life support from as close to the site of injury as possible, through transport and into definitive care. They tasked the U.S. Defense Research Projects Agency (DARPA) to solicit
industry for the development of this ‘trauma pod’. In 1994, DARPA ultimately selected the Northrop Grumman Corporation (NGC) because NGC was a ‘systems integrator’; that is, a company skilled in the art and science of combining discrete devices onto single power and data busses usually within a single platform (e.g., an aircraft cockpit), while at the same time reducing their total weight and volume. NGC moved forward to develop, in collaboration with the Walter Reed Army Institute of Research (WRAIR), the LSTAT patient care platform. After achieving U.S Food and Drug Administration clearance on the LSTAT in 1998, NGC launched a separate company to build, sell and service the LSTAT platform. This new company is Integrated Medical Systems, Inc. The economic and operational user benefits resulting from a systems integration approach have proven well worth the investment in the automotive, aerospace/defense, and computer communities. The purpose of this paper is to suggest the same user benefits can accrue through the systems integration of medical devices, particularly for the weight- and volume-sensitive international military medical applications.

![Image of LSTAT Patient Care Platform](image)

**Figure 1: Life Support for Trauma and Transport (LSTAT) Patient Care Platform**

### 1.1 Research Problem

The research problem to be addressed is whether the benefits of an integrated patient care platform can be extended successfully to multiple environments; multiple vehicles and facilities (air, land and sea); and to multiple international medical communities. Given that such communities are faced with practically the same types and severities of casualties, requiring the same types of treatments, standardization has long been goal, particularly among NATO nations.

#### 1.1.1 Basis for Standardization

According to the NATO Handbook\(^1\), regarding the Committee of the Chiefs of Military Medical Services (COMEDS):

“The objectives of the COMEDS include improving and expanding arrangements between member countries for coordination, standardisation and interoperability in the medical field….”

A similar objective exists for the Partnership for Peace nations. Again, according to the NATO Handbook:

“In 2001, the COMEDS Plenary Meeting set up a Standing Group of Partners Medical Experts. In cooperation with the Strategic Commanders, this will provide a forum where medical assets and capabilities, PfP goals, and medical pre-arrangements will be addressed.”

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\(^1\) NATO Handbook, Chapter 14: Key to Organisations and Agencies and Other Subordinate Bodies, *The Committee of the Chiefs of Military Medical Services in NATO (COMEDS)*. June 2004.
2.0 METHODS

The methods employed were to provide LSTAT platforms to various military (and in limited cases, civilian) medical groups, with a goal of placing LSTATs in evaluations, exercises, and/or operational use in air, land and sea vehicles, and fixed facilities; as well as in a variety of climatic environments. Such assessments included human and non-human use; the latter to confirm mechanical, electrical, gas, logistics and other equipment and process interfaces. Users were then requested to provide quantitative and qualitative written and photographic evidence of their use. Since operational demands often made this difficult, this method was augmented by an LSTAT Users Conference in San Antonio, Texas (USA) in October 2002, where medical personnel made oral presentations on their experience with the LSTAT platform. LSTATs continue to be deployed, and data gathered, as of the date of this publication.

2.1 LSTAT Applications

The types of military medical applications involving LSTATs in evaluations, exercises and/or operational use have included

• Battalion Aid Station (BAS) – Photo courtesy of the U.S Army

• Mobile Army Surgical Hospital (MASH) - Photo courtesy of the U.S Army
Life Support for Trauma and Transport (LSTAT)
Patient Care Platform: Expanding Global Applications and Impact

- Aeromedical evacuation (rotary and fixed wing) – Right photo, courtesy of the U.S Navy

- Forward Surgical Team (FST) - Photo courtesy of the U.S Army

- Amphibious Assault/Casualty Receiving Transport ship – Photos courtesy of the U.S. Navy
• Military support to civilian casualties (landmine victims) - Photos courtesy of the U.S Army

• Civilian private academic medical center (post-anesthesia critical care unit and intra-hospital transport)

• Civilian public medical center - Photos courtesy of LAC+USC Medical Center
3.0 RESULTS

The LSTAT platform has been shown to operate successfully in a variety of environments, on a variety of platforms, by a variety of international military medical personnel, and with a variety of patients along the continuum of care, including surgery, general anesthesia as well as equipment maintenance and repair.

Specific findings repeatedly confirmed include:

1. LSTAT is suitable for use in most healthcare support applications.
2. LSTAT’s data acquisition system relieves much of the burden of documentation.
3. LSTAT’s data acquisition system reduces the number clinical specialists needed bedside and during transport.
4. LSTAT meets or exceeds the requirements of the American College of Critical Care Medicine guidelines for the transport of critical patients.
5. LSTAT weight does not negatively impact mission performance in most operational environments.

4.0 DISCUSSION

Through the convergence of advancing technology and the demands for interoperability, integrated medical platforms hold the prospect of becoming as standardized as the simple litter/stretcher. Ongoing reductions in weight and volume, together with increasing medical, data and utility capabilities, allow medical forces from multiple nations to share the common benefit of earlier, continuous, and flexible care to the combat casualty.

4.1 Significance to NATO

As coalitions increase in size and number, and as they are employed more frequently, efficient and effective combat casualty care will depend on standardization not only of processes and procedures, and not only of simple medical equipment such as bandages and litters, but of medical, data, and utility devices, as well. Just as NATO and PfP nations can share integrated aircraft platforms, they can now share integrated patient platforms.

5.0 CONCLUSIONS/RECOMMENDATIONS

Initial indications are that some international military medical forces prefer the benefits of an integrated patient platform. However, validation of this conclusion, and of the specific clinical and economic benefits, require additional evaluation through demonstrations, exercises and actual operational use, with an emphasis on gathering and analyzing quantitative data.
Hyperbaric Oxygen Therapy – An Adjunct to Optimal Combat Trauma Management

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The views expressed in this article are those of the author and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government.

INTRODUCTION

Although wound statistics from Operation Iraqi Freedom are still being analyzed, a preliminary analysis of casualty data demonstrates that approximately 70% of the injured combatants sustained trauma to their extremities.(Malcolm 2004) During the acute management of these injuries at Echelon II and III medical facilities, approximately 75 - 100 major vascular reconstructions and 180 limb amputations were performed. Anecdotal reports reveal that a number of the wounded combatants required tissue grafting, flap reconstruction, revision amputation or treatment for resistant infection subsequent to their evacuation to tertiary care facilities. Indeed, of the 560 surgical procedures performed for combat related injuries aboard the USNS Comfort, approximately 31% were noted to be sufficiently complicated by persistent tissue necrosis, wound infection, graft failure or delayed wound healing that further surgical management was required.(Helmers 2004) Residual ischemia has been shown to play a role in each of these processes. Hyperbaric Oxygen (HBO2) has been shown to be an effective in reversing ischemia and limiting wound healing complications in hospital-based, clinical care settings. It is proposed that HBO2 therapy could be applied in Echelon II and III operational environments to similarly limit the extent of surgical debridements, improve tissue flap and graft survival, decrease wound infection rates, speed complex wound healing and, ultimately, reduce the morbidity experienced by wounded combatants. This paper discusses the physiologic mechanisms underlying HBO2 therapy’s clinically beneficial effects, examines potential roles for HBO2 in the optimal management of combat-related trauma, and provides practical suggestions for HBO2 treatment chamber deployment into operational environments.

MECHANISMS OF HBO2 THERAPY

Reversal of Acute Ischemia

All wounds are characterized by a state of relative hypoxia.(Hunt, Twomey et al. 1967) Local oxygen tensions in the vicinity of a wound are approximately half the values observed in normal, non-wounded tissue.(Sheffield 1998) Musculoskeletal injuries secondary to crush, blast and penetrating trauma each produce local tissue ischemia, hypoxic gradients from zones of necrotic to healthy tissue and, when inadequately treated, the potential for propagation of ischemic injury into adjacent healthy tissues.(Warriner and Hopf 2003) Clinically, HBO2 therapy can be used to correct tissue hypoxia. This is accomplished when a patient breathes 100% oxygen at elevated atmospheric pressure. Physiologically, a directly proportional
increase in plasma oxygen tensions is produced and, at 2 - 2.5 atmospheres absolute pressure, arterial \( P_{O_2} \) elevations in excess of 1500 mmHg are achieved. (Warri ner and Hopf 2003) As a result, tissue oxygen tensions are also elevated and the diffusion of oxygen into areas of relative hypoxia markedly enhanced. (Strauss 2003; Niinikoski 2004) This significant level of hyperoxygenation allows for the reversal of localized tissue hypoxia and corrects the pathophysiology related to oxygen deficiency. This improved tissue oxygenation translates into the ability to limit the duration and progression of ischemic tissue necrosis (Zamboni, Roth et al. 1989; Bouachour, Cronier et al. 1996; Ramon, Abramovich et al. 1998), improve demarcation between necrotic and viable tissue (Isakov Iu, Atrostchenko et al. 1979; Rosenthal, Benderly et al. 1985; von Schroeder and Botte 1998; Murphy, Banwell et al. 2000), temporize against exceptional blood loss anemia (Hart 1974; Sherman, Sennik et al. 1989; Bitterman, Reissman et al. 1991; Greensmith 2000; MacFarlane, Cronje et al. 2000; Stark, Coatesworth et al. 2003) and enhance the survival of reconstructive tissue grafts and flaps. (Tai, Birely et al. 1992; Gampper, Zhang et al. 2002; Kalani, Jorneskog et al. 2002; Ulkur, Yuksel et al. 2002; Richards, Lineaweaver et al. 2003)

**Reduction of Reactive Edema Formation**

Post-traumatic vasogenic edema develops as a direct consequence of acute soft tissue injury and, as the injured, hypoxic tissues lose their ability to regulate intracellular water, it becomes accentuated by the formation of cytogenic edema. (Strauss 2003) The resultant extracellular fluid accumulation increases diffusion distances between oxygen-carrying capillaries and surrounding cells, reducing total oxygen delivery and perpetuating cytogenic edema formation. In closed tissue compartments, increases in interstitial pressure cause collapse of the microcirculation, further exacerbating existing ischemia and threatening previously uninvolved, healthy tissues. Clinically, this pathologic process is recognized as compartment syndrome. HBO\(_2\) therapy has been shown to be an effective adjunct in the management of compartment syndrome. Three distinct processes contribute to this clinical effectiveness. First, by reversing acute tissue hypoxia, HBO\(_2\) breaks the cycle between cellular ischemia and progressive edema formation, limits the total volume of soft tissue necrosis. (Nylander 1986; Skyhar, Hargens et al. 1986; Nylander, Nordstrom et al. 1988; Zamboni, Roth et al. 1993) Second, HBO\(_2\)-induced vasoconstriction produces a 20% decrease in arterial blood flow. (Bird and Telfer 1965; Bird and Telfer 1966) Edema reduction occurs because filtration of capillary fluid is decreased, while vascular outflow and improved oxygen delivery are maintained. Finally, the affected soft tissues are protected from reperfusion injury, a topic covered more fully in the next section. (Haapaniemi, Sirsjo et al. 1995) From the clinical standpoint, HBO\(_2\) therapy can be used to prophylax against suspected or impending compartment syndrome. (Myers 2000; Strauss 2003) Where a compartment syndrome is already established, HBO\(_2\) can be used to decrease the extent of required surgical fasciotomy, accelerate resolution of residual edema, and speed primary wound closure. (Bouachour, Cronier et al. 1996; Fitzpatrick, Murphy et al. 1998; Lindstrom, Gullichsen et al. 1998; Assenza, Borromeo et al. 2001; Van Poucke, Leenders et al. 2001; Gold, Barish et al. 2003)

**Optimizing Host Antibacterial Defenses**

Beyond the occurrence of frank necrosis, pathologic levels of hypoxia are correlated with increased rates of wound infection and, consequently, delayed wound healing. (Niinikoski 1969; Silver 1977) HBO\(_2\) therapy has been shown to be effective in limiting the incidence of wound infection, speeding resolution of refractory infections, and decreasing the morbidity and mortality associated with malignant infections. Several discrete mechanisms account for the beneficial effects of HBO\(_2\) in controlling infection. First, neutrophils required tissue oxygen tensions of 30-40 mmHg to destroy bacteria by oxidative killing mechanisms. (Mandell 1974; Hohn 1977) Leukocyte-mediated killing of aerobic gram-positive and anaerobic gram-negative organisms is restored when the pathologically low tissue oxygen tensions characteristic of wounded and infected tissues are
increased to physiologic or supraphysiologic levels by HBO₂ treatment. (Mader 1987; Knighton, Fiegel et al. 1990) Second, aminoglycoside and cephalosporin antibiotic transport across the bacterial cell wall does not occur if tissue oxygen tensions are below 20-30 mmHg. Therefore, HBO₂ therapy may enhance transport and augment antibiotic efficacy. (Park, Muhvich et al. 1991; Hirn 1993; Mendel, Reichert et al. 1999) Third, hyperoxygenation of the tissues surrounding areas of malignant infection may be of significance in preventing the extension of invading microorganisms. (Korhonen, Hirn et al. 1998; Korhonen 2000) Clinically, the most dramatic benefits are seen in reducing the morbidity and mortality of necrotizing fasciitis and gas gangrene, where treatment with HBO₂ results in clinical improvement even when standard measures have failed. (Schreiner, Tonjum et al. 1974; Bakker 1985; Riseman, Zamboni et al. 1990) Such benefits have been demonstrated for combat casualties. (Shupak, Halpern et al. 1984; Pailler and Labeeu 1986) Clinical improvements have also been noted in the management of peritonitis. (Bogomolova and Bol'shakov 1996) HBO₂ therapy is useful as a rescue treatment of refractory bone infections. (Aitasalo, Niinikoski et al. 1998; Maynor, Moon et al. 1998; Chen, Shih et al. 2003) In addition, HBO₂ is effective in reducing the need for re-operations in neurosurgical procedures complicated by infected grafts. (Eltorai, Hart et al. 1984; Larsson, Engstrom et al. 2002)

Prophylaxis Against Reperfusion Injury

The body of scientific literature continues to elucidate the multiple biochemical mechanisms behind HBO₂ therapy’s ability to limit and prophylax against ischemia-reperfusion (I/R) injury. These include upregulation of TGF-beta₁ which ameliorates reperfusion injury by up-regulating bcl-2 and inhibiting TNF-alpha production (Yang, Bosco et al. 2001; Grunenfelder, Miniati et al. 2002), catalase induction (Kim, Choi et al. 2001), inhibition of intracellular adhesion molecule (ICAM-1) formation (Buras, Stahl et al. 2000) and stimulation of the fibrinolytic enzymes tissue plasminogen activator (t-PA), urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type one (PAI-1). (Tjarnstrom, Holmdahl et al. 2001) While it is not possible to say at this point which of these mechanisms is most predominant in the beneficial effects of HBO₂ in limiting I/R injury, the preponderance of models consistently demonstrate this effect. (Buras 2000) Such inducible I/R protection has been demonstrated even when HBO₂ treatment was delayed two to eight hours. (Murakami, Horinouchi et al. 2001; Tjarnstrom, Holmdahl et al. 2001; Agir, Mersa et al. 2003) Pretreatment with HBO₂ has also been shown to be protective against subsequent periods of ischemia in neural and musculoskeletal tissues. (Murakami, Horinouchi et al. 2001; Dong, Xiong et al. 2002) Clinically, prophylaxis against I/R injury could be used to help improve post-surgical outcomes in patients undergoing peripheral vascular repairs, flap and grafting, and primary closure of large tissue defects. (Chen, Chen et al. 1998; Mazariegos, O'Toole et al. 1999; Myers 2000)

Improving Tissue Repair Rates

As noted previously, the hypoxic nature of all wounds has been demonstrated and, when pathologically increased, is correlated with impaired wound healing. (Hunt, Twomey et al. 1967; Niinikoski 1969; Gottrup 2004) Fibroblast replication, collagen deposition and angiogenesis are all oxygen sensitive responses that are necessary to proper wound healing. (Hunt and Pai 1972; Knighton, Silver et al. 1981; LaVan and Hunt 1990; Ishii, Miyanaga et al. 1999) Induction of several oxygen dependent growth factors has been elucidated in this process. Specifically, the production of nitric oxide (NO), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), angiopoietin-2 (ANG-2) and their respective tissue receptors are all upregulated in the presence of HBO₂. (Zhao, Davidson et al. 1994; Bonomo, Davidson et al. 1998; Boykin 2000; Sheikh, Gibson et al. 2000; Lin, Shyu et al. 2002; Kang, Gorti et al. 2004) These effects have been shown to persist after patient removal from the hyperbaric environment. (Siddiqui, Davidson et al. 1997) Additionally, the degree of wound healing induced by HBO₂ therapy appears to be
either superior to or synergistic with topically applied growth factors. (Bonomo, Davidson et al. 2000; Boykin 2000; Chen, Lai et al. 2002) In animal models, acceleration of wound healing has been noted for both wounds in both regionally ischemic and normal tissue beds. (Uhl, Sirsjo et al. 1994) Improvements in healing have also been demonstrated for orthopedic and neurosurgical procedures. (Atesalp, Komurcu et al. 2002; Chen, Lai et al. 2002) Clinically, HBO₂ therapy has been shown to improve rates of wound healing and reduce amputation rates in patients sustaining injuries in both civilian and combat environments. (Bialik, Fishman et al. 1987; LaVan and Hunt 1990; Radonic, Baric et al. 1995; Porcellini, Bernardo et al. 1997; Atesalp, Komurcu et al. 2002; Wang, Li et al. 2002; Warriner and Hopf 2003; Zamboni, Browder et al. 2003)

**Hyperbaric Effects Summary**

For hypoxic, complex wounds such as those induced by combat trauma, the net effect of serial hyperbaric exposures is improved local host immune response, increased clearance of infection, enhanced tissue growth, angiogenesis and wound epithelialization. When applied as an adjunct to surgical interventions, antibiotics and other clinically indicated therapies, HBO therapy is effective adjunct in the management of severe and refractory musculoskeletal problems. Indeed, the timely application of hyperbaric oxygen can be a limb- and life-saving therapy. (Wang, Li et al. 2002) More practically, adding HBO₂ treatment to other standard of care therapies can reduce the number and extent of required surgical procedures, decrease the duration of hospitalization and recovery time, improve post recovery function and, ultimately, enhance the wounded patient’s prospects for full recovery.

**Combat-Related Treatment Indications:**

<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Reduce Acute Tissue Ischemia</th>
<th>Minimize Edema Formation</th>
<th>Reperfusion Injury Prophylaxis</th>
<th>Improve Host Antibacterial Defenses</th>
<th>Improve Tissue Healing Rates</th>
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<tr>
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<td><strong>Acute Blood Loss Anemia</strong></td>
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Operational Deployment of HBO₂ Chambers:

The use of HBO₂ therapy for the management of combat related trauma has been suggested by a number of authors.(Cramer 1985; Spichev and Gostev Iu 1987; Rudge 1993; Broome 1997; Radonic, Baric et al. 1997; Fitzpatrick, Murphy et al. 1998; MacFarlane, Cronje et al. 2000) The primary difficulty with employment of this therapeutic modality is providing ready access of combat casualties to hyperbaric chamber facilities. As noted from the mechanistic discussions above, the acute benefits of HBO₂ therapy are best achieved when the therapy is provided within a few hours of injury. However, for the diseases and injuries presented, treatment with HBO₂ is generally considered to be an adjunct to appropriate antibiotic therapy and standard surgical management. Thus, whenever possible, initial treatment with HBO₂ should be timed to occur immediately after definitive surgical interventions have taken place, between planned surgical procedures, or when lack of OR availability allows HBO₂ therapy to be used as a temporizing measure. Unfortunately, logistical issues complicate deployment of hyperbaric chambers into the combat environment. Indeed, size, safety and mobility constraints render co-location of hyperbaric chambers at Echelon I treatment levels impractical. In contrast, at least two hyperbaric systems are available for use at Echelon II level treatment facilities. The most portable of these systems is the single person (i.e. a monoplace chamber) folding hyperbaric stretcher. This foldable, lightweight chamber, called the Emergency Evacuation Hyperbaric Stretcher (EEHS), has received certification for use within DoD. While the majority of these systems were initially intended for use in the remote treatment of decompression sickness and submarine escape and rescue operations, their potential application to combat trauma cases has been noted.(Locklear 2002) Deployment of U.S. Navy's Transportable Recompression Chamber System (TRCS), as was used by the Special Medical Response Team in August 2002 to treat miners trapped in a Pennsylvania coal mine. This later chamber has the advantage of being large enough to allow a medical attendant to accompany the patient during treatment (i.e. a multiplace chamber). At Echelon III level treatment facilities, multiple hyperbaric treatment options could be employed. These could include those chamber systems already mentioned as well as larger chamber systems such as the Fly Away Recompression Chamber (FARC). For maritime operations, a multiplace hyperbaric chamber could be stationed aboard amphibious ships or designated hospital ships, such as USNS Comfort and USNS Mercy. HBO₂ therapy used in support of reconstructive procedures and complex wound management can be arranged by medevac of select patients to regional Echelon IV treatment facilities that have fixed multiplace and monoplace chamber facilities. Such hospital-associated HBO₂ treatment capabilities can be found in most industrialized nations. Many of the chamber complexes are associated with existing tertiary care, military treatment facilities.


Hyperbaric Oxygen Therapy – An Adjunct to Optimal Combat Trauma Management
Biochemical Markers of Brain Injury: Applications to Combat Casualty Care

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1.0 BACKGROUND

The Need For Biochemical Markers Of Brain Injury: Brain injury resulting from traumatic, ischemic and/or chemical etiology is a significant international health concern, representing a potentially catastrophic debilitating medical emergency with poor prognosis for long-term disability. It represents a major problem to military care, accounting for 25% of all combat casualties and is the leading cause of death (approaching 50% incidence) among our wounded soldiers reaching Echelon I medical treatment [1]. In civilian life, the incidence of brain injury and resultant long-term disabilities caused by traumatic insults (gunshots, automobile accidents, sports, etc.) and ischemic events (strokes, cerebral hemorrhage, cardiac arrest, etc.) are several orders of magnitude greater. There are more than 1 million TBI cases that are treated and released from an emergency department annually in the United States resulting in more than 230,000 hospitalizations, 50,000 deaths and 80,000 disabilities. The current estimation is 5.3 million Americans live with TBI-related disability. TBI is the greatest cause of death and disability in young people less than 24 years old [2].

With the exception of diuretics, supportive measures and, when appropriate, recombinant tissue plasminogen activator (tPA) [3], there are currently no approved drug treatments for traumatic or ischemic brain injury. There have been a large number of clinical trials studying potential therapies for traumatic brain injury (TBI) that have resulted in negative findings with a cost of over $200 million [4, 5]. Many investigators have pointed out that the absence of biochemical markers of injury could have contributed to these failures [6]. Unlike other organ-based diseases where rapid diagnosis employing biomarkers (usually involving blood tests) prove invaluable to guide treatment of the disease, no such rapid, definitive diagnostic tests exist for traumatic or ischemic brain injury to provide physicians with quantifiable neurochemical markers to help determine the seriousness of the injury, the anatomical and cellular pathology of the injury, and to guide implementation of appropriate triage and medical management.

Criteria For Biochemical/Surrogate Markers: In the course of research on biomarkers, our laboratories have developed criteria for biomarker development. As reflected in the present proposal, useful biomarkers should employ readily accessible biological material such as CSF or blood (CSF is routinely accessible in severely injured TBI patients), predict the magnitude of injury and resulting functional deficits and possess high sensitivity and specificity, have a rapid appearance in blood and be released in a time-locked sequence after injury. Ideally, biomarkers should employ biological substrates unique to the CNS and provide information on injury mechanisms, a criterion which is often used to distinguish biochemical markers from surrogate markers of injury, which usually do not provide information on injury mechanisms.

Assessments of the sensitivity of markers to specific therapeutic interventions are an effort outside of the scope of the present proposal. Potential gender- and developmentally-related differences in biomarker profiles can also be determined in later studies.

Uses Of Biomarkers: Biomarkers would have important applications in diagnosis, prognosis and clinical research of brain injuries. Triage is a major function of far-forward medical care in a combat environment or during national disasters. Simple, rapid diagnostic tools will immensely facilitate allocation of the major medical resources required to treat TBI and other brain injuries. Accurate diagnosis in acute care environments can significantly enhance decisions about patient management including decisions whether to admit or discharge or administer other time consuming and expensive tests including computer tomography (CT) and magnetic resonance imaging (MRI) scans. Biomarkers could have important prognostic functions especially in patients suffering mild TBI, which make up an estimated 80% of the 2.5 to 6.5 million individuals who suffer from lifelong impairment as a result of TBI [7, 8]. Accurate identification of these patients could facilitate development, of guidelines for return to duty, work or sports activities and also provide opportunities for counseling of patients suffering from these deficits. Biomarkers could provide major opportunities for the conduct of clinical research including confirmation of injury mechanism(s) and drug target identification. The temporal profile of changes in biomarkers could guide timing of treatment. Finally, biomarkers could provide a clinical trial outcome measure obtainable much more cheaply and readily than
conventional neurological assessments, thereby significantly reducing the risks and costs of human clinical trials.

**Current Status Of Research:** Analysis of specific biochemical markers is a mandatory component of diagnosing dysfunction in a number of organs such as myocardial infarction. However, there are no biomarkers of proven clinical utility for TBI and stroke.

TBI is difficult to assess and clinical examinations are of restricted value during the first hours and days after injury. Conventional diagnoses of TBI are based on neuroimaging techniques such as CT scanning, MRI and single-photon emission CT scanning [9-11]. CT scanning has low sensitivity to diffuse brain damage, and the availability of MRI is limited [12-14]. Single-photon emission CT scanning detects regional blood-flow abnormalities not necessarily related to structural damage.

A recent review of biomarkers of TBI highlighted the need for biomarker development [15]. The most studied potential biochemical markers for TBI include creatine kinase (CK), glial fibrillary acidic protein (GFAP), lactate dehydrogenase (LDH), myelin basic protein (MBP), neuron-specific enolase (NSE) and S-100 proteins. The bulk of research in TBI has focused on NSE and S-100β. The specificity of NSE for brain is high [16], sex- and age-related variability is low [17-24], and NSE is rapidly detectible in serum after TBI [25]. However, studies relating NSE serum levels to admission GCS in patients with severe TBI show conflicting results. Similar data have been reported concerning relationships with CT scan findings, ICP and long-term outcomes. In mild TBI, NSE failed to separate patients from controls [26-28]. Thus, NSE is predominantly used as a marker for tumors [29]. NSE is also released in the blood by hemolysis, which could be a major source of error [29].

S-100β has high specificity for brain [16] although it is present in other tissues such as adipocytes and chondrocytes [30]. Investigators have reported S-100β serum levels correlate to both GCS scores, neuroradiologic findings at admission and long-term outcomes [31-33]. However, investigators have recently raised questions about the utility of S-100β reporting that high serum levels of S-100β are detectible in trauma patients not having head injuries, a factor not adequately controlled for in earlier studies [34]. In addition, serum levels of S-100β following mild TBI do not show strong correlations with neuropsychological outcome [35]. Research in this area continues and recent reports have indicated the potential utility of measures of GFAP [36] and cleaved tau protein [37] in blood following TBI.

Investigators have also generally recognized the need for more objective assessments of outcome following stroke, including biochemical markers [38, 39]. The approval of tPA as a treatment for acute stroke has additionally highlighted the potential utility of biochemical markers. Use of tPA may be hindered by diagnostic concerns because neurological deficits accompanying stroke can mimic those seen during transient ischemic attacks, complex migraine, space-occupying lesions and post-ictal paralysis. A reliable biochemical marker might give assurance to physicians considering administering thrombolytic agents for acute stroke [39].

Previously reported biomarkers of cerebral ischemia include NSE, brain specific creatine kinase enzyme (CPK-BB), S-100β and inflammatory cytokines such as IL-6 [39]. NSE and S-100β have been the most studied. After cardiac arrest, NSE elevations in serum and CSF have been correlated with neurological recovery [40-42]. Serum and CSF NSE values were reported to be elevated in rodent models of focal ischemia in proportion to the eventual infarct volume [43-45]. In clinical trials, peak serum NSE values also predicted infarct volumes as shown by CT. Correlating serum NSE values with functional outcome was less successful[43, 44, 46], possibly because functional neurological deficit is influenced by as much by location of brain injury as by infarct size [46]. S-100β protein has been studied most extensively for characterization of ischemic injuries after cardiac surgery, and several reports have documented post-operative serum elevations [47-49]. However, many of these reports do not include careful studies of neurological outcome, and several investigators have recently criticized the diagnostic utility of S-100β during cardiac surgery. [34]
αII-Spectrin Degradation—A Prototype Biomarker: Our research program to develop biomarkers for TBI has focused on α-spectrin degradation as a prototypical biochemical marker [50-52]. αII-spectrin is the major structural component of the cortical membrane cytoskeleton and is particularly abundant in axons and presynaptic terminals [53, 54]. Importantly, αII-spectrin is a major substrate for both calpain and caspase-3 cysteine proteases [55]. Our laboratory has provided considerable evidence that αII-spectrin is processed by calpains and/or caspase-3 to signature cleavage products in vivo after TBI [56-59] and in vitro models of mechanical stretch injury [60]. Immunoblots of αII-spectrin degradation thus provide concurrent information on the activation of calpain and caspase-3, potentially important regulators of cell death following TBI. The calcium sensitivity and low basal levels of calpain optimize its utility as a marker of cell injury. Although not found in erythrocytes and thus robust to confounding by blood contamination, αII-spectrin is not specific to the CNS [54]. We have generated considerable laboratory data on the utility of αII-spectrin degradation as a biomarker for TBI. Preliminary human data are also promising. Recent collaborative studies conducted at the University of Florida and WRAIR have also indicated that αII-spectrin degradation is a useful biomarker for ischemic injury and potentially capable of distinguishing ischemic vs. mechanical brain insults (see below).

1.1 RESULTS OF STUDIES

To date, we have conducted three significant studies examining the potential role of biomarkers following acute traumatic brain injury (TBI) and focal cerebral ischemia. The first study examined accumulation of α-II spectrin and calpain-cleaved α-II spectrin breakdown products in cerebrospinal fluid (CSF) after experimental TBI in rats produced by closed cortical impact [50]. As we previously demonstrated, cleavage of α-II spectrin by calpain and caspase-3 resulted in accumulation of protease-specific spectrin breakdown products (SBDPs) that can be used to monitor the magnitude and temporal duration of protease activation. However, accumulation of α-II spectrin and α-II SBDPs in CSF after TBI had never been examined. Following a moderate level (2.0 mm) of controlled cortical impact TBI in rodents, native α-II spectrin was decreased in brain tissue and increased in CSF from 24 h to 72 h after injury. In addition, calpain-specific SBDPs were observed to increase in both brain and CSF after injury. Increases in the calpain specific 145 kDa SBDP in CSF were 244%, 530% and 665% of sham-injured control animals at 24 h, 48 h and 72 h after TBI, respectively. The caspase-3-specific SBDP was observed to increase in CSF in some animals but to a lesser degree. Importantly, levels of these proteins were undetectable in CSF of uninjured control rats. These results indicate that detection of α-II spectrin and α-II SBDPs is a powerful discriminator of outcome and protease activation after TBI. In accord with our previous studies, results also indicated that calpain may be a more important effector of cell death after moderate TBI than caspase-3.

The second study examined the accumulation of calpain and caspase-3 proteolytic fragments of α-II spectrin in CSF after middle cerebral artery occlusion (MCAO) in rats [61]. This investigation examined accumulation of calpain- and calpase-3-cleaved α-II SBDPs in CSF of rodents subjected to 2 hrs of transient focal cerebral ischemia produced by MCAO followed by reperfusion. After MCAO injury, full-length α-II spectrin protein was decreased in brain tissue and increased in CSF from 24 to 72 hrs after injury. Whereas α-II SBDPs were detectable in sham-injured control animals, calpain but not caspase-3 specific α-II SBDPs were significantly increased in CSF after injury. However, caspase-3 α-II SBDPs were observed in CSF of some injured animals. These results indicated that α-II SBDPs detected in CSF after injury, particularly those mediated by calpain, may be useful diagnostic indicators of cerebral infarction that can provide important information about specific neurochemical events that have occurred in the brain after acute stroke.

The final study examined whether α-II SBDP levels were associated with injury magnitude and predicted lesion size [62]. Injury magnitude following closed cortical impact injury in rats significantly elevated the mean levels of both ipsilateral cortex (IC) and cerebral spinal fluid (CSF) SBDP at 2, 6, and 24 hours after two levels of lateral controlled cortical impact (1.0 mm and 1.6 mm of cortical deformation) in
rats. CSF SBDP levels were significantly higher after severe (1.6 mm) injury than mild (1.0 mm) injury. CSF SBDP levels were significantly correlated to IC levels in individual rats at 2, 6 and 24 hours after TBI. We also assessed the correlation between CSF SBDP levels and lesion size from T2-weighted magnetic resonance images (MRI) at 24 hours after TBI as well as correlation of two additional biomarkers, tau and S100β. Mean levels of CSF SBDP (r = 0.833) and tau (r = 0.693) significantly correlated with lesion size while levels of CSF S100β did not (r = 0.188). In a model to determine which marker or combination of markers (SBDP, tau, S100β) best predicted lesion size, CSF SBDP levels were the only significant predictor of lesion size. Furthermore, larger lesion sizes 24 hours after TBI were negatively correlated with decreased motor performance on days 1-5 after TBI (r = -0.708). Based on this data, we propose that CSF SBDP levels are a novel and promising biomarker of TBI and other acute CNS injuries.

1.1.1 DISCUSSION

Studies to date have demonstrated CSF levels of SBDP have three properties of a good biomarker: 1) association with injury magnitude, 2) reflection of pathophysiology in the brain, 3) significant contribution to prediction of outcome as measured by lesion size. Not only do these studies strongly support the utility of CSF SBDP as a biomarker of acute neuronal injury, they provide further evidence of the relationship between injury magnitude and biochemical outcome measures. This study is also the first rigorous preclinical evaluation of a biomarker of acute neurological injury. The contribution of this work is a foundation for future studies assessing the utility of this marker in human brain injury.

Injury magnitude significantly increased CSF and cortical levels of SBDP over the two control groups, sham-craniotomy and naïve rats. CSF levels of SBDP were significantly higher after severe (1.6 mm) injury than mild (1.0 mm) injury at 2, 6 and 24 hours after TBI reflecting injury magnitude.

Increased levels of calcium after TBI have been shown in several models [77, 87, 96, 97]. After TBI, calcium initiates a cytotoxic cascade of proteases including calpain which breaks down the cytoskeletal protein, spectrin. Higher levels of injury magnitude increased mRNA levels of calpain-1 and calpain-2 in the injured cortex and hippocampus (unpublished data). Similar to our study, varying injury magnitude by depth or by velocity of impact, significantly effected lesion size [79]. Injury magnitude also significantly increased peak intracranial pressure and hippocampal neuron loss in similar models of TBI [76, 79]. Temporal increases in intracellular calcium were correlated with injury magnitude after controlled cortical impact TBI in rats [77]. The corresponding increase in calcium after more severe TBI may explain the association between injury magnitude and SBDP levels in the IC and CSF. In the acute time period following TBI, CSF SBDP significantly correlated with cortical levels of SBDP and both increased with injury magnitude. Calpain-mediated SBDP have been extensively examined and shown to increase in in vivo and in vitro models of neuronal injury [70, 88, 89, 93]. Recently it has been shown that CSF SBDP increased in models of TBI [50] and ischemia [61]. The increased levels of SBDP 150/145 are primarily associated with calpain activation in our CCI model. Although caspase-3 may also cleave spectrin to SBPD 150, similar to prior work in our laboratory [91], the caspase-3 signature SBDP 120 was not significant in our CCI model, suggesting a much less relevant role of caspase-3 in the production of SBDP in this model. Calpain inhibitors have been neuroprotective in models of TBI [73, 94], ischemia [71, 84, 86], and spinal cord injury [69]. The ability of CSF levels of SBDP to reflect the pathophysiology of acute neuronal injury may provide a therapeutic target for treatment of TBI and an effective way to monitor treatment of TBI.

CSF levels of SBDP significantly contributed to prediction of lesion size after TBI. Other biomarkers have shown varying correlations with lesion size. Serum levels of creatine kinase isoenzyme BB did not correlate with CT findings in patients with mild TBI [85]. Two clinical studies of serum levels of S100β revealed a correlation with contusion volume [81, 92], while in a study of mild TBI, serum S100β levels did not correlate with MRI or CT scans [80]. S100β may be released from damaged glial cells, and this variable may not change consistently with the magnitude of injury.
Importantly in multi-trauma patients without head injuries, S100β reached high serum levels after bone fractures and thoracic contusion and also increased after burns and minor bruising [66]. Numerous studies examined the use of S100β to mark cerebral damage after cardio-pulmonary bypass surgery [65] but S100β was found to be released from the mediastinum of cardiopulmonary bypass patients [66]. After stroke, higher serum S100β levels were associated with larger infarcts and more severe neuropsychological deficits [63, 68, 74]. However Hill and colleagues [83] found only 32% of stroke patients had elevated serum S100β on admission. Early identification of stroke is necessary for optimal treatment within three hours.

CSF c-tau levels were significant predictors of outcome measures (intracranial pressure and GOS at discharge) [98] supporting the finding of a significant correlation between CSF tau and lesion size in our study. On the other hand, Franz et al., 2003 [78] showed that CSF levels of total tau did not correlate with injury severity (initial GCS) nor with outcome (GOS). The wide range of tau levels in that study was thought to be due to distance of the white matter lesion from the ventricles. Lesion variability is less in a model of CCI than in a clinical study of TBI. Initial examination of serum c-tau indicated the presence of serum c-tau increased the odds of an intracranial injury and a greater chance of a poor out-come [95], however, later work indicated serum cleaved tau levels did not correlate with outcome measures [75]. After acute stroke, tau increased in the CSF [82] and serum [72], and serum tau levels correlated to lesion size and severity. Similar to S100B, however, it increased in less than 50% of stroke patients [72]. Our study did not examine serum SBDP levels but further work will be important to establish if SBDP crosses the blood-brain barrier and reflects SBDP levels in the CSF and brain.

Changes in high resolution MRI have been shown to correlate well with histology in a lateral fluid percussion model [64] and a closed head injury model [67] of TBI. Areas of hypo-intensity on MRI were associated with hemorrhage or mechanical disruption and areas of hyper-intensity were associated with edema [64]. Twenty-four hours after rats underwent sham-craniotomy, varying amounts of hyper-intensity were noted, most likely due to edema associated with the changes in cranial pressures. In the closed head injury model, areas of hyper-intensity decreased between 2 and 7 days after TBI likely representing resolution of edema [67]. Similarly in our study, the overall size of the lesion decreased between 24 hours and 28 days, although a significant correlation was maintained between lesion size in individual rats at the two time points.

We also examined in vivo lesion size and the correlation to neuromotor function. Higher levels of injury magnitude significantly increased lesion size and decreased motor performance. In a stroke model, lesion size from T2-weighted images at 2 and 7 days after ischemia was significantly correlated with an average of individual neurological score [90]. Similarly in our study, the larger the lesion size, the worse the performance on the motor function test. Because lesion size at 24 hours was highly correlated with lesion size at 28 days and significantly negatively correlated with motor performance, it is suggestive that acute levels of SBDP might correlate with both acute motor performance and chronic lesion size. Because withdrawal of CSF is a terminal procedure in our laboratory at this time, the correlation is only speculative.

In conclusion, the results of these studies show that injury magnitude is associated with the levels of SBDP in the IC and CSF over acute time periods after TBI. We also showed the levels of CSF SBDP correlate with IC SBDP levels supporting the idea that CSF SBDP levels reflect the pathophysiology in the cortex at that time. We further showed that 24 hours after TBI, CSF SBDP significantly correlate with lesion size. In a model to determine which marker or combination of markers best predict lesion size, we found CSF SBDP levels to be the best predictor. αII-spectrin is not found in red blood cells [50] although it is found in very low levels in other organs systems (Pike, Flint, Wang, Hayes, unpublished data). Future work could confirm that CSF levels of SBDP have diagnostic utility for prediction of outcome after TBI in humans.

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Photochemical Tissue Bonding: Photons for Healing


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ABSTRACT

Tissue adhesion can be achieved using lasers in a non-thermal process called photochemical tissue bonding (PTB) as well as by the better known processes in which the tissue is heated to achieve welding or soldering. In PTB the energy of the absorbed photons is used to drive photochemical reactions via activated chemical species. Covalent bonds (“nanosutures”) formed between protein molecules bind the tissue surfaces together. In our PTB approach the photosensitizer used superficially stains the tissue such that effects are limited to the tissue interface. The overall temperature rise and concomitant thermal damage is negligible and structural integrity of the tissue is retained. This technology is generally applicable, but not limited to, collagenous tissues. In recent years we have used PTB for corneal repair, skin incision repair and skin grafts, tendon repair, nerve repair and blood vessel repair. In most cases, in vitro results have been followed by promising in vivo studies in appropriate animal models. Immediate repair strength, long term wound healing and functional recovery are all important factors governing the applicability of PTB in any given tissue and have been a focus of our work. This contribution will summarize our experience in mechanisms behind PTB, factors that influence the efficacy of PTB in various tissues and a comparison to other modalities for tissue adhesion and repair.

1.0 INTRODUCTION

Tissue repair is an obvious requirement following traumatic injury or surgical procedures. For most situations the tried and tested methods of mechanical methodologies, such as sutures, clips and staples, provide a cheap, rapid and efficient means of repair. However, in some situations these approaches are not particularly appropriate. Disadvantages include the placement of a foreign body in the tissue with resultant potential for inflammation and scarring. It is also generally true that the larger the structure to be repaired the easier the use of sutures. The opposite applies in that suture placement in microsurgery is technically more difficult. There are also a number of tissues that do not lend themselves easily to mechanical repair. Cornea is a particular example where suture repair is not optimal for a variety of reasons.[1] Microsurgery in blood vessel and nerve repair is also difficult due to the necessity of tightly sealing off the tissue environment.[2] Thus, there is a possibility that a suitable, less invasive technology for tissue repair will be of value for specific applications.

Laser energy has been used in a number of approaches for tissue repair. Lasers have been used in welding tissues together via a photothermal mechanism, involving conversion of the absorbed light energy to heat and subsequent protein denaturation and modification, resulting in bonding of the tissue.[3] As a thermal mechanism operates there is a high requirement for control of laser parameters such that welding is confined as much as possible to the tissue interface and does not contribute to collateral thermal damage in the tissue, which can cause excessive scarring and loss of structural integrity.[3-5] A means to more readily localize the thermal effects involves the application of a dye to the tissue surfaces that specifically absorbs the laser light; thus, thermal relaxation produces only highly localized temperature increase.[6, 7] Further adaptations of laser thermal welding involve the use of solders (protein or other macromolecules) to increase the bonding effect, but the mechanism is still activated in a thermal manner.[3, 8-10]

The use of lasers to activate tissue repair can also be carried out through chemical, rather than thermal processes.[1, 11] In photochemical tissue bonding (PTB) the light energy activates a photosensitizing dye to produce chemical reactions that result in chemical bonding and subsequent tissue repair. PTB has inherent advantages over thermal mechanisms in that the process does not require tight control of dosimetry because a particular target temperature is not required and by avoiding the higher temperatures involved in laser thermal welding, denaturation can be avoided and structural organization of the tissue could be retained. With the law of reciprocity in photochemistry one is required only to provide a given light and dye dose to achieve the desired effect. As the PTB process is not dependent on thermal effects, the light dose can be delivered in an irradiance independent fashion. However, the irradiance is not without limits since there will generally be some element of heat release, even in an efficient photochemical system. Consequently, a very high irradiance can lead to thermal damage as a side effect.

This paper presents some of our recent experience with PTB in our laboratory, the mechanism by which PTB operates and practical issues that arise in its application to tissue repair and wound closure in different tissues.

2.0 METHODOLOGY

In its simplest conception PTB consists of application of a photoactive dye to the tissue surfaces, followed by intimate approximation of the tissue and illumination of the interface with visible light. The photoactive dye absorbs strongly at the wavelength of the illuminating light and the selective absorption essentially limits the photochemistry to the interface. One corollary is that the dye itself remains superficially bound to the tissue and does not penetrate to a great extent from the surface of the tissue.[12]

![Figure 1: end to end (A), sandwich (B) and circumferential (C) approximation.](image)

In our studies we have used rose bengal (RB), a dye that absorbs in the visible spectrum that has other clinical uses[13], in combination with green light from Argon ion (514 nm) or CW Nd/YAG (532 nm) lasers. RB was applied in all cases as a 0.1% (w/v) solution in aqueous phosphate buffer. Irradiation has been applied as a circular spot, a line of 2 mm diameter and an annulus through the use of a computer-driven scanning device. The beam geometry was chosen for the specific application. The irradiance (W/cm²) of the light was varied in some cases using filters and the fluence (J/cm²) was varied by changing the period of illumination.
3.0 RESULTS

3.1 Corneal Repair

Primary feasibility studies were carried out on cornea.[1] The cornea is particularly attractive for PTB as it is transparent, avascular and largely acellular except for the surface layers and it is comprised predominantly of collagen. Cornea is also not ideal for suture placement as it is the primary refractive component of the eye and uneven suture tension can lead to post-operative astigmatism. In preliminary studies to test the ability of PTB to create an immediate bond in corneal tissue, a full thickness incision was created through the cornea of enucleated rabbit eyes (New Zealand white rabbit) with a 3.5 mm beveled keratome. Rose Bengal dye (20 µl of a 0.1% w/v solution) was applied to the walls of the incision via syringe and the wound was then irradiated with 514 nm light from an argon ion laser under different conditions of fluence and irradiance. Strength of repair was measured by controlled injection of saline into the anterior chamber and measurement of the increase in intraocular pressure (IOP) using a pressure transducer until a pressure was reached where the sealed incision was ruptured and leakage occurred (IOP_L). Figure 2 shows the IOP_L levels measured following repair of corneal incisions as a function of the light fluence used when delivered at a constant irradiance of 0.8 W/cm². An increased repair strength was observed up to a total fluence of 800 J/cm². The reduction seen at fluences higher than 800 J/cm² may indicate thermal damage under these conditions when no cooling was applied to the tissue.

Figure 2: Fluence dependence of strength of repair of corneal incision as a function of total fluence delivered at an irradiance of 0.8 W/cm².

Figure 3 shows the results obtained for the same fluence range but delivered at a 3x higher irradiance. Comparison of figures 2 and 3 shows that the use of increased irradiance in an attempt to deliver the required light fluence as quickly as possible was not successful as the resulting repair strengths were lower. In addition to the reduced efficacy of repair, the cornea underwent shrinkage, indicative of thermal overload to the tissue. This highlights a major consideration in PTB in that there is a balance required between fluence and irradiance.
and that use of high irradiances can be detrimental to the outcome. However, cooling can be employed to minimize the thermal effect and allow for use of higher irradiance.

![Graph showing fluence dependence of strength of repair of corneal incision.](image)

**Figure 3:** Fluence dependence of strength of repair of corneal incision as a function of total fluence delivered at an irradiance of 2.4 W/cm².

These preliminary experiments prompted in vivo tests in the same rabbit model, using the same incision wound. Following repair the intraocular pressure required to rupture the repair was determined by injection of saline into the anterior chamber. The results were more impressive in vivo because the same repair strength required an order of magnitude less fluence (~40 J/cm²); consequently, the irradiation time was reduced to a few minutes. We have found this to be fairly typical in our experiments to date in different tissues. In addition to measurements of immediate repair strength, the time dependence of repair strength and wound healing responses were followed up to four weeks post-treatment. There was no reduction in wound repair strength in the days following treatment, in fact greater strength was observed as healing progressed. There were signs of neovascularization after two weeks but this resolved after four weeks and was initiated by the incision itself rather than the treatment. Thus, the use of PTB corneal surgery has promise.

A further test of PTB for corneal applications was carried out in penetrating keratoplasty (PK, corneal graft). This is a particularly good problem for the PTB approach as the use of sutures to secure a corneal graft is problematic due to the slow healing process in the cornea that requires the sutures to remain in place for a considerable time, thus increasing the possibilities of adverse reaction due to foreign body response and ultimate graft rejection. The placement of sutures also introduces post-treatment astigmatism due to uneven suture tension that requires follow-up outpatient visits for adjustments to be performed. Experimentally, a 7 mm circular graft was harvested from one eye using a trephine. Rose Bengal solution was then applied to the cut surface of the host tissue and to the outer circumference of the graft and the corneal “plug” was then replaced in the host and held in place with 4-8 stay sutures. Irradiation was performed using a CW Nd/YAG laser at 532 nm with the 2 mm diameter spot being scanned to describe a circle of 7 mm diameter. In this
fashion the light was limited to the interface between graft and host with a small border of overlap. Following illumination the intraocular pressure to cause leakage from the sealed graft was measured in the same manner as for incisional wounds. The contralateral eye was treated in similar fashion except that the stay sutures alone were placed without PTB treatment. In all cases the pressure to cause leakage from the graft/host interface was higher when PTB was used to seal the interface. One obvious but important success factor to come out of this work was the need for intimate contact between the tissue surfaces to be bonded. As PTB works through formation of covalent bonds, bonding occurs on a molecular scale and the surfaces must be in tight, intimate contact. In grafts where we did not have good approximation at certain sites in the circumferential interface the seal was incomplete and leakage occurred at lower intraocular pressure. It should also be noted that the IOP to cause leakage from the interface is higher in control eyes for transplants compared to incisional wounds as the sutures do provide a seal, albeit incomplete, that reduces leakage at lower pressures.

![Graph showing increase in IOP from control to PTB-treated corneal transplants in New Zealand white rabbits.](image)

**Figure 4: Increase in IOP$_L$ from control (stay sutures only) to PTB-treated corneal transplants in New Zealand white rabbits.**

### 3.2 Peripheral Nerve Repair

As shown in Fig. 1 there are a number of ways in which tissues can be bonded using PTB. In the corneal examples given above the bonding is of the end to end type (A). For cylindrical structures like nerve or blood vessels PTB is applied to the outer periphery only. Peripheral nerve fibers are enveloped by a sheath of connective tissue called the epineurium. Nerve repair is done at the level of tissue repair rather than cellular repair, with the simplest approach being reconnection at the epineurium, which then protects the environment of the nerve interior to allow healing to occur.[2, 14] When a severed nerve is placed in a solution of rose bengal the dye is taken up exclusively by the collagenous epineurium. The endoneurium and nerve fibers are
not stained. This is ideal for peripheral nerve repair as an epineurial seal can be created using PTB. The approach we have used is shown in Figure 5 where an epineurial cuff is created as shown, to increase the surface area between the approximated tissue surfaces.

**Figure 5: Cartoon showing epineurial cuff approach to peripheral nerve repair using PTB.**

We have used this approach to test the feasibility of PTB for peripheral nerve repair in a rat model. The sciatic nerve was isolated and transected in Sprague-Dawley rats and the following treatments were performed. Ten animals were used for each treatment group.

1. Microsuture repair (current standard of care).
2. No repair (proximal nerve stump was sutured into muscle to prevent any possibility of regrowth).
3. Epineurial cuff alone.
4. Epineurial cuff + PTB using RB and 532 nm light (0.5W/cm², 2.5 minute exposure).

Figure 6 shows a photograph of a rat sciatic nerve that was transected and then repaired using the epineurial cuff and PTB approach. The photo on the left shows a rat sciatic nerve immediately following the PTB treatment and the cuff is clearly visible. The photograph on the right was taken 90 days after treatment and shows the repaired nerve that is free from any signs of external scarring or gross inflammation. The suture in this picture was placed to aid the histologist in locating the repair site.
The end-points studied included retention of gastrocnemius muscle (enervated by the sciatic nerve) and histology for regrowth of myelinated axons in the distal stump. In the former the muscle weight in the treated limb was compared to the weight of the same muscle in the untreated hind limb. At the 90-day end point the muscles were harvested and the ratio of the muscle weights in treated and untreated limbs are presented as a function of treatment in Fig. 7. In the negative control where no regrowth was possible, the gastrocnemius muscle in the treated leg wasted away and a low ratio was obtained. Retention of muscle mass was obtained...
with all other treatments. In the cuff alone group sutures were placed to form the cuff as shown in Figure 5. The cuff alone does not provide a strong seal but provides some benefit in terms of nerve repair. The additional bonding provided by the cuff + PTB approach enhanced the recovery as seen by a higher ratio of muscle weights in treated to untreated limbs approaching the best results obtained using standard of care microsurgery where 6 epineurial microsutures were placed at the repair site. A possible reason for the slightly lower effects seen with PTB is that in formation of the cuff the nerve length is reduced by 1-2 mm, which imparts an increased tension on the nerve, a factor that is known to be detrimental to nerve regrowth. Thus, although PTB seals the nerve environment to allow healing the cuff approach does impart some negative side effect. This could be overcome using a graft approach where the length of the nerve is not reduced overall.

![Histological sections (400x) of nerve harvested at 90 days post-treatment and stained for regrowth of myelinated axons using toluidine blue. A. No repair, distal to repair site. B. PTB + cuff, at repair site. C. PTB + cuff, proximal to repair. D. PTB + cuff, distal to repair.](image)

The best analysis of regrowth following peripheral nerve repair is provided by histology of the repaired nerve by comparison of sections taken at the repair site and at points proximal and distal to the site. The presence of myelinated fibers distal to the repair site is indicative of nerve regrowth. Sections were stained with toluidine blue to detect regenerated myelinated fibers. Figure 8 shows histological sections obtained at different sites following different treatments. In all cases, the section taken proximal to the nerve shows myelinated axons as expected as the deterioration of the nerve occurs distal to the site of injury. Thus, comparison of proximal and distal sections allows analysis of extent of nerve regrowth. In the negative control (A) where no repair
was carried out there is a complete absence of myelinated fibers in the nerve distal to the repair site. Sections C and D show sections from nerve repaired using the PTB + cuff approach at sites proximal and distal to the repair site, respectively. It is clear from section D that a large amount of the axons have regenerated into the distal stump. Section B shows a section through the repair site itself and is notable for a lack of scarring or otherwise irregular histology at the repair site itself. This is an important finding as microsutures can cause significant scarring, which is known to be detrimental to regrowth and functional recovery.[14] In conclusion, PTB used in this fashion has potential for microsurgical applications where sutures can be problematic.

3.3 Blood Vessel Anastomosis

Another example of a circumferential bond is in reanastomosis of severed blood vessels. This area was studied in detail in laser welding research in the past and is also highly appropriate for PTB.[3, 4, 15, 16] We have carried out preliminary studies using pig blood vessels ex-vivo in conjunction with PTB using rose bengal and a CW Nd/YAG laser. We have again used the cuff approach to increase the surface area of overlap to provide for a strong seal.

Figure 9: A. Swine carotid artery showing overlap of proximal and distal ends in cuff (arrow). B. Repaired pig vein on tensiometer before measurement. C. Stretching of vein to 3 times its normal length without rupture of repair.

Figure 9A shows the overlap area in a cuff created in the repair of a severed pig carotid artery ex vivo. The red color of the rose bengal is visible prior to irradiation. For blood vessel anastomosis we have used 532 nm irradiation parameters of 1.5W cm² for 2.5 minutes (450 J/cm²) on each side of the vessel. Figure 9B shows a repaired pig vein mounted on a tensiometer prior to measurement of tensile strength. Figure 9C demonstrates the strength of the repair as the vein stretches to 3-4 times its normal length and resists a force of ~ 2N before the seal is ruptured. These encouraging preliminary results have now led to in vivo studies of anastomosis in the rat femoral artery model where a complete seal is observed following PTB and circulation can be re-established without leakage of blood. Experiments are currently underway to look at longer term effects following PTB repair in blood vessels.
4.0 CONCLUSIONS

Photochemical tissue bonding (PTB) has proven to have potential in the repair of a variety of different tissues, especially those rich in collagen. The examples shown here demonstrate the different types of joints or seals that can be achieved. In addition to these examples of cornea, nerve and blood vessel PTB has also been successfully applied to repair wounds in skin, tendon and larynx. Many other possibilities exist and PTB has potential for being a platform technology that could be widely applied in surgical settings. The important factors for successful PTB include a strong adsorption of the photoactive dye to the target tissues, a minimization of thermal effects through correct dosimetry and external cooling and an intimate approximation of the tissue surfaces such that covalent crosslinks or “nanosutures” can be formed at the interface. PTB is particularly appropriate for small or delicate structures where placement of conventional sutures is physically difficult, time-consuming or liable to cause unwanted side effects.

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6.0 REFERENCES


Center for Military Biomaterials Research: Focus on New Materials for Trauma Technology

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SUMMARY

The Center for Military Biomaterials Research (CeMBR) has been created as a catalyst to develop breakthrough products specifically designed for military healthcare needs. CeMBR’s initial programs focus on combat wound care and protection of soldiers from chemical and biological agents. CeMBR is building a network of industrial, academic and military partners to conduct rapid development of biomaterial-based products. CeMBR is focused on meeting the needs of the military not only for these products but also for education to understand and better utilize the emerging technologies.

Introduction

The Center for Military Biomaterials Research (CeMBR) is a new program of the New Jersey Center for Biomaterials. CeMBR’s mission is to create a network among academia, industry and the military that provides rapid and effective pathways for the identification, development and utilization of biomaterial-based technologies and products for the military’s health care needs. The initial, principal focus of CeMBR is on biomaterials for combat casualty care.

The impetus for the founding of CeMBR comes from a study of the U.S. Army’s need to understand and take full advantage of the emerging biomaterial technologies and products. This is delineated in a 2001 study by the National Academies of Science (NAS), titled “Opportunities in Biotechnology for Future Army Applications,” [1], also known as the “BAST Report.” The recommendations of this NAS study are: that the Army needs to partner with the existing biomedical research establishment; must have technical professionals capable of translating biomedical findings into engineering practice; and should study the potential impact of biomedical research on future military operations. CeMBR has been designed to provide the Army and other services with the means to meet these recommendations. CeMBR’s first military partner is the Telemedicine and Advanced Technology Research Center (TATRC), a unit within the US Army Medical Research and Materials Command. As CeMBR’s activities mature, it will reach out to the other military services.

Development of medical devices based on new biomaterials is extraordinarily complex and only a broadly based research and development organization with strong academic ties can bring together all of the science and engineering needed for a focused biomaterials development effort. Expertise is required in materials science, synthetic organic chemistry, polymer chemistry, surface chemistry and physics, pharmaceutics, immunology, tissue engineering, biochemistry, medicine, and mechanical and biomedical engineering. Additionally, there is a intricate set of regulatory requirements that must be met to bring a biomaterial-based product to commercialization. Because CeMBR is a program within the framework of the established New Jersey Center for Biomaterials, CeMBR already has access to over 60 associated faculty members at Rutgers -
the State University of New Jersey, the University of Medicine and Dentistry of New Jersey, and the New Jersey Institute of Technology, who can provide much of the required expertise. In addition, CeMBR has attracted faculty from other leading research universities around the country who further enhance its capabilities to meet the military’s needs.

**Business Model**

CeMBR has adopted a “market driven” business model [2,3] with three core functions: 1) project management, 2) applied research and product development, and 3) education for the military. CeMBR will facilitate the development of industrial prototypes that are already in the product pipeline to reach commercialization in a form that meets the Army’s product specifications. This means working with industrial partners to advance their technologies through development contracts that take the product through the successive stages from the laboratory to production. CeMBR has also been asked by the military to review underutilized intellectual property already funded and owned by the Department of Defense so that the potential value of these concepts can be realized. In cases where there is no available enabling technology or industrial prototype product to meet a certain military requirement, CeMBR will conduct applied research programs to develop the necessary technology. As recognized in the BAST Report, the military must build its internal capabilities to act on emerging opportunities in the biomaterials field. Because CeMBR is part of an academic partnership organization, it has in place the educators to bring the appropriate training to the military.

To accelerate product development so that biomaterial technologies can be moved quickly to battlefield applications, two cycles of interactions will be linked through CeMBR (Figure 1).

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**Figure 1: CeMBR Links Academia and Industry with the Military To Accelerate Product Development**
In one cycle, product requirements defined by the military will be brought to the associated faculty to obtain their best ideas and expertise. Funding and support from the Department of Defense will flow to the faculty to drive this. Simultaneously, in a second cycle, product concepts and commercial expertise at associated industrial partner companies will be linked to the military’s requirements so as to create collaborative prototype development. Hence, CeMBR will facilitate the flow of biomaterial product concepts from the faculty and the industrial partners who can commercialize these concepts. Prototype products that result from these interactions will be brought to the military to gain funding and support for later stages of product development that include pre-clinical and clinical trials.

The key to success of this linked process is an effective network of industrial and military partners. Already, CeMBR has attracted the interest of several companies and is establishing a series of individual industrial partnerships. Military organizations that are already working with CeMBR include TATRC, the U.S. Army Medical Research Institute of Chemical Defense, the U.S. Army Institute of Surgical Research, the U.S. Army Research Institute of Infectious Disease, and Picatinny Arsenal. Additional partners will be sought based on the specific needs of the military as they are identified.

The management structure of CeMBR has been set up to ensure that all parts of the network are active participants. The executive committee is composed of Professor Joachim Kohn, the principal investigator, Professor David Devore, the chief operating officer, Professor Bozena Michniak, chair of the Commercialization Advisory Board, Professor Michael Jaffe, chair of the Scientific Advisory Board, and Carole Kantor, associate director of the NJ Center for Biomaterials. The Commercialization Advisory Board has members from the military, industry (including small-cap, mid-cap and large-cap companies) and academia, and also includes experts in patent law, licensing and technology transfer, business development and FDA regulatory requirements.

Federal funding for CeMBR is coordinated through TATRC. For the first two years of operations, CeMBR has received $2.5 million and it has requested $6.8 million for the third year in order to establish a pipeline of diversified biomaterial products that can be brought to the military market in a time frame of two to seven years. It is anticipated that the development costs for these products will be substantial, and hence, products must find dual use in the larger, civilian market in order to justify the investments required.

**Identifying Military Needs**

The first, and most critical, objective of CeMBR is to establish the specific needs of the military for biomaterial-based products. This is being accomplished through a collaboration with the National Academies of Science’s National Materials Advisory Board (NAS/NMAB), which began with a “Biomaterials Science and Technology Roadmap Workshop” held in February, 2004. Participants in the workshop included 15 senior military representatives, 27 senior research and business managers from industry (Table 1), and 40 research faculty members in science, engineering and medicine from several universities around the country. Funding for the Workshop was provided by TATRC. The participants met for three days to identify the major biomaterial-related needs of the military. The NAS/NMAB has assembled an expert committee, chaired by James Anderson, MD, PhD, of Case Western Reserve University and the NAS Institute of Medicine, to create a report based on the Workshop. This “roadmap” document, which will provide a coordinated set of recommendations to expedite military acquisition of biomaterials-related healthcare products, will be available in the summer of 2004.
Table 1
Industry Participants in the Biomaterials Roadmap Workshop

- Advanced Ceramics Research – Tucson AZ
- BioCure Inc. – Norcross GA
- Celgene, Inc. – North Brunswick NJ
- Center for Biomaterials and Advanced Technology, Ethicon Inc. – Somerville NJ
- Corium International Inc. – Redwood City CA
- ECI Biotech – Worcester MA
- FMC Corporation – Philadelphia PA
- Hale & Dorr – Princeton NJ
- Johnson & Johnson Wound Management – Somerville NJ
- Laureate Pharma LP – Princeton NJ
- LifeCell Corporation – Branchburg NJ
- Ortho Clinical Diagnostics – A Johnson & Johnson Company – Raritan NJ
- Osteotech, Inc. – Eatontown NJ
- Polymerix Corporation – Piscataway NJ
- Tepha, Inc. – Cambridge MA
- TyRx Pharma, Inc. – New Brunswick NJ
- Vectramed Inc. – Princeton NJ
- Vincogen Inc. – North Brunswick NJ

The Roadmap Workshop participants identified four major areas of importance to the military: 1) wound care, 2) agent delivery, 3) tissue regeneration, and 4) sensors and diagnostics. Biomaterials for wound care may lead to products that ultimately provide wound closure and limb stabilization, including dressings that stop bleeding while delivering antibiotics and pain relief. Agent delivery includes such biomaterial applications as vaccine adjuvants and barrier creams providing prophylaxis for exposure to biological and chemical agents. Tissue regeneration is a broad area, involving neural, vascular and orthopedic applications; examples include conduits to channel regrowth of severed nerves, biomaterials with extracellular matrix components for nerve regeneration, resorbable anti-thrombogenic implantable vascular devices, antibiotics in bone grafts, and weight bearing bone substitutes that remodel into the host bone. Such products are anticipated to have several major effects, including saving limbs, restoring function, and enabling faster return of warfighters to active duty.

Biomaterials Research Programs

In keeping with the objective to rapidly advance products, CeMBR has already initiated an applied research program that targets areas known from the BAST Report to be essential to the Army’s needs. The first project is being conducted by BioCure, Inc. (Norcross, GA) on a fast-polymerizing, spray-on wound dressing for battlefield use. The initial version of the dressing will provide a robust, abrasion resistant, adhesive dressing that will be applicable to complex anatomical geometries. Later versions of the dressing will include drug delivery for treatment of pain, infection and bleeding.

The second project is being conducted by Professor Bo Michniak at UMDNJ on a human skin model for evaluating protection from chemical warfare agents. Professor Michniak has begun a collaboration with Dr. Ernest Braue, Jr., U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, to identify chemical warfare agents that are of the greatest concern to the military and to select simulants for these agents that mimic their chemical and physical behavior so as to enable testing and development of predictive chemical information. In addition, CeMBR is working with the U.S. Army Medical Research Institute of Infectious Diseases to develop projects directed at vaccines and drug delivery for defense against major biological weapons threats such as anthrax and botulinum.
Once the NAS’ Roadmap Report is complete, CeMBR’s Commercialization Advisory Board will begin to review proposals for new projects and solicit industrial partners so as to target the most critical military needs. By the third year of funding, CeMBR will launch educational programs developed with its military advisors to enable the military to better understand and utilize biomaterial technologies. Also, CeMBR will begin a review of the military’s “orphaned” intellectual property in order to bring valuable concepts back into the product development cycle.

**Intellectual Property and Funding**

There are at least three sources of intellectual property (IP) that CeMBR will use to create value for the military: University IP, Industrial IP, and Military (DoD) IP. CeMBR will match the IP to military healthcare needs and create partnerships with the owners of the IP to advance the technologies to meet military requirements. Whenever appropriate, CeMBR will develop licensing agreements for the IP and may assist in forming spin-out companies to produce the products. This will enable funds to flow back to the inventors of the IP as well as providing funding to CeMBR in the form of contracts or IP licenses.

CeMBR will allocate its funds to support industrial partners as they develop their prototype products to reach rapid commercialization. CeMBR will also allocate funds to support research or development contracts for university faculty members who are working on new biomaterials science and technology that will be required to meet military requirements, and also for development of their existing advanced pre-commercial prototypes. CeMBR funds will also be allocated for development and delivery of educational programs on biomaterials for the military.

CeMBR’s organization and network are in place and growing. In March, 2005, CeMBR will move into its new $14 million building on the Rutgers campus in Piscataway, which will provide laboratory, clean room, pilot scale-up and office facilities (Figure 2).

![Figure 2. CeMBR’s New Home in the Life Sciences Building at Rutgers](image-url)
Status

CeMBR’s management team is in place and the Commercialization Advisory Board has convened. Active, enthusiastic support has come from several U.S. Army organizations, including Picatinny Arsenal, US Army Medical Research Institute of Chemical Defense, US Army Medical Research Institute of Infectious Disease, and US Army Institute of Surgical Research. It is anticipated that CeMBR will grow its military interactions to include the other service branches and CeMBR will begin to operate as a national center, bringing in the leading companies and university faculty in the biomaterials field. Because the goal of CeMBR is rapid commercialization and utilization of biomaterials-based products by the military, CeMBR has also initiated discussions with the US Food and Drug Administration so that FDA requirements will be defined at the onset of each CeMBR program to help speed the development process. The Biomaterials Roadmap Workshop will be an annual process in which the needs of the military are reviewed in detail, along with all product development and research programs; these programs will be modified or new ones initiated as required to address the most current needs.

References

Tactical Medical Logistics Planning Tool: Modeling Operational Risk Assessment

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TACTICAL MEDICAL LOGISTICS PLANNING TOOL: MODELING OPERATIONAL RISK ASSESSMENT

The Tactical Medical Logistics Planning Tool (TML+) is a software program designed for Navy and Marine Corps medical planners as a tool that (1) models the patient flow from the point of injury through more definitive care, and (2) supports operations research and systems analysis studies, operational risk assessment, and field medical services planning. TML+ is designed with a user-friendly graphic user interface, an open architecture, and four program modules. The casualty generation module uses an exponential distribution to stochastically generate wounded in action, disease, and nonbattle injuries. The care providing module uses generic task sequences, simulated treatment times, and personnel, consumable supply, and equipment requirements to model patient treatment and queuing within a functional area. The network/transportation module simulates the evacuation (including queuing) and routing of patients through the network of care via transportation assets. The reporting module produces an Access database detailing various metrics, such as patient disposition, time-in-system data, and consumable, equipment, personnel and transportation utilization rates, which can be filtered according to the user’s needs. TML+ can be used before deployment as a deliberate planning tool or during deployment as a crisis-action tool that assists planners in responding to the rapidly changing wartime environment.

1.0 INTRODUCTION

The United States Marine Corps (USMC) has changed its doctrine and policy to achieve more flexible and effective combat operations. To accomplish this goal (as expressed in Operational Maneuver from the Sea), Concept of Naval Force Medical Protection for the 21st Century, Joint Vision 2020, Marine Corps Strategy 21, and Sea Power 21 illustrate the need for highly mobile medical units with improved responsiveness. This doctrine requires new tools to assist in deliberate and crisis-action tactical and medical planning for the rapidly changing warfighting environment.

In answer to this need, the Naval Health Research Center (NHRC) and Teledyne Brown Engineering (TBE) developed the Tactical Medical Logistics Planning Tool (TML+). TML+ is a software program designed for Navy and Marine Corps medical planners as (1) a simulation tool that models the flow of patients from the point of injury through more definitive care, and (2) a research tool that supports operations research and systems analysis studies, operational risk assessment, and field medical services planning.

1.1 BACKGROUND

In response to changing warfighting doctrine, the Marine Corps Combat Development Command (MCCDC) and the Marine Corps Systems Command (MARCORSYSCOM) developed the Forward Resuscitative

Surgery System (FRSS). Marine Corps concepts and doctrine state that warfighting will require increased mobility and dispersion, and reflect a higher tempo of operations among combat elements. These goals require medical assets to achieve greater mobility and faster response without compromising the high level of care traditionally administered.

The FRSS is designed as a highly mobile, rapidly deployable, trauma surgical unit that provides emergency surgical interventions required to stabilize casualties who might otherwise die or lose limbs before reaching treatment. FRSS may be used as the initial surgical capability ashore in the traditional amphibious assault, or it may be the only surgical capability ashore, as in an Operational Maneuver From the Sea scenario. FRSS is designed to respond to the demands of rapid phase changes that require the ability to shift operational objectives quickly and efficiently. To meet this mission, FRSS requires a small logistical footprint that supports early introduction into the operating area, rapid movement, deployment, and re-deployment in forward areas.

Introducing this new capability into the Marine Corps continuum of care raised many questions as to its impact on medical treatment and resources. Therefore, MARCORSYSCOM sponsored an effort to represent the medical assets required to support FRSS in all stages of operations. Assisting in this effort, NHRC developed TML+ as a tool to research how FRSS could most efficiently use medical resources such as supplies, personnel, and transportation to provide patients with the best protection and medical care possible. As TML+ was designed, it was expanded to model additional medical treatment facilities, including the First Responder, Battalion Aid Station (BAS), FRSS, Shock Trauma Platoon (STP), Surgical Company (SC), and Casualty Receiving and Treatment Ship (CRTS), and is currently being expanded to include additional theater platforms both shipboard and ashore.

1.2 LITERATURE REVIEW

Modeling and simulation software has long been integral to the Navy’s preparation for contingencies. The Navy has designed programs addressing issues specific to warfighting and specific to the medical needs of a mission. Examples of the Navy’s warfighting modeling software include the Joint Warfare System (JWARS), and Joint Semi-Automated Forces (JSAF).

JWARS is a program developed to model the warfighting requirements within a joint theater of operations, simulating the combat, maneuvering, and movement of units and supplies across land, air, and sea. Using a decision tree structure, JWARS models direct and indirect fire engagements, the formations of units when moving, assembling, attacking, and defending, and communications across units. In addition, JWARS models the supply and resupply requirements necessary to sustain a warfighting mission, scheduling supply delivery of fuel and ammunition via transportation assets according to how the scenario unfolds within the simulation (Joint Warfare Systems Office, 2003). JWARS is valuable for operational planning and execution, force assessment studies, systems effectiveness and trade-off analyses for a warfighting mission (Stone & McIntyre, 2001).

JSAF is a program designed to model the complex integration of all branches of the military (Army, Air Force, Marines, and Navy) in the execution of a warfighting mission. JSAF generates elements of a contingency, such as troops, tanks, ships, airplanes, munitions, buildings, and sensors, which interact within the constraints of a combat environment. The synthetic environment is a representation of terrain, oceans, and weather conditions that affect the decision, interactions, and capabilities of joint forces (“Information”). JSAF was later expanded to include a medical component called Joint Medical Semi-Automated Forces (JMedSAF), which provides medical planning and rehearsal within a joint environment. JMedSAF simulates force-on-force interactions and models the treatment, transportation, and evacuation of the resulting casualties according to joint doctrine (Hardy et. al., 2001).
However, to ensure the success of a warfighting mission, the Navy must have the required medical resources necessary to support the operation. As a result, the Navy has developed software that models the medical component of a contingency so that medical planners and logisticians can research how to provide the best medical treatment possible within the constraints of a scenario. Such medical programs include the Ground Casualty Projection System (FORECAS), the Estimating Supplies Program (ESP), and the Medical Analysis Tool (MAT).

FORECAS is a software program developed by NHRC that is designed to provide medical planners with the estimates of the average daily rates of wounded in action (WIA) and nonbattle injury (NBI) patients during a specific scenario. NHRC developed these rates primarily based on the analysis of historical accounts of ground operations. A deterministic model, FORECAS assists medical providers by projecting the distribution of injuries and illnesses likely to occur within different warfighting environments (Blood et. al., 2003).

ESP is a program developed by NHRC for three purposes. First, ESP can be used as an estimation tool that projects the quantities (including weight, cube, and cost) and combinations of consumable supplies and equipment necessary to support the needs of a patient stream throughout the continuum of care. Second, ESP can be used as a decision tool that evaluates inventory readiness by assessing which supplies are missing and how these missing supplies affect medical treatment options. Third, ESP is a mapping and training tool that illustrates the relationship among PCs, tasks, supplies, and areas of care. As a deterministic model, ESP is most useful for generating the supplies needed to treat a user-defined patient distribution (Tropeano & Konoske, 2002).

MAT is designed as planning tool for the joint environment. Medical planners use MAT to both generate the medical requirements required to support patient treatment within a joint warfighting operation as well as develop and evaluate courses of action for this operation. As a tool for both deliberate and crisis-action planning, MAT determines the number of beds, the number of operating room tables, number and types of personnel, and the amount of blood required to treat the casualty stream. MAT also identifies bottlenecks within the system and assesses risk associated with the designated medical treatment facilities (Marghella, 2003).

Each of these programs provides useful information for preparing for a mission; however, there is still the need to model the flow of casualties within a specific network of treatment facilities from the generation of casualties through the treatment system, simulating the treatment times and demands on consumable supplies, equipment, personnel, and transportation assets in the far-forward environment. TML+ fills this need specifically for the Marine Corps and Navy, and could be expanded to include the data for a joint environment.

1.3 DESCRIPTION OF TML+

To simulate the flow of patients, the user enters the length of the scenario, enters the mean numbers of WIA, disease (DIS), and NBI expected to occur, and builds a treatment network by selecting the types and locations of levels of care and the transportation assets expected to evacuate patients. With these inputs, TML+ uses stochastic processes to model patient arrivals, treatment, and outcomes as they flow from the point of injury (POI) through a network of care facilities. TML+ currently:

- Generates a stream of patients occurring randomly in time and space among POIs.
- Generates the specific patient conditions (PCs) for each patient.
- Prioritizes the treatment and evacuation of patients based on the severity of injuries.
• Models mortality as killed in action, died of wounds as a function of complications, and died of wounds due to a delay in treatment.
• Simulates patient flow through levels of care (LOCs), including arrival times, wait times, and treatment times.
• Models the routing and utilization of transportation assets.
• Generates dynamic reports in graph and tabular formats that show the status of the medical treatment facilities, patient disposition, and resource utilization.

To successfully execute these functions, TML+ has a significant amount of underlying data that includes over 400 PCs developed by the Joint Readiness Clinical Advisory Board and NHRC; medical treatment tasks; task sequences; treatment times; consumable supplies and equipment; weight, cube, and cost of each supply item; died of wounds due to time; died of wounds due to complications; type, speed, and capacity of transportation assets; levels of care and their respective functional areas; number of personnel; and personnel skill sets.

2.0 METHODOLOGY

The methodology used to develop TML+ has three principal features: a Windows-based graphic user interface, an open architecture, and a four-module program structure.

2.1 GRAPHIC USER INTERFACE (GUI)

TML+ was designed as a user-friendly program with a Windows-based interface (see Figure 1). The GUI has four primary parts: the Scenario Explorer, the Common Data Explorer, the Properties box, and the Network View. The Scenario Explorer displays the user’s inputs for the treatment network while the Common Data Explorer shows the programs predefined levels of care and transportation assets as well as their underlying data. The Properties box displays specific attributes of the selected item and the Network View provides a graphic representation of the treatment network.
The user drags and drops the LOC and transportation icons from the Common Data Explorer onto the Network view, then connects these assets by drawing lines between the icons to establish the primary and secondary routes the patients travel. Once this treatment network is built, the user uses the Scenario Explorer in conjunction with the Properties box to input the length of the scenario, the theater of operations, and the number of WIA, DIS, NBI, and KIAs expected to occur during the scenario.

2.2 ARCHITECTURE

TML+ was designed as a non-proprietary program to be distributed to a multitude of users. In addition, TML+ has an open architecture built using C++ programming and the C#.net framework that can be expanded to include additional aspects of medical modeling (such as new treatment facilities, transportation assets, and personnel). This flexible architecture allows TML+ to be tailored to any type of warfighting environment.

2.3 FOUR TML+ MODULES

TML+ is a discrete event simulation program in which discrete units of traffic (in this case, patients) move from point to point in the system while competing with each other for the use of resources (personnel, equipment, consumable supplies, and transportation assets). TML+ has four modules: casualty generation, care providing, network/transportation, and reporting.
2.3.1 Module 1 - Casualty Generation

There are three ways to generate casualties: repeatable rate, table rate, and a user-defined casualty stream. The repeatable rate is the number of casualties expected to occur within a certain number of hours. The user enters the type of casualties (WIA, DIS, NBI), the mean number of casualties, and the time period within which they are expected to occur. This rate is repeated for the length of the scenario. For example, if a user enters a mean number of 12 WIAs every 24 hours, then approximately 36 WIAs are stochastically generated for a 72-hour scenario. TML+ assumes that casualties occur randomly in time according to a Poisson process where the inter-arrival time—the time between patient arrivals—is given by a random variable. TML+ stochastically generates the inter-arrival time based on an exponential distribution.

Exponential random variables are often associated with a waiting time that precedes the occurrence of certain specific events. For instance, the time that precedes an injury is a random variable that may reasonably be assumed to be exponential (Strait, 1983). Abramowitz and Stegun (1972) provide the derivation of the equation for randomly generating an exponentially distributed number.

The table rate allows the user to input different casualty rates across a collection of time periods, providing the ability to model the pulses and pauses in patient flow. Unlike the rate option, the numbers the user enters are generated once. Table 1 is a sample table rate casualty generator. From this input, TML+ would stochastically generate a mean of 2 casualties from hour 0 to hour 24:00, a mean of 3 from hour 24:01 to hour 48:00, and mean of 10 from hour 48:01 to hour 50:00.

<table>
<thead>
<tr>
<th>Casualty Type: WIA</th>
<th>Mean Number of Casualties</th>
<th>Ending Time Period (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Sample table rate casualty generator

The user-defined casualty generator offers the greatest control of the patient stream for the user. The user defines each casualty type, the specific PC code of the casualty, when it arrives into the system (in minutes), and the POI at which it occurs. Table 2 shows a sample user-defined casualty generator.

<table>
<thead>
<tr>
<th>Casualty Type</th>
<th>Arrival Time</th>
<th>Patient Code</th>
<th>POI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIA</td>
<td>2.0</td>
<td>68</td>
<td>POI1</td>
</tr>
<tr>
<td>WIA</td>
<td>5.0</td>
<td>178</td>
<td>POI1</td>
</tr>
<tr>
<td>WIA</td>
<td>25.0</td>
<td>55</td>
<td>POI2</td>
</tr>
<tr>
<td>NBI</td>
<td>38.0</td>
<td>32</td>
<td>POI1</td>
</tr>
<tr>
<td>DIS</td>
<td>39.1</td>
<td>166</td>
<td>POI1</td>
</tr>
</tbody>
</table>

Table 2. Sample user-defined casualty generator
Killed in action (KIA) casualties are determined by two criteria: the user-defined ratio and the Bernoulli probability function. First, the user enters a ratio of the amount of killed troops per group of injuries (for example, 1 KIA per 10 WIA). To appropriately simulate battle, KIA is drawn only from WIA casualties. Then, within the parameters of the identified KIA ratio, TML+ uses the Bernoulli probability function to determine when a WIA casualty is simulated to be KIA.

Casualties generated by the repeatable rate and table rate methods (the first two methods discussed above) that are not designated KIA are assigned a PC. In randomly generating the PC code of each patient, TML+ uses probabilities developed by NHRC where the individual PC probabilities sum to unity. To develop these rates, NHRC extracted data from historical accounts of ground operations, adjusted the data for factors such as recency of operation and medical advances, and computed the rates for battle intensity levels light, moderate, heavy, and intense (Blood et al., 2002). TML+ simulates a PC outcome for a particular casualty by comparing a randomly chosen value of the uniform distribution to the cumulative probability distribution of PC values determined by NHRC.

2.3.2 Module 2 - Care Providing

Once the casualty stream is generated, TML+ simulates the treatment of patients through a network of levels of care with varying medical capability. A level of care is defined as a facility with one or more functional areas while a functional area is defined by the personnel, equipment, and consumable supplies assigned to establish its medical capability. Figure 2 is a representation of the care providing process. There are two primary aspects of the Care Providing module: treatment and queuing. The patient arrives at the functional area and queues for treatment. When the required assets become available, the patient proceeds into the functional area to receive treatment.
Treatment is defined as the series of medical tasks required to treat a specific PC. Each PC is linked to a set of medical tasks, and each medical task is linked to the consumable supplies, equipment (including quantity, weight, cube and cost) and personnel required to accomplish that task. When the personnel and equipment required to treat the specific PC are available, treatment begins.

Each functional area has a generic task sequence (GTS), which defines the order in which medical tasks are performed to provide treatment to a casualty. In other words, the GTS orders all the medical tasks performed on all the PCs treated at that functional area. When a patient arrives at the functional area, the tasks associated with the specific PC are correlated against the functional area’s GTS; the subset of medical tasks required to treat the specific PC are performed in the order designated by the GTS. As the patient proceeds through the GTS, TML+ assigns the personnel and equipment required by each task for the duration of that task’s completion so that it cannot be assigned to another patient. When the task is complete, those resources are freed and returned to the equipment and personnel pool. In addition, the consumable supply quantities are decremented from the functional area inventory. When the inventory is exhausted, TML+ continues to track inventory consumption into negative amounts to make visible the quantity the inventory was short in treating the patient stream.

Treatment time in a particular functional area or LOC is defined as the total time to perform a set of tasks plus the waiting time that may be associated with each task. The simulated treatment time of the patients is based on three factors. First, each task has an average task time assigned to it. As the patient proceeds through the GTS, a random task time is calculated for each task based upon the exponential distribution using the average task time for that specific task (the same algorithm used to generate casualty inter-arrival times). Second, there are four types of tasks within the GTS: sequential, concurrent, continuous, and repeated:

- **Sequential tasks** are those that are performed one after another.
- **Concurrent tasks** are those that are completed simultaneously.
- **Continuous tasks** are those that use equipment for the patient’s length of stay in that functional area.
- **Repeated tasks** are those that are performed more than once during the patient’s stay at the functional area (for example, checking blood pressure every 4 hours in post-operative care).

The types of tasks completed to treat the PC influence the aggregated total treatment time across the tasks in a functional area. Third, treatment time is also influenced by the availability of assets. If the intensity of the casualty flow is high, the patients must wait for equipment and personnel assets. This wait time increases the average treatment time, indicating that the treatment network requires more assets to alleviate the accumulation of patients waiting for treatment.

Patient queuing occurs in two places within the care providing module: before treatment begins and during treatment. Before treatment begins, when a patient arrives at a functional area, TML+ determines if the personnel and equipment are available to perform the first task. If the medical resources are assigned a busy status, the patient is placed into a waiting pool. TML+ offers two types of service disciplines (who enters service first if several casualties are waiting); the user can either choose to treat patients on a first-in-first-out (FIFO) basis, which treats the casualty who has been waiting the longest, or a Priority basis, where the casualty with the highest severity is selected to receive treatment first. If FIFO is selected, patients entering the waiting pool are rank ordered based on arrival time. If Priority is selected, patients are rank ordered on the severity of the PC and arrival time.
When the assets needed to perform the first task are available, the patient moves from the waiting pool into the functional area and task treatment begins. For each subsequent task in the GTS, TML+ performs the same test to determine the availability of the required personnel and equipment assets—a patient cannot proceed to the next task until the necessary assets are free. Each task is assigned two personnel types, the default provider, which is the best-case provider, and the minimum provider, who has the skills necessary to complete the task. If the default provider is busy, TML+ determines if the minimum provider is available. If the minimum provider is also busy, the patient goes back into the waiting pool. Likewise, if the equipment items necessary to treat the PC are busy, the patient is placed back into the waiting pool, ordered by the severity of the PC and arrival time.

The patient disposition is checked at two points in the functional area, at the beginning and end of treatment. TML+ determines at the beginning of the GTS if the patient has died of wounds due to a delay in the start of treatment. An algorithm, developed from subject matter expert data that analyzed the deterioration of a patient who goes without treatment, gives a probability of survival (death) that can be compared to a simulated uniform random number. Based on the PC code and time since injury, the algorithm models the survival (or death) of the casualty at that specific point in the scenario.

Second, after treatment is completed, the final set of tasks in the GTS, called disposition tasks, determines the patient’s outcome (died of complications, returned to duty, transferred to the next functional area, or evacuated to the next level of care). Each disposition task for a PC has a probability of occurrence and the set sums to unity. A simulated uniform random number compared to the cumulative distribution of these four outcomes determines which disposition task is chosen for that specific patient.

2.3.3 Module 3 – Network/Transportation

The network/transportation module has four aspects: a treatment network definition that connects the LOCs, transportation assets that link these LOCs, transportation routing, and patient queueing for transport.

Treatment Network Definition

The user defines a treatment network by first selecting one of five canned configurations:

1. First Responder to BAS to CRTS
2. First Responder to BAS to SC to CRTS
3. First Responder to BAS to FRSS to CRTS
4. First Responder to BAS/STP to CRTS
5. First Responder to STP/FRSS to CRTS

The user can use the original five configurations or build on them by adding or modifying LOCs to represent more sophisticated and realistic treatment networks for various tactical scenarios. All configurations in TML+ assume the surviving casualty proceeds from a POI, where Self/Buddy Aid is received, to a First Responder location.
Transportation Assets

The user identifies the transportation assets for each LOC added to the treatment network. Each transportation asset is defined by an empty speed, a loaded speed, a maximum litter capacity, a maximum ambulatory capacity (2 ambulatory seats equal 1 litter space), patient loading and unloading time, and a maximum wait time that the transporter remains grounded waiting for a full patient load. The user can modify each of these aspects of the transporter.

Transportation Routing

The patient flows through the treatment network via transportation assets according to two types of routing rules: primary and secondary. The primary routes are established by the user’s definition of the treatment network and the rules defined in TML+ data restrict the path patients may travel based on the medical capabilities of the selected LOCs and functional areas as defined by Navy and Marine Corps doctrine. For example, a primary routing rule restricts patients from being evacuated from POI to BAS; it is assumed patients always receive treatment from a First Responder before evacuation. The secondary routes are determined by three steps. First, TML+ checks to determine if patient’s PC is treated at the next LOC. If no, the patient is queued for evacuation as described in the next section. If yes, TML+ next checks to determine if the LOC has a busy status, meaning that the patient capacity of the LOC has been reached and a certain quantity of patients (as designated by NHRC according to doctrine) are waiting for treatment. For example, if both beds in FRSS operating room are busy and two patients are waiting for treatment, FRSS has a busy status and patients are routed to the next LOC. If the LOC is free, the patient is queued for evacuation. If the LOC is free, TML+ stochastically determines by a random draw whether the patient is treated at that LOC. Because not all PCs are treated at every functional area, this random draw is based on the percentage of patients with a specific PC that are treated at a specific functional area.

Patient Queuing for Transport

Patient queuing begins when the evacuation call for the patient occurs. An evacuation call is placed when the patient reaches the task in the GTS that determines an evacuation disposition. TML+ checks the availability of transportation assets, which are prioritized by empty speed and capacity with the fastest and largest being called first to evacuate patients. In addition, the availability of the asset is determined by assessing all the demands made on that asset; the asset services the LOC with the highest priority casualty waiting to be evacuated. If the asset is busy, the patient queues for transport according to the service discipline, either FIFO or Priority. Patients entering the waiting pool are ranked based on arrival time. If the asset is available, the patient is assigned a claimed status and the capacity of the asset is decremented based on the PC’s designation as a litter or ambulatory. When the transportation asset reaches full capacity, it is assigned a busy status, which precludes it from being called for a subsequent evacuation run until its present assignment is completed. Travel time to the LOC is determined by dividing the distance to be traveled by the transporter’s unloaded speed.

2.3.4 Module 4 - Reporting

There are three layers of data in the Reporting module in TML+: raw database tables, stored queries, and queries within the GUI. TML+ outputs raw results to a Microsoft Access database. This database is then interpreted by stored queries in the report database. These stored queries then present the data in the report viewer in the GUI; the report viewer is dynamic, allowing the user to easily manipulate the output to view the desired data through drop-down menus and filtering options.
The general reports provide summary information on user inputs. In addition, TML+ creates an audit report, a detail intensive listing of each process completed by TML+. This report is not only useful for debugging purposes but can be used to obtain information currently not provided by other reports. In addition, TML+ provides assumption reports that make visible the assumptions of the program’s simulation processes and data. The casualty generation reports include the numbers and types of patients, the disposition of patients at each LOC, the distribution of PCs across patient categories. The reports are useful for determining if the treatment network is adequately providing the personnel mix and treatment facility capabilities necessary to treat the patient stream. The care providing reports include time-in-system information, the number of patients being treated at a functional area at one time, patient arrival time and treatment times, and consumable, equipment, and personnel usage. These reports are valuable for providing visibility on which LOCs may have congestion, for determining if the treatment network is treating patients quickly and efficiently, and for assessing whether the network has enough personnel, consumables, and equipment. The transportation reports include the number of evacuation trips per asset, trip times, average wait time for evacuation. These reports are valuable for assessing if the network has enough assets to meet the evacuation needs of the patient stream, and if they can be better distributed throughout the network. Importantly, the reports described above are only a subset of the possible reports that TML+ can provide. Because of the open architecture of the program and the filtering and sorting capabilities of Access, the TML+ reporting module can be tailored to report any information the user deems valuable.

3.0 CONCLUSION

The use of TML+, as a program that models the flow of patients through a network of treatment facilities, has at least two implications for the medical planning process. First, TML+ is an analysis tool that can perform different types of planning. In deliberate planning, TML+ can be used before deployment to determine the medical assets and the medical treatment network that would optimally treat the expected patient stream. In crisis-action planning, TML+ can be used during deployment to reconfigure the medical treatment network in response to contingency events. In near real-time planning, TML+ can be used to track how patient treatment and evacuation events proceed as the mission is performed. This versatility provides medical planners and providers with a well-rounded view of the medical requirements for a particular mission as well as helps planners respond quickly and efficiently to the rapidly changing warfighting environment.

Second, TML+ is a research tool that can help identify the best course of action for the anticipated patient stream with the projected assets. For example, TML+ can be used to determine whether a particular medical treatment facility can successfully handle a specific patient stream; how the relocation of a treatment facility affects patient treatment; which and how much of the supply, personnel, and transportation assets are utilized by the expected patient stream; and whether one network of care facilities is more efficient for treating patients than another network of care facilities. Such research can be used to both determine how to best configure and employ those assets already secured and to justify the procurement of additional assets to ensure a high standard of medical care.

REFERENCES


The Medical Triage Assistant: A Diagnostic Sensor Suite for Far Forward Medical Care

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ABSTRACT

A new method for obtaining critical physiologic data in combat injured war fighters is presented. The device is called the Medical Triage Assistant (MTA). This method uses a wearable glove format that has embedded sensors for electrocardiogram (ECG), pulse oximetry and body temperature. Data is collected in real-time. Results are presented applying this device to measure physiologic changes in an anesthetized pig model subjected to hypoxia, cold induced hypothermia, and pharmacologic induced bradycardia and tachycardia. MTA data is compared to data simultaneously measured by Edwards Lifesciences Explorer, Mallinckrodt digital thermometer and Nellcor pulse oximetry. Excellent correlation was noted between data obtained by the MTA and that by traditional methods.

1.0 INTRODUCTION

In the civilian sector, clinical management of trauma has evolved into a well established approach. It begins with early intervention by well trained paramedics or emergency medical technicians (EMTs). The key element of this prehospital care is immediate stabilization and rapid evacuation to a Level 1 trauma center. At the trauma center, highly skilled medical and nursing specialists intervene to provide definitive care. Vast resources in manpower, diagnostics and therapeutics are expended on each patient. The time from injury to advanced care is expected to be less than 1 hour, the “golden hour”, an approach that has been credited with improving the clinical outcome from traumatic injury.[1]
Although battlefield care has been traditionally based on the principals of civilian care, the application of civilian trauma to military trauma is differentiated by several key factors, and the ability to apply the principles of “golden hour” is often not possible[2]. While the military has improved forward care through the introduction of expeditionary medical units and forward surgical teams, the initial triage and management remains the responsibility of the military first provider (combat medic).

Military conditions are austere. Medical care must be administered in a resource limited, chaotic environment. Not only must the combat medic contend with multiple casualties requiring simultaneous care, care must be rendered under ground combat conditions, which can dramatically extend evacuation times. Furthermore, the combat medic generally does not have the same level of training and re-training of that of the civilian paramedic. Another confounding issue is the available resources. Highly variable evacuation time and patient load requires prudent dispensation of scarce medical resources. Medical supplies are limited to what medics can carry on their back; typically this is restricted to two 1-liter I.V. fluid bags, bandages and some pharmacologic agents such as analgesics. The availability of objective physiologic data would enable the judicious management of medical resources.[3]

An important advance in trauma management has been the development of the Advanced Trauma Life Support (ATLS) [4] and Advanced Cardiac Life Support (ACLS) guidelines[5]. Physiologic data is required to properly execute these algorithms, as these treatments are clinical response driven. Civilian paramedics have portable physiologic monitors to obtain this data such as Propaks. Unfortunately, these devices are too large and heavy for practical use by combat medics. Furthermore, interpretation of this data (ECG, pulse oximetry) is not taught to the combat medic. Although the Army is in the process of implementing training to increase the combat medic skill level requirements, it is not anticipated this transition will be completed until FY 09.

In the following, we describe a new device called the Medical Triage Assistant (MTA). It measures appropriate physiologic data (i.e., ECG, pulse oximetry, body temperature) that can be applied to well-established clinical algorithms for trauma management. A potential configuration for the sensor suite is a wearable glove with processing and display capabilities on a forearm worn device. To reduce medic training requirements, automated algorithms will be used to interpret the data signals and to recommend a course of action.

The need for a portable, noninvasive inexpensive device that can provide rapid reliable physiologic data is clear. The MTA device described here is designed for use by first responders such as combat medics and civilian EMTs. The clinical utility of the MTA is expanded, as only one device is needed to care for multiple patients. Vital signs provided by the device will allow first responders to make earlier patient management decisions and initiate well established clinical guidelines, thereby optimizing the potential for positive patient outcome.

2.0 METHODS

Male adult Yorkshire pigs weighing from 30-50 kg (n=6) were used in these studies. Animals were housed and cared for under the guidelines of the NIH standards for laboratory animal care. This protocol received full approval by the USUHS Institutional Animal Care and Use Committee (IACUC).

Each pig was sedated with Ketamine (10 mg/kg, I.M.) and Xylazine (2 mg/kg, I.M.). Next, a 20g. angiocatheter was placed into an ear vein to permit administration of an intravenous anesthetic dose of pentobarbital (20mg/kg) and blood sampling. Femoral vein and artery catheters were placed. Pigs remained fully anesthetized throughout study.
For respiratory studies only, pigs were paralyzed with Pancuronium (0.1 mg/kg, I.V.) so that the rate could be varied with the ventilator setting. These pigs were intubated with an endotracheal tube and placed on mechanical ventilation. Oxygen was delivered at a FiO2 of 0.35. A Nellcor pulse oximeter was placed on the ear, a Mallinckrodt digital thermometer was placed on the pit of the left foreleg. Standard ECG surface leads were placed over the chest and connected to an Edwards Lifesciences Explorer physiologic monitor. Repeated doses of pentobarbital (5 mg/kg, I.V.) were administered every 15 minutes to maintain anesthesia. MTA sensors were placed in corresponding locations.

Bradycardia was induced with Esmolol and tachycardia induced with atropine. Hypoxia was induced by decreasing ventilator rate. Hypothermia was induced by administering cold (4°C) saline, I.V.

At the conclusion of study, while still under anesthesia, pigs were euthanized with an I.V. injection of 50cc of saturated KCl solution.

Data from the traditional monitoring methods was compared to MTA data using paired t-test. ECG data was collected as discrete digital data and was also compared using the paired t-test. In addition, ANOVA was applied to data. Significant difference was predetermined to be p<0.05.

3.0 RESULTS

The results of this study are seen in the following figures.

The ECG profile obtained by the MTA during pharmacologically induced bradycardia is shown in Figure 1, and correlates with that from the Edwards Lifesciences Explorer. Analysis showed no significant difference between the 2 groups (p>0.05).
The ECG profile obtained by the MTA during pharmacologically induced tachycardia is shown in Figure 2, and correlates with that from the Edwards Lifesciences Explorer. Analysis showed no significant difference between the 2 groups (p>0.05).

The pulse oximetry data obtained by the MTA is shown in Figure 3, and closely parallels that data obtained from the Nellcor pulse oximeter. Analysis showed no significant difference between the 2 groups (p>0.05).
Figure 4, which shows the temperature data obtained from the MTA, correlated well with that obtained from the Mallinckrodt digital thermometer. Analysis showed no significant difference between the 2 groups (p>0.05).

4.0 CONCLUSIONS

This study in anesthetized pigs demonstrates that the Medical Triage Assistant (MTA) is able to detect and record ECG, pulse oximetry and body temperature over physiologically relevant ranges. The data obtained by the MTA is the same as that measured by traditional methods. In addition, the data is real-time. The major difference between the MTA and currently used devices is that the MTA is much smaller and lighter.

5.0 ACKNOWLEDGEMENTS

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6.0 REFERENCES

Non-Invasive Hemoglobin Monitoring during Hemorrhage and Hypovolemic Shock

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ABSTRACT

Background: Serial blood draws for the assessment of a trauma patient’s hemoglobin (sHgb) and hematocrit (sHct) is standard practice. In the event of multiple casualties this process can be time consuming and lead to the inefficient use of valuable resources. A device that would allow for continuous real-time, non-invasive monitoring of hemoglobin and tissue perfusion would not only improve the utilization of scarce and valuable resources but would also improve triage efforts. Purpose: We developed a device utilizing the technology of Diffuse Optical Spectroscopy (DOS) to obtain non-invasive measurements of tissue hemoglobin concentration (THC) and oxygen consumption in an animal model of hypovolemic shock induced by successive blood withdrawals. Measured DOS results were compared against invasive systemic physiological measurements to demonstrate that DOS provides a reliable non-invasive measurement of tissue THC, and also quantifies various degrees of hemorrhage induced systemic hypovolemia and subsequent tissue perfusion decreases.

Methods: Intubated New Zealand White rabbits (N=16) were hemorrhaged via a femoral arterial line every 10 minutes until a 20% blood loss (10-15 cc/kg) was achieved to attain hypovolemia. A DOS probe was placed on the inner thigh to measure muscle concentrations of oxygenated-Hgb (Hb-O2) and deoxygenated-Hgb (Hb-R) during bloodletting. THC and tissue hemoglobin saturation (S\textsubscript{T}O\textsubscript{2}) were calculated using oxygenated and deoxygenated hemoglobin concentrations. DOS-measured values were compared against traditional invasive measurements, systemic hemoglobin (sHgb), arterial oxygen saturation (S\textsubscript{AO2}), and venous oxygen saturation (S\textsubscript{VO2}) drawn from arterial and central venous blood. Systemic blood pressure (mAP), heart rate (HR) and S\textsubscript{AO2} were monitored throughout the entire experiment. Results: DOS and traditional invasive measurements versus blood loss were closely correlated (R=0.98 and R=0.97, respectively) showing a decline in both. S\textsubscript{T}O\textsubscript{2} and Hb-O\textsubscript{2} followed similar trends with hemorrhage whereas an increase in Hb-R was observed. Conclusion: DOS provides a potential platform for reliable non-invasive measurements of tissue oxygenated and deoxygenated hemoglobin and may accurately reflect the degree of systemic hypovolemia and compromised tissue perfusion.

INTRODUCTION

Hemorrhage remains a leading cause of death in combat and major trauma [1]. In multiple traumas, rapid assessment of victims that are critically volume depleted is necessary to reduce morbidity and mortality associated with hypoperfusion and gauge resuscitation. However, during the acute hemorrhage, systemic

hematocrit (sHct) and hemoglobin (sHgb), may be artificially normal or increased due to the inherent lag time in the body’s fluid shifts[2]. Therefore, observation in peripheral perfusion may be a more representative indicator of volume status. A means for a quick and portable non-invasive assessment of tissue hemoglobin concentration (THC) and perfusion would increase efficiency in diagnosing patients in greatest need for volume replacement and may aid in the assessment of those patients undergoing resuscitation. In the hospital setting, evaluation of the critically ill patient often consists of systemic hemodynamic monitoring by invasive central venous access and by serial blood draws. Therefore, having the ability to assess tissue hemoglobin and perfusion parameters non-invasively in real time may reduce the cost and complications associated with invasive monitoring techniques.

To address these concerns, tissue oxygen hemoglobin saturation monitoring with near infrared spectroscopy (NIRS) has been proposed as a possible alternative to invasive monitoring [3-10]. These NIRS devices exploit blood chromophore properties of light absorption at characteristic frequencies. Accordingly, the amount of light absorbed is directly proportional to the chromophore concentration. These instruments, however, are limited by their ability to measure only light absorption and do not account for light scatter that occurs in complex tissues. While NIRS devices are able to monitor relative changes in tissue chromophore concentration, this results in significant limitations, with the inability to accurately differentiate absolute concentration of oxygenated tissue hemoglobin (Hb-O₂) from deoxygenated tissue hemoglobin (Hb-R).

Diffuse optical spectroscopy (DOS) is a novel technique that is able to simultaneously measure both light absorption as well as light scatter in turbid media and tissue [11-15]. Instrumentation based on this theory is not bound by the restrictions seen in NIRS, and as a result has the potential to accurately measure absolute tissue chromophore amounts; especially those of considerable importance, Hb-O₂ and Hb-R. Using a prototype instrument developed in our laboratory [11, 12, 16, 17], we were able to demonstrate that DOS-derived physiologic hemoglobin properties correlated with invasive measurements of cardiac output (CO), mean pulmonary artery pressure (mPAP), mean systemic arterial pressure (mAP), and arterial oxygen saturation (SₐO₂) [17]. In this report, to address how DOS reflects changes of sHgb during blood volume depletion, we compared hemoglobin obtained from a complete blood count (CBC) drawn invasively to non-invasive tissue hemoglobin DOS measurements (THC = Hb-O₂ + Hb-R) with emphasis in delineating Hb-O₂ from Hb-R.

**MATERIAL AND METHODS**

This protocol was approved the University of California, Irvine Animal Research Committee, protocol No. 2000-2218.

*Anesthesia and Intubation.* Male New Zealand White rabbits (N=16) (Myrtle Rabbitry Inc., Thompson Station, TN) weighing 4.0 ± 0.4 kg were anesthetized with a ratio of Ketamine HCI (100mg/ml) (Ketaject, Phoenix Pharmaceutical Inc., St. Joseph, MI):Xylazine (20mg/ml)(Anased, Lloyed Laboratories, Shenandoa, IA) at a dose of 0.75 cc/kg IM. After the IM injection a 22-24 gauge 1 inch catheter was placed in animal’s marginal ear vein to administer IV anesthesia and secured with 1 inch standard porous adhesive tape. Maintenance anesthetic was dosed at 0.3cc of 1:1 mixture of Ketamine:Xylazine IV (Ketamine 100 mg/ml:Xylazine 20 mg/ml) as needed. Depth of anesthesia was monitored according to established guidelines [18]. Animals were intubated with a 3.0 endotrachael tube and mechanically ventilated (Harvard apparatus dual phase control respirator: South Natick, MA) at respiration rate of 32/min and a tidal volume of 50cc and FiO₂ of 100%. Pulse oximetry was accomplished with a probe placed on the forlimb to measure SₐO₂ (Biox 3700 Pulse Oximeter, Ohmeda, Boulder, CO) and compared to arterial blood gas measurements.
Cardiac Output and Pulmonary Artery Pressure. After adequate anesthesia a median sternotomy was performed to expose the heart. A calibrated flow transducer (T106 small animal flow meter, Transonic System, Inc, Ithaca, NY) was placed around the ascending aorta to determine cardiac output (CO). The mean CO was determined from a 10 second sample. Pulmonary artery pressures were obtained by placement of an 18-gauge catheter in the pulmonary artery and connected to a calibrated pressure transducer (TSD104A transducer and MP100 WSW System, Biopac Systems, Inc, Santa Barbara) and collected digitally. Mean, systolic, and diastolic pressures were determined from 5-10 second tracings.

Blood Gas Analysis and Complete Blood Count. A right femoral arterial line was placed for arterial blood draws and systemic pressures. After all blood draws lines were flushed with less than 0.5 cc of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) to prevent line thrombus occlusion. Arterial blood samples were measured with a blood gas analyzer (IRMA Series 2000 Blood Analysis System, Diametrics Medical Inc., St Paul, MN). Mixed venous blood samples were drawn from the pulmonary artery.

Complete blood counts (CBC) were obtained from collected samples of venous blood and sent to an outside facility (Antech Diagnostics, Irvine, CA) for measurements.

Non-Invasive measurements (Diffuse Optical Spectroscopy). Detailed analysis of DOS has been described previously in detail [11, 12, 16]. Briefly, a multi-wavelength, frequency domain instrument (FDPM) was combined with a steady state near infrared (NIR) spectrometer for the non-invasive in vivo measurement of tissue chromophore concentration. Broadband DOS employs six laser diodes (661, 681, 783, 823, 850, and 910 nm) and a fiber-coupled avalanche photo diode (APD) detector (Hamamatsu high-speed APD module C5658). The APD detects the intensity-modulated diffuse reflectance signal at modulation frequencies between 50 to 550 MHz after propagating through the tissue. The absorption and reduced scattering coefficients are measured directly at each of the six laser diode wavelengths using the frequency-dependent phase and amplitude data [11-13, 16]. The reduced scattering coefficient is calculated throughout the NIR by fitting a power-law to these six reduced scattering coefficients [19-21]. The steady-state acquisition is a broadband reflectance measurement from 600 nm to 1000 nm that follows the FD measurements using a tungsten-halogen light source (FiberLite lamp) and a miniature spectrometer (Ocean Optics USB2000). The intensity of the steady-state (SS) reflectance measurements are calibrated to the FD values of absorption and scattering to establish the absolute reflectance intensity. The absolute steady-state reflectance spectra are then analyzed to calculate $\mu_a$ spectra. Finally, the tissue concentrations of Hb-O$_2$, Hb-R, and H$_2$O are calculated by a linear least squares fit of the wavelength-dependent extinction coefficient spectra of each chromophore.

Experimental Model. After completion of the sternotomy, baseline measurements of the above mentioned variables were obtained, and non-invasive assessment of Hb-O$_2$ and Hb-R was completed. The first hemorrhage was accomplished by withdrawing blood via a femoral arterial line (15cc). The blood drawing and measurement process was repeated every 20 minutes until a 20% blood loss (15 cc/kg) was achieved to attain hypovolemia. An entire experiment lasted for approximately 60 minutes. At completion of the experiment each animal was euthanized with an intravenous injection of Eutha-6 (1.0 -2.0 cc) through the marginal ear vein catheter according to animal laboratory guidelines (Institutional Laboratory Animal Care and Use Committee, University of California, Irvine ARC protocol No. 2000-2218).

RESULTS

Diffuse optical spectroscopy versus cardiac output and systemic mean arterial pressure. DOS measurements of THC and tissue oxygen saturation ($S_tO_2 = \frac{[Hb-O_2/THC]}{100\%}$) demonstrated similar trends with systemic
arterial pressure and cardiac output (Figs 1 and 3). All parameters displayed a decreasing trend with successive blood withdrawal.

Diffuse optical spectroscopy versus systemic hemoglobin values. Both measurements of DOS THC and systemic hemoglobin (sHgb) displayed a downward trend with blood volume withdraw (Fig 2). DOS demonstrated a greater percent change when compared to systemic values. Significant differences compared to baseline (p<0.05) were noted in DOS THC and not sHgb at the initial blood draw. DOS THC and sHgb versus blood loss were closely correlated (R=0.98 and R=0.97, respectively).

Diffuse optical spectroscopy tissue oxygen saturation versus systemic arterial and venous oxygen saturation. Arterial oxygen saturation (SaO2) was maintained above 97% throughout the experiment. Systemic venous oxygen saturation (SvO2), however, decreased during blood volume withdrawal (Fig 3). Similarly, a decline of about 20% in DOS tissue oxygen saturation (STO2) was observed. At the end of the hemorrhage period STO2 appeared to be in equilibrium equivalent to SvO2 measured directly.

Diffuse optical spectroscopy tissue oxygenated and deoxygenated hemoglobin. When delineating tissue Hb-O2 and Hb-R components from the combined THC, both Hb-O2 and Hb-R showed a decline in an absolute amount during hemorrhage (Fig 4 and 5, respectively). This, however, was primarily due to the drop in the THC. When normalizing for this decrease in THC by dividing these parameters by the total tissue hemoglobin it was observed that the relative oxygenated hemoglobin (Hb-O2/THC) displayed a significant drop (Fig 4). In contrast, an increase in the relative deoxygenated hemoglobin (Hb-R/THC) was observed (Fig 5). When taking a ratio of the absolute concentration of Hb-O2 and Hb-R a decline of about 33% from baseline was noted (Fig 6).

DISCUSSION

These results demonstrate that DOS can non-invasively quantify Hb-O2 and Hb-R. DOS measured decreases in THC, which paralleled sHgb, during hemorrhage. The high sensitivity of DOS is realized by greater percent changes in DOS-measured tissue hemoglobin concentration compared to changes in systemic invasively measured hemoglobin concentrations, which take time to equilibrate.

Previous NIRS studies have been applied in the use of monitoring tissue oxygen saturation [3-7]. These devices, however, are limited because tissue optical properties change under varying degrees of hypovolemia and, as a result, confound observations that are associated with the baseline measurements. While all NIRS devices can measure tissue oxygen saturation, many lack the ability to measure absolute concentrations of oxygenated and deoxygenated hemoglobin in tissue. This limitation arises from an inability to measure tissue light scattering, which is unfortunate because scattering is the dominant effect in NIR light transport [22]. To compensate for this, NIRS often uses calibration curves or average path length calculations derived from healthy subjects. These corrections can provide reliable measurements for hemodynamically stable patients. However, as stated above, since both tissue scattering and absorption change during volume depletion, these results become unreliable when acute systemic changes occur. In addition, photon path lengths display a high degree of intra-subject variation, which complicates absolute comparisons in both individuals and across populations [23]. DOS compensates for this by measuring both tissue absorption and scattering properties directly, and can therefore measure absolute tissue deoxygenated and oxygenated hemoglobin concentrations without the need to generate a calibration curve for each series of observations.

This added functionality serves well in assessing acute hemorrhage. Often systemic hemoglobin measurements do not reflect volume loss until compensation by the intracellular and interstitial fluid
compartment occurs [2]. Furthermore, vasoconstriction mechanisms by skin and muscle microvasculature compensate for hypovolemia by shunting blood centrally, and as a result, further decrease peripheral tissue hemoglobin. DOS provides a unique advantage by detecting these decreases in tissue hemoglobin sooner than that which would be observed by systemic hemoglobin measurements (Fig 2). Therefore, DOS may detect these peripheral changes initiated by hypovolemia before systemic signs are present and may provide further insight in regard to the patient’s perfusion state.

Although volume depletion may be observed early with DOS, this does not imply the body is in oxygen debt. Previous studies have shown that the peripheral tissue is able to adjust metabolic demand to the available oxygen supply [24]. In the initial acute hemorrhage, compensation mechanisms allow for a continual oxygen supply for the vital organs. During more advanced stages of acute blood loss, however, the body may exhaust its oxygen reserve and compensating mechanisms, resulting in a decrease in SvO2 (Fig 3), and produce an oxygen debt. Non-invasive measurements of tissue hemoglobin oxygen saturation in conjunction with systemic SvO2 values may elucidate this critical point. Since pulse oximetry relies mainly on arterial oxygen saturation and is mainly dependent on lung function and not the body’s metabolic state, non-invasive measurements of SvO2 may also provide a significant compliment to these traditional measurements especially in environments where blood gas or central venous monitoring may not be available.

A critical advantage DOS has over previously described NIRS devices is the ability to measure absolute concentrations of Hb-O2 and Hb-R. Out in the field, the initial assessment of a trauma victim is limited to available monitoring devices. As a result it is difficult to assess the degree of hypovolemia or more importantly, hypoperfusion. NIRS devices are limited in these situations because of the need to start with a baseline measurement and compare future measurements with these over a given time period or perturb tissue using phylesmographic analysis. Although these devices may be useful in the assessment of the resuscitation period, they lack the ability to assess the patient’s initial perfusion state. DOS measurements provide the capability of giving an initial assessment to the perfusion state of the patient, by providing Hb-O2 and Hb-R that is derived from absolute hemoglobin concentrations. For example, decreased ratios of Hb-O2 to Hb-R suggest oxygen debt whereas increasing ratios are more suggestive of an oxygen reserve (Fig 6). Applying this data with the vital signs and clinical observation one may be able to provide a rapid assessment of the patients perfusion state, and depending on the available resources, initiate the resuscitation effort, or as in the case of multiple traumas rapidly move to assess the next injured party.

A significant amount of resources are needed for assessment of volume depletion in the critically ill patient, often consisting of surveillance serial blood draws. This is not only time consuming but occurs at a substantial price. A non-invasive, continuous real time assessment would be a more affordable and efficient way to monitor signs of hypovolemia and of possible hypoperfusion. DOS has the potential to offer these capabilities at the initial exam and over a continuous time period. If proven efficacious in the critical trauma setting, the eventual goal of the device would be a hand held portable system that would allow for transfer of data from medics in the field to physicians at a base if patients were not accessible to immediate health care. This device would not only free up valuable resources that are needed for mass causality but could provide potential information and instruction from distant bases to assess those who are in most urgent need of care.

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Non-Invasive Hemoglobin Monitoring during Hemorrhage and Hypovolemic Shock


Figure 1: Total tissue hemoglobin concentration (THC) and systemic parameters versus volume loss. Cardiac output (CO) and mean arterial pressure (mAP) declined with hemorrhage when compared to baseline measurements. THC followed a similar trend.
Both sHgb and THC demonstrated a drop with progressive hypovolemia. A significant (p<0.05) reduction in THC during the initial blood draw was observed when compared to baseline measurements. This was not apparent with sHgb, although by the second blood draw both had significantly decreased.
Figure 3: Arterial ($S_aO_2$), venous ($S_vO_2$), and tissue oxygen ($S_TO_2$) saturation versus hemorrhage. $S_aO_2$ was maintained constant while a decline in $S_vO_2$ occurred with blood loss. $S_TO_2$ obtained by DOS showed a 33% reduction when compared to baseline.
Figure 4: Non-invasive measurements of tissue oxygenated hemoglobin (Hb-O₂). A reduction in both THC and Hb-O₂ hemoglobin occurred with hemorrhage. When normalizing for the systemic drop (Hb-O₂/THC) a notable decline was still present.
Figure 5: Non-invasive measurements of tissue deoxygenated hemoglobin (Hb-R). A drop in both THC and Hb-R occurred with blood loss. However, when normalizing for the systemic drop (Hb-R/THC) a relative increase of Hb-R was observed.
Figure 6: Absolute concentrations of tissue oxygenated (Hb-O$_2$) and deoxygenated (Hb-R) hemoglobin. Hb-O$_2$ decreased relative to Hb-R of about 33% from baseline.
Time-Critical Decision Making in Casualty Care during Special Operations – A Proposed Tactical Combat Casualty Care (T3C) Flowchart-System as a Learning Tool

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SUMMARY

The in Tactical Emergency Medical Support interested but civilian anaesthetist author tried to create an algorithm for the management of casualties in tactical setting. During this work was he confined over the current civilian pre–/hospital trauma textbooks only to the freely accessible printed and electronic military sources.

An electronic correspondence with two experts of this topic [Col.(ret.) Clifford C. Cloonan MD, FACEP and Capt. Frank K. Butler, Jr, MD] pointed out that the traditional civilian emergency medical approach is suitable for this case only with important restrictions and modifications due to necessities of the special operations. Their advises have changed from an originally pathophysiologically oriented, strictly designed, and slightly complicated algorithm to a more practical, loose and simple flowchart.

Unfortunately there is not any official and organized interest in Hungary for TEMS, therefore the author can’t report concrete results but he would get gratefully any feedback from the professional providers.

1.0 INTRODUCTION

The Combat Casualty Care is perhaps the most exciting type and biggest challenge of the pre–hospital emergency medicine. The injury management during special operations could be even more complicated than in conventional warfare (e.g. need for an advanced but covert trauma care because of the permanent tactical threat, by a non–physician provider with spread function, with remarkably limited sources, until an extremely delayed evacuation, mission’s concern etc.). [1]

1.1 Time–Critical Decision Making (TCDM)

The term of TCDM derives from informatics. Originally it covers merely those decision processes where the state of the object is permanently changing and the end–state depends on the time of the right decisions. The management of an seriously injured casualty [2] — especially in tactical setting — is its excellent example: critically important decision, within a critically short time, under critically difficult circumstances).

1.2 The Role of the Flowcharts in the Emergency Medicine

During the solving of problems we try often to reduce them from difficult to a simple ‘yes/no’–pattern. If we can weight the problems and find the right priority, we can easily manage even life–threatening medical problems (e.g. BLS in cardiac arrest). At least from educational reasons is advisable to make

simple flowcharts even for non–physician medical personnel to memorize the right sequences of these important tasks, even if it seems to oversimplify the problem.

1.2.1. Basic Life Support Algorithm (BLS)
The Peter Safar’s ABC of Life Saving (i.e. Airway–Breathing–Circulation) is since 1960 the standard of the basic level cardiopulmonary resuscitation. The International Liaison Committee on Resuscitation uses since 1997 BLS–flowcharts. The current BLS–Algorithm of the Hungarian Resuscitation Council [3] shows a good example of a decision aid.

1.2.2 Flowcharts for Management of Seriously Injured Casualties
The use of algorithms in critical care (including trauma management) is associated with the scientific activity of William C. Shoemaker [4]. One of his European follower, the German trauma surgeon Karl–Georg Kanz makes excellent flowcharts about both pre– and hospital trauma care [5]. According its philosophy was based the original version of the T3C–Algorithm on it. The recent (5th) edition of PHTLS — Basic and Advanced Prehospital Trauma Life Support textbook contains didactic flowcharts too.

1.3 The Medical Problem
The management of major traumas is unfortunately not so simple as the BLS. Instead of successive ‘step–by–step’ approach are often simultaneous interventions necessary; as well as is the classic A–B–C sequence often only theoretically the best answer.

1.3.1 Current Advanced Trauma Life Support Programs
Although have the existing various Trauma Life Support education programs essentially similar ideology, they are not always to interchange.

1.3.1.1 Hospital vs. Pre–Hospital Approaches
The classical Advanced Trauma Life Support™ approach is suitable only for the treatment in hospitals. Its prehospital derivatives (such as the Prehospital Trauma Life Support™ and the Basic Trauma Life Support™) concern in treatment at the scene of trauma without enormous additional danger but with relatively good logistical background (medical material, equipment, rapid transport and appropriate receiving facility). Although all the three programs can be a part of the military medical curriculum, they are not a real solution for the battlefield casualties.

1.3.1.2 Civilian vs. Military Community
Also some Armies have own trauma programs of different level (e.g. Combat Lifesaver Course, Trauma AIMS, Battlefield Advanced Trauma Life Support™ [6] etc.). These courses covers then either the Self–/Buddy–Aid or the +2nd Echelon Care. For lack of an appropriate solution for the Special Operations wrote Butler, FK jr. et al. their famous and often cited article about the Tactical Combat Casualty Care in Special Operations [7]. The analyse of the SOF casualties wounding and given treatment provides the scenario–base of training for further SOF–medics. [8;9]

1.3.1.3 European vs. American View
There are some differences in the prehospital trauma care policy between USA and Europe, as well as among the European countries (e.g. equipment supply and skill competence of provider, physician presence at the scene, treatment’s time restriction, accessibility of Level I/II trauma centres, trauma epidemiology etc.).
1.3.2 Evidence Based Medicine (EBM)

In time of EBM is the lack of scientific evidences particularly troublesome. The traditional prehospital emergency medicine is rather ‘common sense’–based and the apparently logical answers could be debated even on base of results provided by necessarily restricted combat casualty care. Unfortunately it is very difficult to make scientific investigations in prehospital, even in combat setting.

2.0 THE T3C FLOWCHART–SYSTEM

Though is the current version of the T3C–Algorithm approximate to the other usual civilian and military pre–/hospital trauma treatment guidance, is it based on Tactical Combat Casualty Care [1;7–9].

2.1 Structure

The main flowchart (Figure 1) follows the classic triple division of the treatment according to the tactical situation (Care Under Fire, Tactical Field Care and Tactical CASEVAC). In its perpendicular axis align the questions. The symbols with double frame could be opened into separate sub–algorithms. The decisions are supported with various check–up lists and matrices.

2.1.1 Care Under Fire Phase

The upper half depicts the first tasks supplemented by additional flowcharts (an operational risk assessment and management algorithm for the tactical extrication; a Simple Triage and Rapid Treatment™ like or other type solution for multiple casualties and a flowchart about the use of tactical tourniquet).

2.1.2 Tactical Field Care and CASEVAC Phase

For the recognizing of a significant injury requiring an immediate treatment was assembled a specially modified ABC as a mnemonic. Its categories are simple to judge without any instrument even by a non–medic. At the same time — if advanced equipment present (e.g. portable vital sign monitor, continuous vital sign sensing) — is the loose diagnostic framework easily to augment.

2.2 The Part of Advanced Trauma Care

At the bottom of the flowchart are shown parallel the allusions to the advanced supportive treatment. The circulatory support remains centred: after the possible stopping of life–threatening external bleeding helps a left–to–right shift (from E to C) the stabilization. Without a rapid CASEVAC has a demand for advanced respiratory support — caused by either a permanent severe hypotension despite the appropriate volume replacement or a serious traumatic brain injury — a very bad prognoses and poor chances for the casualty in a tactical setting.
2.3 Available Sub-algorithms (not shown here):

2.3.1 Chest Decompression at Suspected Tension Pneumothorax

2.3.2 Airway and Ventilatory Management (with emphasized using of supraglottic airways)

2.3.3 Cervical Spine Clearance and Immobilization

2.3.4 IV/IO–Access

2.3.5 Fluid Replacement — with a 3–D Combat Fluid Matrix

2.3.6 Treatment of the Skeletomuscular Injuries

The Flowchart–System with its cross references as a learning tool is currently for presentations purpose only but with a supervised, validated content and in an appropriate format (e.g. PDA) could it be perhaps a real–time decision aid too.
Check-Up: Injury Mechanism

- High Energy Impact Trauma, e.g.:
- Fall from Significant Altitude
- All High Speed (Vehicle) related Accident
- Blast Injury
- Penetrating or Blunt Injuries Proximally from Elbow/Knee w. Severe Tissue Damage and/or Functional Impairment of:
  - Cardiorespiratory System
  - Central Nervous System
- Obvious or Hidden Massive Blood Loss
- Special Significant Environmental Emergencies e.g.:
  - Temperature related
  - Altitude and Water related
  - Venom and Other Intoxication

*The Chosen Methods & Used (Issued and/or Improvised) Equipment depend on:
- Tactical Situation (CUF vs. TFC vs. TEC)
- Competence & Skill Practice of Given Provider

Probable Diagnosis: Serious Combat Injury (in Tactical Setting)

Rapid Assessment of the Situation

Still Living Casualty (in all probability?)

Care Under Fire Conditions?

Massive External (e.g. Limb) Bleeding?

Systematic Focused Body Check-Up

Request for Immediate CASEVAC?

Stabilized Casualty?

Advanced Wound & Fracture Management* (P.R.N.)

Advanced Circulatory Support* (P.R.N.)

Advanced Respiratory Support* (P.R.N.)
[1] Butler, FK, Jr.: Medical support of special operations. In Burr, RE; Bellamy, RF [Eds.]: *Medical operations in harsh environments (Textbook of Military Medicine)*


**Acknowledgement:**

I have to thank Capt. Butler, F.K. jr. and Col. Cloonan, C.C. for their advises and enormous help specially.
DARPA Soldier Self Care: Rapid Healing of Laser Eye Injuries with Light Emitting Diode Technology

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Abbreviations: LED, light-emitting diode; ERG, electroretinogram; LRRI, log relative retinal illumination; RGC, retinal ganglion cell; TTX, tetrodotoxin.

Photobiomodulation by light in the red to near infrared range (630-1000 nm) using low energy lasers or light-emitting diode (LED) arrays has been shown to accelerate wound healing, improve recovery from ischemic injury and attenuate degeneration in the injured optic nerve. At the cellular level, photoradiation at low fluences can generate significant biological effects including cellular proliferation and the release of growth factors from cells. Mitochondrial cytochromes have been postulated as photoacceptors for red to near-infrared (NIR) light energy and reactive oxygen species or mitochondrial redox changes have been advanced as potential mediators of the biological effects of this light.

We hypothesize that the therapeutic effects of red to near infrared light result, in part, from intracellular signaling mechanisms triggered by the interaction of NIR light with the mitochondrial photoacceptor molecule cytochrome oxidase which culminate in improved cellular mitochondrial energy metabolism and

antioxidant production. In support of this hypothesis, we have demonstrated in primary neuronal cells that NIR-LED photo-irradiation (670 nm at 4 J/cm²) increases the production of cytochrome oxidase in cultured primary neurons, reverses the reduction of cytochrome oxidase activity produced by metabolic inhibitors and attenuates cyanide-induced apoptosis. We have also shown that the action spectrum of NIR light for stimulation of cytochrome oxidase activity parallels the near-infrared absorption spectrum of the oxidized form of cytochrome oxidase. More recent studies have provided evidence for the therapeutic benefit of NIR-LED treatment in the survival and functional recovery of the retina and optic nerve in vivo after acute injury by the mitochondrial toxin, formic acid generated in the course of methanol intoxication. The prolonged effect of brief NIR-LED treatment implies that it induces a cascade of events leading to the stimulation of gene expression, protein synthesis, and oxidative metabolism. Gene discovery studies conducted using microarray technology have provided additional insight into the mechanism of NIR-LED action in the retina. These studies have documented a significant upregulation of gene expression in pathways involved in mitochondrial energy production and antioxidant cellular protection. We also have preliminary data indicating that 670 nm LED treatment promotes retinal healing and improved visual function following high intensity laser-induced retinal injury in adult non-human primates and improves peripheral visual function in LHON patients with central vision loss. Importantly, there was no evidence of damage to the retina or optic nerve following 670 nm LED treatment in either the experimental or clinical studies. Based on these findings we propose that NIR-LED photobiomodulation represents an innovative and non-invasive therapeutic approach for the treatment of retinal injury and disease.

In summary, studies by our research group in the last year of funding have demonstrated that NIR-LED treatment: (1) heals poisoned neurons by stimulating cytochrome oxidase activity; (2) protects against retinal damage and improves the recovery of retinal function in a rodent model of mitochondrial poison-induced blindness and (3) promotes retinal healing and improved visual function following high intensity laser-induced retinal injury in adult non-human primates.

VISION

Non-invasive treatment of retinal and optic nerve injury using light-emitting diode (LED) arrays delivering monochromatic light in the red to near infrared range (630-1000 nm).

BACKGROUND:

Photobiomodulation by light in the red to near infrared range (630-1000 nm) using low energy lasers or light-emitting diode (LED) arrays has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured optic nerve (19-24). At the cellular level, photoirradiation at low fluences can generate significant biological effects including cellular proliferation, collagen synthesis and the release of growth factors from cells (22, 25, 26). Our previous studies have demonstrated that LED photoirradiation at 670 nm (4 J/cm²) stimulates cellular proliferation in cultured cells and significantly improves wound healing in genetically diabetic mice (19, 24). Despite its widespread clinical application, the mechanisms responsible for the beneficial actions of photobiomodulation have not been elucidated. Mitochondrial cytochromes have been postulated as photoacceptors for red to near infrared light energy and reactive oxygen species have been advanced as potential mediators of the biological effects of this light (25, 27).

The present studies were initiated to test the hypothesis that that the therapeutic effects of red to near infrared light result, in part, from the stimulation of cellular events associated with the interaction of NIR-LED light with the photoacceptor molecule, cytochrome oxidase. Task 1 documented the involvement of
cytochrome oxidase as a primary photoacceptor molecule for near-infrared light. **Task 2** studies examined the effects of LED photobiomodulation retinal function and retinal gene expression in rodents subjected to mitochondrial toxicity. **Task 3** studies were conducted to begin to investigate LED treatment in laser eye injury in rabbit and nonhuman primate animal models.

**METHODS**

**Materials.** Light-emitting diode (GaAlAs LED) arrays (8 x 10 cm) at wavelengths of 670 nm, 728 nm, 770nm, 830 nm and 880nm were obtained from Quantum Devices, Inc. (Barneveld, WI.). Methanol (HPLC grade) obtained from Sigma Chemical Co. (St. Louis, MO) was diluted in sterile saline and administered as a 25% w/v solution. Thiobutabarbitol sodium salt (Inactin) was purchased from Research Biochemicals International (Natick, MA). Atropine sulfate was obtained from AmVet Pharmaceuticals (Fort Collins, Colorado). Hydroxypropyl methylcellulose (2.5%) drops were acquired from IOLAB Pharmaceuticals (Claremont, CA). Atropine sulfate ophthalmic solution drops were purchased from Phoenix Pharmaceutical, Inc. (St. Joseph, MO). All other chemicals were reagent grade or better.

**Cell Culture.** To determine the optimal wavelength for activation in neurons, GaAlAs LEDs at 670nm, 728nm, 770nm, 830nm and 880nm were tested in cultured neurons, which were inactivated by TTX at a power intensity of 50 mW/cm² and energy density of 4J/cm². Studies of retinal ganglion cells and retinal pigment epithelial cells measuring DNA synthesis in response to LED treatment confirmed the effectiveness of these wavelength, power and energy parameters, thus paving the way for in vivo rodent retinal healing studies.

**Animals.** Long-Evans rats, Dutch Belted Rabbits and Cynomolgus monkeys were used in these experiments. All animals were supplied food and water ad libitum and maintained on a 12 hr light/dark schedule in a temperature- and humidity-controlled environment. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**Methanol-Intoxication Protocol** Rats were placed in a thermostatically controlled plexiglas chamber (22 x 55 x 22 cm; maintained at 22 - 23° C) and exposed to a mixture of N₂O/O₂ (1:1; flow rate 2 liters/min) for the duration of the experiment. N₂O/O₂ exposure produces a transient state of tetrahydrofolate deficiency in the rat resulting in formate accumulation following methanol administration (12). Methanol (25% w/v methanol in saline) was administered (i.p.) to N₂O/O₂ treated rats at an initial dose of 4 g/kg, followed by supplemental doses of 1.5 g/kg at 24 and 48 hours. This methanol intoxication protocol has been shown to produce a state of prolonged formic acidemia with formate concentrations between 5-8 mM in methanol-intoxicated rats resulting in visual dysfunction (6, 7). Moreover, similar concentrations of blood formate over similar time periods have been shown to produce ocular toxicity experimentally in monkeys and have been associated with visual toxicity in human methanol intoxication (8, 9, 11). Formate concentrations were determined from tail vein blood samples by fluorometric analysis (6, 7, 13).

**light-emitting Diode Treatment.** *In vitro:* GaAlAs LEDs at 670nm, 728 nm, 770 nm, 830nm, and 880nm were tested in cultured neurons which were inactivated by TTX at a power intensity of 50 mW/cm² and energy density of 4J/cm². *In vivo:* Rats were placed in a plexiglas restraint device (12.7 x 9 x 7.6 cm). The LED array was positioned directly over the animal at a distance of 1-inch exposing the entire body. Treatment consisted of irradiation at 670 nm for 2 min and 24 sec resulting in a power intensity of 28 mW/cm² and an energy density of 4 joules/cm² at 5, 25 and 50 hours after the initial dose of methanol. These stimulation parameters (670 nm at an energy density of 4 J/cm²) were previously demonstrated to be...
beneficial for wound healing and for stimulating cellular proliferation and cytochrome oxidase activity in cultured visual neurons (19, 28).

**Rodent ERG Procedures and Analyses.** ERG experiments were performed as previously described (6, 7). The light stimulation apparatus consisted of a three beam optical system. All three beams were derived from tungsten-halide lamps (50W, 12V). Beam intensity was controlled by using neutral density step filters. ERG recordings were differentially amplified and computer averaged. The amplified signal was processed through a two stage active narrow bandpass filter (the half voltage of this filter was 0.2 times the center frequency). To ensure that any transients in the response that occur at the onset of the stimulus pulses were not included in the average, the initiation of signal averaging was delayed by a preset number of stimulus cycles (typically a minimum of 20). The resulting ERG is an extremely noise-free, single cycle, sinusoidal waveform. The averaged responses were measured (peak-to-trough amplitude) from a calibrated digital oscilloscope display.

Prior to ERG analysis, ophthalmoscopic examination confirmed that all eyes were free of lenticular opacities or other gross anomalies. Rats were anesthetized with thiobutabarbital sodium salt (100 mg/kg, i.p.), positioned in a Kopf stereotaxic apparatus and placed on a heating pad to maintain core body temperature at 37°C. Atropine sulfate (0.05 mg/kg, s.q.) was administered to inhibit respiratory-tract secretions. The pupil of the eye to be tested was dilated by topical application of 1% atropine sulfate. Methylcellulose was topically applied as a lubricant and to enhance electrical conduction. A circular silver, wire recording electrode was positioned on the cornea, a reference electrode was placed above the eye, and a ground electrode was placed on the tongue. Recordings were obtained under ambient light conditions from cool white fluorescent room lights approximately 100 cd/m² at the rat’s eye. Flickering stimuli (light: dark ratio = 0.25: 0.75) were presented. Responses to 60 successive flashes were averaged for each stimulus condition. At each test wavelength, a minimum of four stimulus intensities spaced at intervals of 0.3 log unit, were presented. The stimulus intensity yielding a 5µV criterion response was determined by extrapolating between the two intensity points that bracketed the 5µV response for each animal. All sensitivity measures were made in triplicate.

Two experimental protocols were employed to evaluate retinal function. (1) 15 Hz/510 nm ERG Response: ERGs were recorded in response to a 15 Hz flickering light at a wavelength of 510 nm over a 3 log unit range of light intensity. For these studies, the unattenuated stimulus (log relative retinal illumination = 0) had an irradiance of 25 µW distributed over the 70° patch of illuminated retina. This can be calculated to produce retinal illumination equivalent to about 10⁴ scotopic trolands. These recording conditions disadvantage rods; however, since at least 97% of rat photoreceptors are rods and Ergs are recorded at luminance intensities ranging from 10¹ - 10⁴ scotopic trolands, it is likely that the responses to the 15 Hz/510 nm light are drawn from both rods and medium wavelength cones (M-cones) (6, 7, 32). (2) 25Hz/UV ERG Response: UV-sensitive cone responses were elicited by a 25 Hz flickering ultraviolet light (380 nm cut off) in the presence of an intense chromatic adapting light (445 µW) which eliminated responses mediated by rods and M-cones (32). 25Hz/UV ERG responses were recorded over a 1.5 log unit range of light intensity. For these studies, the unattenuated stimulus (log relative retinal illumination = 0) had an irradiance of 25 µW distributed over the 70° patch of illuminated retina. This can be calculated to produce retinal illumination equivalent to about 10⁴ scotopic trolands in the rat eye.

**Rabbit and Primate Visual Function Assessments** Prior to testing, each animal received a complete ophthalmological examination, including visualization of the fundus using indirect ophthalmoscopy, intraocular pressure (IOP) measurement, and examination of the anterior chamber by slit-lamp biomicroscopy. Stereo color photographs were obtained from each eye using a digital fundus camera. The integrity and thickness of the retinal layers in the macular region also were assessed by optical coherence tomography (OCT). The functional integrity of the macula was assessed by mERGs. Two mERG stimuli were used: 1) 103 equal sized hexagonal elements each subtending 4.4 deg; and 2) 241 equal sized hexagonal elements each subtending 2.2 deg.
Laser-induced Injury: Following initial screening and baseline testing, a grid of laser burns was applied to the macula by Dr. T. M. Nork, M. D., a board-certified retinal surgeon. The individual spots were approximately 75-150 microns in diameter, 75-120 mW in strength and 0.05-0.2 sec in duration. The grid extended to about 10 arc degrees from the foveal center in all directions (20° diameter).

NIR-LED treatment: One hour following macular grid laser and mERG assessment, LED treatment was delivered. The treatment consisted of 670 nm light delivered for 1 min 45 sec, at an intensity of 38 mW/cm², resulting in an energy density of 4 J/cm² at the target tissue. LED treatments will be repeated at 24, 48, 72 and 96 h following creation of macular laser wounds. Functional testing (mERG and VEP) of both eyes will be repeated at 72 h and 100 h following creation of macular laser wounds. At 100 h the stereo color retinal photographs and OCT images will be obtained from each eye.

Retinal and Visual Function Analysis: The mERG results in a ‘first-order kernel’ voltage v. time waveform that consists of a series of negative and positive waves. Each wave has an associated amplitude and implicit time (delay). The mERG software allows the individual mERG waves to be averaged into groups suitable for the design of a particular experiment. The waves corresponding to the macular region where the laser wound was produced were averaged. The amplitude and implicit time of the first four waves (n1, p1, n2, p2) of the first-order kernel were submitted to an analysis of variance (ANOVA) with Time (baseline, 1 h post, 100 h post), Eye (laser, fellow), and Group (treated, untreated) as factors. Appropriate repeated measures statistical procedures (Greenhouse-Geiser corrections) were used. In addition, means, error estimates percent recovery statistics were graphically presented.

Histopathologic analysis. Retinal tissue was prepared for light and electron microscopic analysis as described by Seme et al. (2001). The retina, LGN and visual cortex of laser-injured and NIR-LED-treated animals was processed for cytochrome oxidase histochemistry at the Medical College of Wisconsin to determine potential benefits of LED-therapy on retinal and cortical neurons.

DNA microarray gene discovery studies: Specific genes that show altered expression were identified using DNA microarray technology at WRAIR Molecular Pathology Laboratory using either commercial gene arrays or glass slides or using our custom human chips for monkey samples. For glass slides fluorescent labeling was performed and scanned in the AXON scanner. The data was analyzed with Gene Pix software. Statistical, clustering, sorting parameters were applied by using Gene Spring and Partek software.

Statistical Analysis. All values are expressed as means ± SEM. A one-way ANOVA with Bonferroni’s test was used to determine whether any significant differences existed among groups for blood formate concentrations. For ERG studies, a two-way ANOVA was performed. In all cases, the minimum level of significance was taken as P< 0.05.

RESULTS

Task 1: To document the involvement of cytochrome oxidase as a primary photoacceptor molecule for near-infrared light.

Hypothesis: We hypothesize that the therapeutic effects of red to near infrared light result, in part, from the stimulation of cellular events associated with increases in cytochrome c oxidase activity.

Milestone: LED light at wavelengths corresponding to absorption peaks of copper centers in the cytochrome oxidase molecule are most effective in stimulating cytochrome oxidase activity and stimulating ATP synthesis.
Results and Discussion: In NIR-LED therapy there are four important treatment variables that must be addressed to optimize any therapeutic regimen. (1) Energy density or fluence, (2) NIR-LED wavelength (3) number of treatments and (4) treatment interval. With respect to energy density, we are confident that treatment at an energy density of 4 J/cm² is within the optimal range. This confidence is based on a large body of evidence from our studies and those of other investigators which documents that exposure to near-infrared light at energy densities (fluence) between 2 – 10 J/cm² promotes mitochondrial energy metabolism, cell division, wound healing, protects against retinal damage and improves the recovery of retinal function in following retinal damage by a mitochondrial poison and promotes retinal healing and improved visual function following high intensity laser-induced retinal injury. With respect to LED wavelength, the majority of our studies have been conducted using 670 nm LED light and we have substantial evidence that NIR-LED treatment at 670 nm is beneficial both in vitro and in vivo. In studies investigating additional NIR wavelengths, we have shown that the recovery of neuronal cytochrome oxidase activity and cellular ATP content correlates with the cytochrome oxidase absorption spectrum (Figure 1). These studies showed that LED light at wavelengths corresponding to the absorption peaks of the copper centers in the cytochrome oxidase molecule (670 nm, 830nm and 880 nm) were most effective in promoting the recovery of cytochrome oxidase activity in cultured primary neurons.

Figure 1: Recovery of Neuronal Cytochrome Oxidase Activity and Cellular ATP Content Correlates with Cytochrome Oxidase Action Spectrum

We also have evidence that multiple treatments are more effective than a single treatment in stimulating cytochrome oxidase activity and the three NIR-LED treatments administered at 5, 25, 50 hours following toxin exposure protects against and reverses toxin-induced retinal toxicity in rat. In the monkey, 5 treatments administered 1, 24, 48, 72 and 96 hours post-injury were effective in ameliorating high-energy laser-induced retinal injury.

Deliverable to DARPA: In vitro evidence that the cytoprotective effects of red to near infrared light result, in part, from the stimulation of cellular events associated with increases in cytochrome oxidase activity.
**Task 2:** To examine the effects of LED photobiomodulation retinal function and retinal gene expression in an animal model of toxin induced retinal mitochondrial dysfunction.

**Hypothesis:** The prolonged effects of brief LED treatment result from the induction of a cascade of events leading to the stimulation of gene expression, protein synthesis, and oxidative metabolism.

**Milestones:**
1. Three brief NIR LED treatments produced rapid recovery of retinal function following toxic retinal injury.
2. NIR LED-treatment induced the expression of genes which code for proteins involved in oxidative metabolism and cellular protection. (collaboration with Dr. Marti Jett at WRAIR, Wash. D.C.)

**Results and Conclusions:** *Retinal Function Studies:* Methanol intoxication produces toxic injury to the retina and optic nerve resulting in blindness. The toxic metabolite in methanol intoxication is formic acid, a mitochondrial toxin known to inhibit the essential mitochondrial enzyme, cytochrome oxidase. Studies were undertaken to test the hypothesis that exposure to monochromatic red radiation from light-emitting diode (LED) arrays would protect the retina against the toxic actions of methanol-derived formic acid in a rodent model of methanol toxicity. Using the electroretinogram as a sensitive indicator of retinal function, we demonstrated that 3 brief (2 min. 24 sec.) 670 nm LED treatments (4 J/cm²), delivered at 5, 25 and 50 hours of methanol intoxication, significantly attenuated the retinotoxic effects of methanol-derived formate during intoxication (Figure 2) and profoundly improved the recovery of retinal function following intoxication (Figure 3). We further show that LED treatment protected the retina from the histopathologic changes induced by methanol-derived formate (ref or figure). These findings provide a link between the actions of monochromatic red to near infrared light on mitochondrial oxidative metabolism *in vitro* and retinoprotection *in vivo.* They provide the basis for phase II studies directed at examining laser injury in a rodent model.

**Figure 2**

**Figure 3**

*670 nm NIR-LED Protects Against Retinal Toxicity*  
*670 nm NIR-LED Improves Recovery*
Gene Expression Studies: We have compared gene expression profiles in the neural retina of untreated rats with those from the neural retina of methanol-intoxicated rats and LED-treated methanol-intoxicated rats. Results from these studies indicate that methanol intoxication and LED treatment altered the retinal expression of nearly 80 genes. At least 26 of these genes that were up-regulated in the retinas of methanol intoxicated rats were correspondingly down-regulated by in the retinas of LED treated methanol intoxicated rats and vise-versa (Figure 5). Several functional subcategories of genes regulated by NIR-LED were identified in retinal samples, including those encoding DNA repair proteins, antioxidant defense enzymes, molecular chaperones, protein biosynthesis enzymes, and trafficking and degradation proteins. Striking differences were observed in genes from cytochrome oxidase family, peroxiredoxin family and genes involved in cell growth and maintenance (Figure 6). Differential expression of selected genes was confirmed at the level of RNA. We intend to further substantiate these findings using real time PCR, Northern and Western analysis and to investigate the roles of several of these genes in cellular energy production and cellular survival.
Figure 5: Up and Down Regulation of Genes in the retinas of methanol-intoxicated vs NIR-LED-treated methanol-intoxicated rats.

Figure 6: Gene families regulated by NIR-LED treatment in rat retina
In summary, our studies to date have revealed a great many potential markers and have provided some explanations as to the mechanisms by which LED exposure may enhance the wound healing process. The next phase of this project will build on these initial efforts and focus on the military relevant problem of retinal repair. The team assembled by Dr. Whelan includes highly experienced civilian and military ophthalmologists who will measure various parameters in order to quantitatively determine functional retinal repair +/- LED treatments. Using our sophisticated molecular techniques as impartial markers, we will further characterize the process and optimize healing of retinal injuries to understand the chain of events that lead to improved wound healing.

**Deliverable to DARPA:** Evidence that brief LED treatment up-regulates the expression of genes important in cellular survival and down-regulates the expression of genes involved in cell death pathways.

**Task 3** To investigate NIR-LED treatment in laser eye injury in rabbit and non-human primate animal models.

**Hypothesis:** NIR-LED treatment will improve retinal healing and visual function following high intensity laser injury.

**Rationale/Approach:** These studies were conducted by the team at the University of Wisconsin, Madison (J. Ver Hoeve) in collaboration with the MCW group on cynomolgus monkeys (Macaca fascicularis). Following baseline assessments (two baseline mERGs/VEPS) (multifocal ERGs/visual evoked potentials), all monkeys will receive laser wounds to the macula of one eye. The fellow eye will not be lasered. Treated monkeys will receive LED treatment to the lasered eye and the fellow eye. LED treatment will consist of 1 min 45 sec of 670 nm light at 4 J/cm² delivered at 1, 24, 72, and 96 h following the laser wound. Other LED parameters will also be used, as dictated by the results of Tasks 1 & 2. Additional monkeys will serve as controls (no LED treatment). The functional effect of LED treatment will be assessed by comparing mERG and VEP parameters in the treated eyes with the untreated eyes of the control monkeys immediately following the laser wound and at 72 h and at 100 h, prior to sacrifice. In addition, the possibility of deleterious functional effects of the LED treatment will be assessed by comparing the fellow (non-lasered) eyes with baseline values and with the non-lasered eyes of the control monkeys. For logistical reasons, monkeys will be tested in pairs (treated and control).

**Preliminary Data in the Rabbit Model:** To optimize LED treatment parameters *in vivo*, we have examined laser retinal injury in a rabbit animal model. To date we have performed one experiment using this animal model. Two rabbits were used in this study- one lasered without LED treatment and one lasered with LED (treatment at 24,48 and 72 h post injury. A laser grid was created in the central retina of right eye of each animal (Figure 7). The LED treated rabbit fundus had fewer distinct laser spots 1-week following laser injury (Figure 7). These preliminary findings are indicative of improved retinal healing following LED treatment in laser injured rabbit model.
Preliminary Data in Nonhuman Primates: We have initiated studies of laser retinal injury in a nonhuman primate model. To date, we have performed two experiments using this animal model. In each experiment one monkey was lasered without LED treatment and one lasered with LED treatment (670 nm, 4 J/cm²). A laser grid (128 spots delivered to the macula and perimacula) was created in the central retina of right eye of each animal (Figure 8). This grid consisted of grade I and II burns, photocoagulating the photoreceptors and outer nuclear layer of the retina. Multifocal ERG was performed to assess the functional state of the retina. In the first experiment, the LED-treated monkey was treated at 1, 24, 72 and 96 h post injury. ERG amplitude in both LED treated and untreated monkeys was temporarily increased shortly after laser injury and this increase was greater in the LED-treated monkey. Assessment of the severity of the laser burn in LED treated and untreated animal demonstrated a greater than 50% improvement in the degree of retinal healing at 1 month post-laser in the LED-treated monkey (Figure 9). In addition, the thickness of the retina measured at the fovea by optical coherence tomography did not differ from the pre-laser thickness in the LED-treated animal whereas it was 50% thinner in the untreated animal (Figure 8). Importantly, LED treatment prevented the loss of cytochrome oxidase staining in the lateral geniculate nucleus (Figure 10) clearly showing that the brain was responding to visual input from the “healed” retina in the LED-treated animal much more effectively than in the untreated animal.

In the second study, the LED-treated animal was treated once per day for 11 days and mfERG recordings were recorded. Again, shortly after laser injury, the ERG amplitude was temporarily increased in both LED treated and untreated animals. However, in this experiment the increases were comparable. (Figure 11) At 4 days post laser injury, the mfERG responses in LED treated and untreated animals had decreased to pre-laser amplitudes. However, by day 11 post laser, the mfERG response in the LED treated monkey was more than 50% greater than that measured in the untreated (sham) monkey. In both experiments, these preliminary findings are indicative of improved retinal healing and visual cortical function following LED treatment in laser injured primate model. It must be stressed that these findings are preliminary and it essential that this study be replicated and expand upon.
Figure 8: Laser grid appearance and foveal thickness in control (left) and NIR-LED (right) treated monkey retina.

Figure 9: NIR-LED Treatment Stimulates Retinal Healing following laser injury.
Grading Scale: Higher values are indicative of less complete healing. 0 = spot faded, no distinct borders; 0.5 = portion of Border visible; 1.0 = borders of entire spot visible. (t-test)
Figure 10: Quantitative Analysis of Cytochrome oxidase histochemistry in LGN of control laser-treated monkey and NIR-LED treated laser treated monkey
Deliverable to DARPA: Evidence that Photobiomodulation may provide an innovative and non-invasive therapeutic approach for the treatment of retinal and optic nerve injury.

**DISCUSSION**

Low energy photon irradiation by light in the far red to near infrared spectral range (630-1000 nm) using low energy lasers or light emitting diode arrays has been found to modulate various biological processes in cell culture and animal models (22-25). This phenomenon of photobiomodulation has been applied clinically in the treatment soft tissue injuries and to accelerate wound healing (19, 22). The mechanism of photobiomodulation by red to near infrared light at the cellular level has been ascribed to the activation of mitochondrial respiratory chain components resulting in initiation of a signaling cascade which promotes cellular proliferation and cytoprotection (25, 27, 28). A comparison of the action spectrum for cellular proliferation following photoradiation with the absorption spectrum of potential photoacceptors lead Karu and colleagues to suggest that cytochrome oxidase is a primary photoreceptor of light in the red to near infrared region of the spectrum (25). We have confirmed and extended these observations showing that the NIR absorption spectrum of cytochrome oxidase correspond with the action spectrum for upregulation of cytochrome oxidase activity and cellular ATP content.
Studies conducted in primary neuronal cultures by our research group have shown that 670 nm LED photobiomodulation reversed the reduction in cytochrome oxidase activity produced by the blockade of voltage–dependent sodium channel function by tetrodotoxin and up-regulated cytochrome oxidase activity in normal neurons (28). Additional studies have extended these investigations to an in vivo system to determine if 670 nm LED photobiomodulation would improve retinal function in an animal model of formate-induced mitochondrial dysfunction. Results of this study demonstrate the therapeutic benefit of photobiomodulation in the survival and functional recovery of the retina in vivo after acute injury by the mitochondrial toxin, formic acid generated in the course of methanol intoxication. We provide in vivo evidence that 3 brief post-methanol-intoxication treatments with 670 nm LED photoradiation promotes the recovery of retinal function in rod and cone pathways and protects the retina from the histopathologic changes induced by methanol-derived formate. These findings provide a link between the actions of red to near infrared light on mitochondrial oxidative metabolism in vitro and retinoprotection in vivo.

Low energy laser irradiation has documented benefits in promoting the healing of hypoxic, ischemic, and infected wounds (19, 22). However, lasers have limitations in beam width, wavelength capabilities, and size of wounds that can be treated (19). Heat generated from the laser light can damage biological tissue, and the concentrated beam of laser light may accidentally damage the eye. Light-emitting diode (LED) arrays were developed for NASA manned space flight experiments. In comparison to lasers, the patented LED technology generates negligible amounts of heat, is clinically proven to be safe, and has achieved non-significant risk status for human trials by the FDA (19). The wavelength, power, and energy parameters used in the present study are based on their beneficial effects for wound healing in human’s (19) and stimulation of CO activity in cultured neuronal cells (28).

The prolonged effect of 3 brief LED treatments in mediating the cytoprotective actions in cultured retinal cells and the retinoprotective actions in methanol intoxication suggests that 670 nm LED photostimulation induces a cascade of signaling events initiated by the initial absorption of light by cytochrome oxidase. These signaling events may include the activation of immediate early genes, transcription factors, cytochrome oxidase subunit gene expression and a host of other enzymes and pathways related to increased oxidative metabolism (25, 28, 44). In addition to increased oxidative metabolism, red to near infrared light stimulation of mitochondrial electron transfer is also known to increase the generation of reactive oxygen species (25). These mitochondrially generated reactive oxygen species may function as signaling molecules to provide communication between mitochondria and the cytosol and nucleus and thus play an important signaling role in the activation of retinoprotective processes following LED treatment (45).

The results of this study and others suggest that photobiomodulation with red to near infrared light augments recovery pathways promoting neuronal viability and restoring neuronal function following injury. Importantly, there was no evidence of damage to the normal retina following 670 nm LED treatment. Based on these findings, we suggest that photobiomodulation may represent an innovative and novel therapeutic approach for the treatment of retinal injury as well as the treatment of retinal diseases, including age-related macular degeneration, glaucoma, diabetic retinopathy, and Leber’s hereditary optic neuropathy.

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REFERENCES


DARPA Soldier Self Care: Rapid Healing of Laser Eye Injuries with Light Emitting Diode Technology
Development of Liposome Encapsulated Hemoglobin (LEH) and Studies of Hemorrhagic Shock by Use of Imaging Studies with Oxygen-15 and Other Radiotracers

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SUMMARY

Liposome-encapsulated hemoglobin is under development by our group as an artificial oxygen carrier for use in combat casualty resuscitation. Encapsulating hemoglobin inside a protective lipid membrane, which mimics a red blood cell, has the advantages of decreasing the toxicity of the free hemoglobin, increasing its circulation time, and permitting the co-encapsulation of hemoglobin protectants to prevent conversion of oxy-hemoglobin to met-hemoglobin. We have recently developed a LEH formulation with an increased hemoglobin concentration as well as improved biological tolerability. Our group has developed several novel methods of assessing the circulation and efficacy of LEH formulations through the use of radiotracers and small animal imaging. These tracer studies are based on the physiologic imaging techniques of single photon emission computed tomography (SPECT) and positron emission tomography (PET) that are currently used in clinical nuclear medicine. Recently, small animal imaging systems have been developed that have very high resolution which permits the imaging of small animals. These imaging techniques provide a very powerful assessment of quantitative regional physiology by non-invasive imaging.

1.0 NEED FOR ARTIFICIAL OXYGEN CARRIERS

It is well documented and generally recognized that the demand for red blood cells as transfusable oxygen carriers cannot always be met under the current blood donation system, especially during natural disaster and war [Kaufman 1991; Tomasulo 1995]. A readily available oxygen transporting volume expander that does not require cross matching and which could be given within 5-10 minutes after the start of an acute traumatic hemorrhage could save many lives [Winslow 2000; Stowell 2001; Winslow 2002]. Obviously, this combined oxygen transporting volume expander would be particularly valuable to the military.

2.0 LIPOSOME-ENCAPSULATED HEMOGLOBIN (LEH)

Liposome-encapsulated hemoglobin is under development by the United States Navy and others as an artificial oxygen carrier for use in combat casualty resuscitation [Rudolph 1991; Cliff 1992; Rabinovici 1993; Phillips 1999; Sakai 2001; Awasthi 2003; Awasthi 2004; Sakai 2004b]. LEH has many important advantages compared to unencapsulated hemoglobin which include the following: 1) Decreased Renal Toxicity. LEH has shown no significant nephrotoxic effects [Rudolph 1995; Phillips 1999; Sakai 2004a]. 2) Potential to

Coencapsulate Allosteric Modifiers and Antioxidants with Hemoglobin. Allosteric modifiers can be coencapsulated with the hemoglobin during LEH manufacture in order to control the oxygen affinity (P_{50}) [Farmer 1988; Sakai 1998]. Hemoglobin protectants can also be encapsulated in the liposome in order to retain the hemoglobin in the oxy-hemoglobin state [Stratton 1988; Takeoka 1997].

3) Decreased Vasoactivity. Because LEH has physical properties closer to red cells, it produces less of a hypertensive response than that observed with cell-free hemoglobin [Nakai 1994; Rudolph 1997; Flower 1999]. Recent studies demonstrate that the vasoconstrictor activity of LEH is 60 times less than that of unencapsulated Hb [Rudolph 1997].

4) Diffusive Properties Closer to Red Cells. The rate of release of the oxygen from LEH in rapid mixing experiments is slower than from cell-free hemoglobin and closer to the rate of release from intact red cells [Sakai 2003]. This slower release may also be an advantage over unencapsulated hemoglobin products currently undergoing clinical testing. Rapid oxygen release from unencapsulated Hb has been hypothesized to cause hypertension secondary to autoregulation at the level of the arterioles [Winslow 2003].

5) Metabolism by RES Similar to Red Cells. LEH is metabolized by the RES of the liver and spleen in the same manner as red cells [Rudolph 1995; Sakai 2004a].

6) Decreased Likelihood of Neurotoxicity. Neurotoxicity has been described with unencapsulated hemoglobin blood substitutes [Panter 1994; Rogers 2003]. It has been hypothesized that there will be less chance for this to occur with LEH because of the protective lipid encapsulation of the hemoglobin with LEH.

The current LEH formulation produced by our group has the following features [Awasthi 2004]:

1) It is a homogeneous LEH formulation that is approximately 0.25 microns in diameter, unlike the originally described LEH formulation which contained large particles of > 1 micron (~ 30% of the population). 2) LEH is now a volume expander due to the addition of albumin to the new formulation and the use of a polyethylene glycol (PEG) coating of LEH. Recent research demonstrates that intravascular volume expansion is a very important additional aspect of a resuscitative fluid (i.e. no oxygen transport is possible without adequate intravascular volume)[Awasthi 2004; Sakai 2004b].

3) The addition of PEG to the LEH formulation has greatly increased the circulation persistence half life of LEH from 18 hours up to 65 hours [Phillips 1999].

4) Prior LEH and other liposome formulations have been reported to cause an acute thrombocytopenic response [Goins 1997; Phillips 1997a; Szebeni 1999]. Coating the surface of LEH with PEG as well as greatly reducing the negative lipid component from 10% to 2% of the formulation has also greatly reduced the thrombocytopenic response in small animals as determined by studies performed in our laboratory.

3.0 CURRENT LEH MANUFACTURING PROCESS

Our laboratory is currently producing 1 liter batches of LEH containing stroma free human hemoglobin (Figure 1). Hemoglobin is separated from outdated human packed red cells using sterile conditions. After lysis, hemoglobin is processed and concentrated to 31.6 g/dL by ultrafiltration through a series of filters (0.65 um, 0.1 um, 500 KDa and 10 KDa). The final hemoglobin product is stored at –80°C until needed for LEH manufacture.

LEH is manufactured by dissolving lipids in chloroform:methanol (2:1) and removing the solvent by rotary evaporation to form a lipid film. After overnight desiccation of the dried lipid film, the lipids are rehydrated in a solution containing pyridoxal-5-phosphate, catalase and β−NAD reduction mixture. The suspension is shell frozen and then lyophilized to form a dried powder. The dried lipid powder is then rehydrated with stroma free human hemoglobin at pH 7.1. This mixture is shaken by oscillation for 2-4 h before microfluidization using 400 um interaction chamber at 20 psi for 15 passes. The microfluidized LEH is then separated from unencapsulated hemoglobin by microfiltration through 0.05 μm filter. The clarified LEH is PEGylated with PEG-5000-DSPE for 1 hour at 37°C. Ultrafiltration of the pegylated LEH product is then performed to further remove uninserted PEG^[DSPE] and unencapsulated hemoglobin. The final PEG-
LEH product is characterized using various assays including laser light scattering particle sizing and endotoxin analysis. Figure 1 shows the typical parameters obtained for a recent PEG-LEH batch containing 2% anionic lipids.

<table>
<thead>
<tr>
<th>LEH Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin and Culture</td>
<td>&lt; 5 EU/ml</td>
</tr>
<tr>
<td></td>
<td>No growth</td>
</tr>
<tr>
<td>Hemoglobin Concentration</td>
<td>6.5 g/dL</td>
</tr>
<tr>
<td>MethHb %</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>P 50</td>
<td>31.83 mmHg</td>
</tr>
<tr>
<td>Lipid Estimation</td>
<td>125.90 mg/dL</td>
</tr>
<tr>
<td>Oncotic Pressure (without albumin)</td>
<td>4.5 mmHg</td>
</tr>
<tr>
<td>Osmolality</td>
<td>0.282 Osmol/kg</td>
</tr>
<tr>
<td>Particle Sizing</td>
<td>247.65 nm</td>
</tr>
</tbody>
</table>

Figure 1: Current LEH Formulation and Current LEH Characteristics

4.0 SMALL ANIMAL IMAGING

Our group has pioneered the use of imaging for the development and evaluation of LEH and other liposome-based formulations. Radiotracers used in these imaging studies include the traditional single photon emission computed tomographic (SPECT) imaging agent, technetium-99m (99mTc) for studying the distribution of LEH [Rudolph 1991; Phillips 1999; Awasthi 2004] as well as the short lived positron emitting (PET) agent, oxygen-15 (15O) for assessing oxygen delivery by LEH [Phillips 1997b; Goins 1998]. The rapid ability to assess a variety of LEH formulations using imaging has greatly aided in the development of a LEH formulation with improved properties.

4.1 Studies with SPECT agents

Using our novel method of labelling liposomes, LEH was labelled with 99mTc method and whole body imaging was performed to track the distribution of the LEH [Phillips 1992; Goins 1993]. This excellent tracking method greatly assisted in the development of a long circulating LEH formulation. The long
circulation was achieved by placing a coating of polyethylene glycol on the surface of the liposome. Imaging with these agents made it easy to study a wide variety of PEG concentrations and methods of inserting the PEG so that a long circulation would be maintained while developing an LEH formulation that had the maximum amount of persistence in circulation [Phillips 1999; Awasthi 2003; Awasthi 2004]. In addition to imaging, blood samples were also collected for radioactivity counting to determine circulation persistence of the LEH. For the most ideal formulation that had a high concentration of hemoglobin, the clearance half-life of LEH was 53 to 65 hours in rabbits and 39 hours in rats [Phillips 1999; Awasthi 2004] (Figure 2). Such circulation times are likely to translate into a T1/2 of about 5 days in humans. These results demonstrate that compared to unencapsulated modified hemoglobin preparations, LEH shows promise as a non-toxic, longer circulating oxygen carrier that is tolerated even at 25% blood volume and that may be developed as a product for transfusion.

Figure 2: Labeled $^{99m}$Tc-LEH was administered to rabbits and imaged with a standard clinical gamma camera. It can be observed that liposomes with PEG and a Neutral lipid formulation had the greatest amount of activity remaining in the heart and circulation at 24 hours. LEH with 10% anionic lipid had decreased amount of activity remaining in the heart. These images can be readily quantitatively analyzed for comparisons at all time points from 0-24 hours.

The LEH imaging studies described above were performed with a standard clinical gamma camera that had not been optimized for small animal imaging studies. In the last year, a commercial vendor has introduced a new imaging system that is dedicated to SPECT imaging of small animals. Our department recently purchased this dedicated microSPECT/CT imaging system (Gamma Medica, Northridge, CA) for the study of small animals (Figure 3). The resolution of this system for rats and mice is at least 10 times greater.
than previously available clinical imaging systems. This system is ideally suited to image SPECT agents of a variety of energies including technetium-99m of 140 kiloelectron volts and indium-111 of 240 kiloelectron volts. This system has also been designed to image the very low photon energies (30 kiloelectron volts) of iodine-125 which can be used for mice only.

![Image of MicroSPECT/CT system](image)

**Figure 3:** The MicroSPECT/CT system can perform high resolution images of mice and rats. It can readily track agents such as LEH and platelets labeled with SPECT radiotracers as well as perform high resolution computed tomographic images for anatomic detail.

### 4.2 Studies with Oxygen-15 PET Imaging

Oxygen-15 ($^{15}$O) studies have great potential for the study of hemorrhagic shock and red cell substitutes. The ability to image oxygen metabolism after inhalation of oxygen-15 labeled oxygen gas can provide significant information about the physiology and function of artificial oxygen carriers. Our group has pioneered the use of oxygen-15 to study oxygen delivery and carrying capacity of LEH [Phillips 1997b; Goins 1998]. Initial studies were performed prior to the advent of small animal microPET imaging systems and they used probes placed over a particular organ to quantify oxygen delivery to the organ. Small animal microPET imaging systems have become commercially available in the last 3 years (Figure 4). In this article, we introduce the use of oxygen-15 for the regional assessment of oxygen delivery by LEH as well as the assessment of the physiology of oxygen metabolism during hemorrhagic shock using microPET (Concorde, Knoxville, TN). Oxygen-15 has a short half life of 2 minutes, which is the longest half-life of any available radioisotope of oxygen. This short half life of oxygen requires that these oxygen-15 studies be performed in close proximity to a cyclotron.
After inhalation, the oxygen-15 gas is absorbed from the lungs into the blood and is carried by the red blood cells to the tissues where it becomes converted to carbon dioxide and water in the mitochondria of cells. The carbon dioxide is rapidly cleared so that the initial images represent the oxygen uptake phase while a gradual washout of the oxygen represents post-metabolic water as illustrated below in figure 5.

**Oxygen-15 Kinetics**

\[
\begin{align*}
15^O2 & \quad \leftrightarrow \quad \text{Blood} \\
15^O2 & \quad \leftrightarrow \quad \text{Tissue} \\
H_215^O & \quad \downarrow \\
\end{align*}
\]

Figure 4: Photograph of the MicroPET system used to image oxygen-15. The picture on the right shows a rat that is covered with a water blanket for temperature control during imaging.

Figure 5: Diagram outlining the distribution of oxygen-15 associated with oxygen gas or water. After uptake by red cells in the lungs, the oxygen-15 moves to the tissues and the mitochondria where it is converted into metabolic water.
4.2.1 Methods for Oxygen-15 Studies of Hemorrhagic Shock

Sprague-Dawley rats (250 g) with an indwelling femoral artery catheter placed two days prior to the oxygen-15 study are anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) cocktail intramuscularly in thigh. Rats are weighed to calculate blood volume. A 23 ga butterfly catheter is placed in tail vein for infusion of resuscitative fluid and maintenance of anesthesia during the entire study by intravenous injection of a diluted solution of 1 part ketamine/xylazine cocktail to 9 parts saline. Next the rats are intubated using modified angiocatheter. The rat is placed on imaging bed of microPET. Warming pad is used to maintain body temperature. The rat is connected to physiological monitoring equipment to measure mean arterial pressure, temperature, heart rate and respiration. Baseline measurements are taken. The rat is then positioned inside microPET camera and insufflated with 5 ml $^{15}$O-oxygen gas with the lungs expanded for 5 seconds. Serial 1 min images are acquired. After this baseline image, the rat undergoes a withdrawal of 50% of its blood volume (based on body weight) at 0.5 ml/min. At 10 min post-hemorrhage, the rat is insufflated with 5 ml $^{15}$O-oxygen gas and a second set of images acquired. The rat is then infused with resuscitative fluid through 23 gauge tail vein butterfly catheter at 0.5 ml/min using syringe pump. Physiological monitoring is continued. At 10 min post-re-infusion the rat is insufflated with 5 ml $^{15}$O-oxygen gas and a third set of images acquired. Final physiological parameters are recorded.

4.2.2 Results

The images depicted in figure 6 show an obvious change in oxygen metabolism from baseline to 50% blood withdrawal. The oxygen metabolism in the nose, eyes and salivary glands is severely decreased after 50% blood withdrawal compared to both baseline and after reinfusion of the shed blood. Transverse tomographic images demonstrate a change in distribution of oxygen metabolism within the brain itself (Figure 7). Quantitative analysis of oxygen metabolism reveals an approximate 40% decrease in the oxygen activity within the brain after the 50% blood withdrawal compared to baseline and an increased oxygen metabolism of the brain above baseline levels after reinfusion of the shed blood (Figure 8).

![Baseline Images](image1.png) ![Post-50% Blood Withdrawal](image2.png) ![Post Shed Blood Reinfusion](image3.png)

Figure 6: Note the significant decrease in oxygen metabolism in the nose and in the salivary glands following the 50% hypovolemic shock. Less noticeable in these images is the slight change of oxygen metabolism in the brain. Quantitative analysis reveals a decrease in activity within the brain as a whole.
Figure 7: These images demonstrate how the analysis can be performed around specific regions of the brain. Note how the images show decreased oxygen metabolism in the brain during hypovolemic shock. A region is placed over the cerebrum. The quantitative results from the region of interest (ROI) analysis are shown in Figure 8 below.
5.0 POTENTIAL OF OXYGEN-15 STUDIES FOR INVESTIGATION OF SHOCK AND BLOOD SUBSTITUTES

The use of PET imaging for the performance of physiologic studies of oxygen metabolism has the potential to provide much new information about shock that would be of value for resuscitation therapy. The advantages of this technique are the following: 1) repeat studies can be performed of dynamic processes so that the same animal can be used as its own control, 2) the protocol for assessing oxygen metabolism in specific organs is simplified so that microsurgery is not required to sample blood going into and out of each organ studied, 3) oxygen metabolism can be observed in organs that could not be studied with previous catheterization techniques such as the nose, muscle, the salivary glands and the spleen, 4) oxygen-15 can also be used in the form of carbon monoxide (C\textsuperscript{15}O) which after inhalation attaches to red blood cells for studies of the effect of hemorrhagic shock on blood volume and 5) observations can be made of intraorgan changes in oxygen metabolism such as our preliminary observation of regional changes of oxygen metabolism in the brain.
brain. Potential studies for which oxygen-15 imaging in small animal models could prove useful include 1) studies dedicated to the assessment of artificial oxygen carriers and the effect of various formulation changes on oxygen delivery, 2) use of oxygen-15 for the assessment of a wide variety of resuscitation protocols and 3) basic investigations into changes in oxygen metabolism during shock.

### 6.0 SUMMARY

There has been significant progress in development of LEH as an artificial oxygen carrier. This progress has been greatly aided by the use of small animal imaging systems to track the distribution of LEH as well as to determine the efficacy of LEH as an artificial oxygen carrier. Recent progress in the development of small animal imaging systems has the potential to increase understanding of basic physiologic changes that occur in shock.

### 7.0 ACKNOWLEDGMENTS

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### 8.0 REFERENCES


Hypertonic Saline Resuscitation Modulates Neutrophil Adhesion Molecule Expression of Post-Traumatic Hemorrhagic Shock Patients

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SUMMARY

Context - Fluid resuscitation for traumatic hemorrhagic shock remains controversial since current protocols using large-volume crystalloid may exacerbate post-traumatic inflammation and organ dysfunction. Experimental data suggests that hypertonic saline/dextran (HSD, 7.5%NaCl in 6%dextran-70) exerts anti-inflammatory and immunomodulatory effects, reduces multiorgan dysfunction and improves outcome. Objective – First trial examining the immunomodulatory properties of HSD in humans. Based on experimental findings, this study proposes to determine whether a single bolus of HSD might modulate the inflammatory response to hemorrhagic shock.

Design - Randomized, double-blinded, placebo-controlled clinical trial.

Setting - Largest tertiary-care university-affiliated Trauma Centre in Canada.

Patients - 27 adult patients, victims of blunt trauma and hypotension due to hemorrhage. 13 patients received HSD, 2 excluded for failing inclusion criteria and 1 for refusing participation.

Intervention – Upon arrival, eligible patients received a single 250-ml bolus of either HSD or placebo (0.9%NaCl) from unmarked bags. Blood samples were collected prior to infusion and over subsequent 24 hours. All patients underwent standard resuscitation.

Main Outcomes - HSD markedly altered shock-induced changes in key adhesion molecules on circulating neutrophils compared to placebo. HSD abolished shock-induced upregulation of CD11b and caused extensive CD62L shedding. Leukocyte counts were similar, except for lymphocytes where HSD prevented the lymphopenia detected in the control group.

Other Results – HSD patients required less crystalloid and blood during the first 24 hours. HSD patients were liberated from mechanical ventilation 24 hours earlier than control. ICU stay, organ dysfunction, infections and mortality did not differ between groups. HSD modestly increased serum sodium and osmolarity. No complications were associated to HSD.

Conclusions – A single bolus of HSD during early resuscitation alters shock-induced inflammatory response by blunting neutrophil activation and preventing post-shock lymphopenia. These findings are consistent with experimental data and provide “proof of principle” for larger clinical trials.

1.0 INTRODUCTION

Hemorrhagic shock and ensuing inflammatory response are prime contributors to morbidity and mortality in trauma.(1-3) While current resuscitation protocols using large-volume crystalloids can correct the shock state, they simultaneously appear to exacerbate systemic inflammation and the development of organ injury.(4-6) Specifically, abnormal activation of polymorphonuclear neutrophils (PMNs) and altered PMN-endothelial interactions have pivotal roles in post-resuscitation multiorgan dysfunction (MOD).(7)
Both human and experimental animal studies demonstrated that a single small-volume (4 ml/kg) infusion of hypertonic saline plus dextran (HSD, 7.5%NaCl 6%dextran-70) effectively restores hemodynamics by osmotically driving extravascular fluid into the vasculature, immediately correcting systemic ischemia and lessening organ edema.\(^8,9\) Early randomized controlled trials (RCT) proved that HSD is effective and safe\(^10-12\), but lacked power to demonstrate more than trends toward better patient outcome.

Recent experimental data demonstrating the anti-inflammatory and immunomodulatory properties of hyperosmolar solutions, particularly with respect to neutrophils, renewed the interest in HSD.\(^4,13,14\) In vivo experimental models, hypertonicity prevented PMN activation, adhesion and transmigration into tissues by altering surface expression of key adhesion molecules (CD11b and CD62L), thus attenuating inflammation and MOD.\(^13,15-19\) Hypertonicity also enhanced lymphocyte proliferation, reverting post-traumatic immunosuppression.\(^15,16,20,21\) All these effects remain unsubstantiated in human trauma patients.

This is the first RCT investigating the immunomodulatory properties of HSD in hemorrhagic-shock patients. Consistent with experimental observations, HSD alters shock-induced inflammation by blunting neutrophil activation and preventing post-shock lymphopenia, providing “proof of principle” for larger clinical trials.

### 2.0 METHODS

#### 2.1 Study Population

This prospective, randomized, double-blinded, placebo-controlled trial of HSD resuscitation for traumatic hemorrhagic shock was conducted at Sunnybrook Women’s College Health Sciences Centre between April 2001–August 2002 (Figure 1). Institutional Review Board approved the study with provisions for delayed informed consent obtained within 24 h.

![Figure 1. Number of patients assessed and enrolled in the trial.](image)
Table 1 displays inclusion/exclusion criteria. Upon arrival, eligible patients randomly received a single 250-mL intravenous bolus of either HSD (7.5%NaCl 6%dextran-70) or placebo (0.9% NaCl) from identical unidentified bags. Resuscitation otherwise adhered to ATLS® guidelines. Patients were followed until hospital discharge or death.

Table 1: Inclusion and exclusion criteria according to the study protocol.

<table>
<thead>
<tr>
<th>Inclusion (all of the following)</th>
<th>Exclusion (any of the following)</th>
</tr>
</thead>
<tbody>
<tr>
<td>adults (16 years or older)</td>
<td>refusal to participate</td>
</tr>
<tr>
<td>blunt trauma</td>
<td>more than 6 hours after trauma</td>
</tr>
<tr>
<td>at least 1 episode of hypotension</td>
<td>vital signs absent or</td>
</tr>
<tr>
<td>(systolic BP = 90 mmHg)</td>
<td>not expected to survive 24 hours</td>
</tr>
<tr>
<td>evidence of blood loss (external,</td>
<td>pregnancy or stigmata of chronic</td>
</tr>
<tr>
<td>thorax, abdomen, retroperitoneum)</td>
<td>disease</td>
</tr>
</tbody>
</table>

2.2 Laboratory Procedures

Blood samples were collected before infusion, 1, 3, 6 and 24h after. As described(13), 100μL aliquots of unstimulated and endotoxin-stimulated (1 μg/ml *Escherichia coli* 055:B5, Sigma) whole-blood were stained with anti-CD11b-FITC and anti-CD62L-PE monoclonal antibodies and analyzed by flow cytometry (BD). Soluble CD62L was measured by sandwich ELISA (Bender MED-Systems).

2.3 Outcome Measures

Primary outcome measure was change in neutrophil activation as measured by surface expression of adhesion molecules (CD11b and CD62L). Secondary outcomes included changes in leukocyte counts, serum sodium/osmolarity, and 24h fluid/blood requirements. Additional data collected prospectively included serum chloride, potassium, creatinine, lactate, bicarbonate, PT/PTT, ICU/hospital stay, mechanical ventilation, mortality, organ dysfunction measured by changes in multiorgan dysfunction score (∆MOD) (ICU admission minus mean daily ICU scores)(22) and pneumonia (fever, leukocytosis, radiological infiltrate, sputum culture).

2.4 Statistical Analysis

Treatment comparisons for continuous variables used repeated measures ANOVA and Pearson $\chi^2$ or Fischer's Exact test for categorical variables. Data are expressed as means ± standard deviation (SD) with a .05 significance level.

3.0 RESULTS

3.1 Study Population

During study period, among 1,121 trauma patients admitted, 94 (8.4%) fulfilled inclusion criteria and 27 enrolled with 13 receiving HSD and 14 placebo. Three HSD patients were excluded, 2 lacking required hemorrhage and 1 refusing participation (Figure 1). Both groups had similar characteristics and pre-admission interventions (Table 2). Patients randomized to HSD received more crystalloid before enrolment ($p=.04$), reflecting longer pre-hospital time and greater likelihood of referrals from other hospitals (ns, not significant).
Hypertonic Saline Resuscitation Modulates Neutrophil Adhesion Molecule Expression of Post-Traumatic Hemorrhagic Shock Patients

No significant differences in clinical outcome were detected. However, HSD patients required less crystalloid in the Emergency Room (ns) and half the amount of blood (ns) and colloids ($p=0.02$) during initial 24h. All patients required ICU admission with similar length of stay. HSD patients were liberated from mechanical ventilation 24h earlier than control (ns). MOD and pneumonia affected both groups equally. Two control patients died, shortening the group’s hospital stay (Table 2).

Table 2 – Baseline characteristics and outcomes of the study patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>HSD (n=10)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>47.5 (15.9)</td>
<td>49.3 (16.7)</td>
<td>.75</td>
</tr>
<tr>
<td>Gender, male, no. (%)</td>
<td>9 (64%)</td>
<td>7 (70%)</td>
<td>.76</td>
</tr>
<tr>
<td>ISS, mean (SD)</td>
<td>25.9 (10.3)</td>
<td>26.3 (11.4)</td>
<td>.83</td>
</tr>
<tr>
<td>Mechanism injury – MVA, no. (%)</td>
<td>9 (65%)</td>
<td>8 (80%)</td>
<td></td>
</tr>
<tr>
<td>Fall, no. (%)</td>
<td>1 (7%)</td>
<td>2 (20%)</td>
<td></td>
</tr>
<tr>
<td>Other, no. (%)</td>
<td>4 (28%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Transferred from other institution, no. (%)</td>
<td>5 (36%)</td>
<td>7 (70%)</td>
<td>.09</td>
</tr>
<tr>
<td>Time - pre-hospital, mean (SD), min</td>
<td>110.5 (66.9)</td>
<td>172.4 (82.9)</td>
<td>.49</td>
</tr>
<tr>
<td>in Emergency Room, mean (SD), min</td>
<td>114.6 (53.5)</td>
<td>164.5 (95.7)</td>
<td>.24</td>
</tr>
<tr>
<td>Lowest systolic BP in ER, mean (SD)</td>
<td>90 (22.7)</td>
<td>80 (15.6)</td>
<td>.31</td>
</tr>
<tr>
<td>Highest HR in ER, mean (SD)</td>
<td>114 (17.1)</td>
<td>110 (12.6)</td>
<td>.29</td>
</tr>
<tr>
<td>Crystalloid - pre-hospital, mean (SD), ml</td>
<td>835 (855)</td>
<td>2144 (1343)</td>
<td>.048 #</td>
</tr>
<tr>
<td>ER, mean (SD), ml</td>
<td>4542 (2758)</td>
<td>3689 (1865)</td>
<td>.28</td>
</tr>
<tr>
<td>total first 24h, mean (SD), ml</td>
<td>8080 (2736)</td>
<td>7796 (3189)</td>
<td>.75</td>
</tr>
<tr>
<td>Blood - pre-hospital, mean (SD), units</td>
<td>0.5 (1.16)</td>
<td>1.22 (1.7)</td>
<td>.27</td>
</tr>
<tr>
<td>ER, mean (SD), units</td>
<td>1.56</td>
<td>1.5</td>
<td>.62</td>
</tr>
<tr>
<td>total first 24h, mean (SD), units</td>
<td>4.36 (6.77)</td>
<td>2.2 (2.9)</td>
<td>.38</td>
</tr>
<tr>
<td>Colloids - total first 24h, mean (SD), ml</td>
<td>696 (773)</td>
<td>361 (377)</td>
<td>.02 #</td>
</tr>
<tr>
<td>LOS - total hospital stay, mean (SD)</td>
<td>27.4 (11.7)</td>
<td>36.9 (43.7)</td>
<td>.048 #</td>
</tr>
<tr>
<td>- ICU stay, mean (SD)</td>
<td>8 (8.2)</td>
<td>7.9 (6.8)</td>
<td>.3</td>
</tr>
<tr>
<td>Patients operated first 24h, no. (%)</td>
<td>10 (71.4%)</td>
<td>6 (60%)</td>
<td>.55</td>
</tr>
<tr>
<td>Number surgical procedures/patient, mean (SD)</td>
<td>2.6 (2)</td>
<td>2.2 (2)</td>
<td>.21</td>
</tr>
<tr>
<td>Complications – vent time, mean (SD), days</td>
<td>5.3 (6.2)</td>
<td>4.3 (7.2)</td>
<td>.91</td>
</tr>
<tr>
<td>pneumonia, number patients</td>
<td>1.43 (.51)</td>
<td>1.3 (.48)</td>
<td>.22</td>
</tr>
<tr>
<td>$\Delta$ MOD score, mean (SD)</td>
<td>1.9 (4)</td>
<td>1.68 (2.4)</td>
<td>.16</td>
</tr>
<tr>
<td>Death</td>
<td>2 (14.3)</td>
<td>0</td>
<td>.21</td>
</tr>
</tbody>
</table>

SD = standard deviation, no. = number, ISS = injury severity score, MVA = motor vehicle accident, min = minutes, ml = milliliters, ER = emergency room, ICU = intensive care unit, vent = mechanical ventilation, $\Delta$MOD = delta multiple organ dysfunction (Marshall et al, Crit Care Med 1995; 23(10):1638-1652), # = statistically significant.
3.2 Primary Outcome

Hemorrhage/resuscitation caused a marked and progressive increase in CD11b expression up to 24h (Figure 2). HSD inhibited shock-induced CD11b upregulation, sustaining it below baseline for the entire 24h period.

![Figure 2](image)

**Figure 2.** Neutrophil CD11b surface expression. Neutrophils were isolated from patients receiving a single 250 mL dose of HSD (7.5% NaCl 6%dextran-70, “O”) or placebo (0.9% NaCl, “■”), labelled with anti-CD11b-FITC monoclonal antibody and analysed by flow cytometry. Y-axis measures mean fluorescence intensity (MFI) in arbitrary units (a.u.). BL = baseline (prior to infusion); *significantly different from BL; †significantly different from control.

Hemorrhage/resuscitation per se caused no change in PMN CD62L expression (Figure 3). By contrast, HSD caused progressive reduction in CD62L expression, reaching a nadir at t=3h and remaining low over the entire experimental period.

![Figure 3](image)

**Figure 3:** Neutrophil CD62L surface expression. Neutrophils were isolated from patients receiving a single 250 mL dose of HSD (7.5% NaCl 6%dextran-70, “O”) or placebo (0.9% NaCl, “■”), labelled with anti-CD62L-PE monoclonal antibody and analysed by flow cytometry. Y-axis measures mean fluorescence intensity (MFI) in arbitrary units (a.u.). BL = baseline; *significantly different from BL; †significantly different from control.
Endotoxin stimulation elicited expected changes in both CD11b/CD62L from all patients, attesting PMN viability/functionality (Figure 4A,B). The reduction of CD62L surface expression paralleled an increase in serum soluble CD62L (Figure 5), suggesting activation-induced shedding mechanisms.

**Figure 4**: Neutrophil surface expression of CD11b (A) and CD62L (B) after endotoxin (LPS) stimulation. A, B – to attest viability and functionality, neutrophils from HSD (○) and placebo (■) groups were stimulated with 1µg/ml E. coli 055:B5 for 1 hour, labelled with specific monoclonal antibodies then analysed by flow cytometry. Y-axis measures mean fluorescence intensity in arbitrary units. BL = baseline, *significantly different from BL, †|significantly different from control group.

**Figure 5**: Serum soluble CD62L was measured to confirm that the reduction in CD62L surface expression was due to activation-induced shedding, by ELISA. Y-axis measures serum concentration (ng/mL) in HSD (○) and placebo (■) groups. BL = baseline, *significantly different from BL, †|significantly different from control group.
3.3 Secondary Outcomes

All patients experienced a drop in total leukocyte and neutrophil counts. HSD abolished the marked lymphopenia observed in the control group, without affecting other counts (Table 3). HSD modestly increased serum sodium/osmolarity, with normalization by 24 h (Table 4). No differences in serum chloride, potassium, creatinine, lactate, bicarbonate and PT/PTT were detected. HSD did not affect blood typing/crossmatching.

Table 3: Total leukocyte, granulocyte and lymphocyte counts at baseline and after resuscitation.

<table>
<thead>
<tr>
<th>Counts (x10^9/L)</th>
<th>Sample Time</th>
<th>*P Value compared to Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>1h</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.75 ± 0.92</td>
<td>14.41 ± 1.82</td>
</tr>
<tr>
<td>HSD</td>
<td>13.09 ± 0.96</td>
<td>14.41 ± 1.82</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.01 ± 0.82</td>
<td>12.18 ± 1.50</td>
</tr>
<tr>
<td>HSD</td>
<td>10.92 ± 0.80</td>
<td>10.59 ± 0.92</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.75 ± 0.26</td>
<td>1.37 ± 0.29</td>
</tr>
<tr>
<td>HSD</td>
<td>1.41 ± 0.24</td>
<td>1.12 ± 0.14</td>
</tr>
</tbody>
</table>

BL = baseline (prior to infusion); *significantly different from baseline.

Table 4: Changes in serum sodium and osmolarity at baseline and after resuscitation.

<table>
<thead>
<tr>
<th>Sample Time</th>
<th>BL</th>
<th>1h</th>
<th>3h</th>
<th>6h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>141.0 ± 3.5</td>
<td>139.2 ± 5.7</td>
<td>139.0 ± 4.6</td>
<td>140.2 ± 4.9</td>
<td>140.0 ± 4.2</td>
</tr>
<tr>
<td>HSD</td>
<td>141.1 ± 3.4</td>
<td>146.7 ± 2.8**</td>
<td>145.5 ± 4.3**</td>
<td>146.1 ± 3.8**</td>
<td>144.0 ± 3.9</td>
</tr>
<tr>
<td>Osmolarity, (mosmol/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>297.6 ± 8.1</td>
<td>299.0 ± 2.8</td>
<td>299.3 ± 4.2</td>
<td>288.5 ± 5.7</td>
<td>288.7 ± 1.2</td>
</tr>
<tr>
<td>HSD</td>
<td>298.3 ± 7.8</td>
<td>304.1 ± 11.3</td>
<td>310.5 ± 2.1**</td>
<td>309.0 ± 1.4**</td>
<td>289.8 ± 5.1</td>
</tr>
</tbody>
</table>

BL = baseline (prior to infusion), *significantly different from baseline, †significantly different from control.
4.0 DISCUSSION

Neutrophil sequestration in various target organs following shock/resuscitation contributes to the pathogenesis of multiorgan dysfunction. Two adhesion molecules, CD11b and CD62L are central to the events leading to PMN-endothelial adhesion with resultant endothelial damage, increased capillary leak and PMN transmigration with oxidative/proteolytic organ damage. The present double-blinded RCT investigated the effect of a single dose of HSD on one critical parameter of PMN activation, the surface expression of adhesion molecules CD11b and CD62L, in patients undergoing hemorrhagic shock resuscitation. HSD caused progressive CD62L shedding as evidenced by reduced surface expression and the presence of soluble CD62L in the blood. Furthermore, HSD prevented shock/resuscitation-induced rise in surface CD11b. These effects mirror recent experimental data and are comparable to reports from normal volunteers. While these effects did not translate in beneficial outcomes, the study was clearly not designed to test this possibility but rather to establish the principle that this intervention might prove salutary in larger patient trials. Our results further corroborate earlier trials demonstrating that HSD represents a safe additive to resuscitation.

The anti-inflammatory effects on PMN we observed are consistent with studies suggesting that HSD has organ protective properties. In a study by Simmas, no children with post-traumatic intracranial hypertension treated with HS developed ARDS versus 30% following conventional treatment. Wade et al demonstrated that survival was twice as high for hypotensive severe head injury patients resuscitated with HSD. Mattox reported less pneumonia, while the HSD-treated patients in this trial required shorter ventilatory support.

Besides attenuating inflammatory damage, HSD also reduces fluid requirements, immediately restores organ perfusion and reduces post-shock organ edema, all factors that might lessen MOD. In contrast, standard large-volume resuscitation may increase brain edema, prolong mechanical ventilation and worsen outcome.

In experimental models, hypertonicity inhibits PMN activation in both animal and isolated human cell preparations, while return to isotonicity restores competency. We found that HSD exerted prolonged effects on PMN CD11b/CD62L expression (24 h), even after normalization of serum osmolarity. Possible explanations include osmotic effects on different cells and tissues, whereby interactions with PMNs, prolong HSD anti-inflammatory properties. Another possibility, compatible with a prolonged effect, is that HSD reduces the initial inflammatory outburst, such that even after the amplification cascade, the overall result is of an attenuated inflammation. Furthermore, attenuated initial inflammation generates less anti-inflammatory compensatory mechanisms, potentially reducing late immunosuppression. Both reduced initial systemic inflammatory response and reversal of late immunosuppression are well described effects of HSD in experimental models.

Trauma/hemorrhage suppresses lymphocyte proliferation and function, resulting in immunosuppression, sepsis and MOD. In experimental models HSD rescues T-lymphocytes, restoring their proliferative capability, decreasing sepsis/MOD. We found that HSD reduced post-traumatic lymphopenia, suggesting similar effects on human lymphocytes.
5.0 CONCLUSION

This trial substantiates experimental observations on the effects of osmolarity in neutrophils and lymphocytes, translating basic science investigations into the clinical situation. It demonstrates for the first time the immunomodulatory effects of HSD in humans. The potential association of such effects with reduced organ dysfunction and better outcome provides proof of principle for further investigations. HSD may in fact prove to be an effective immunomodulatory agent in the broader range of conditions caused by ischemia/reperfusion injury.

Acknowledgements

We thank Sheila Petrongolo for expert technical assistance. This work was supported by Defence R&D Canada.

6.0 REFERENCES


Hypertonic Saline Resuscitation Modulates Neutrophil Adhesion Molecule Expression of Post-Traumatic Hemorrhagic Shock Patients


Hypertonic Saline Resuscitation Modulates Neutrophil Adhesion Molecule Expression of Post-Traumatic Hemorrhagic Shock Patients


Changes in Interstitial Metabolic Parameters during Hemorrhagic Shock

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ABSTRACT

Decompensation in hemorrhagic shock is the critical stage after which resuscitative efforts may prove futile. One mechanism for decompensation may be potassium-mediated vasodilation and/or loss of cardiac contractility, and thus a method of measuring interstitial potassium may be a crucial part of future metabolic monitoring efforts. Anesthetized rats underwent controlled hemorrhage to a constant mean arterial pressure of 40 mmHg. Microdialysis probes were implanted in skeletal muscle, vein, and liver for continuous assessment of potassium, glucose, lactate, pyruvate, and glycerol concentrations. Arterial blood samples were drawn at 30-minute intervals, until late (decompensatory) hemorrhagic shock was reached. Potassium concentrations in muscle interstitium were significantly higher in hemorrhaged animals than controls (2.34 times baseline vs. 1.24, p < 0.05), this difference was not reflected in blood values. These data may provide clues into new ways to monitor and treat victims of hemorrhagic shock on the battlefield.

1.0 INTRODUCTION

Decompensation is the critical “point of no return” in hemorrhagic shock. It is defined as sustained hypoperfusion leading to irreversible cardiovascular collapse (1), and is manifested clinically as the loss of ability to mount a hemodynamic response to aggressive fluid resuscitation. It is generally stated that once decompensation is reached, further resuscitative efforts are futile (1). Several chemical substances have been proposed as markers for tracking the evolution of hemorrhagic shock and impending collapse; these include pH, lactate, pyruvate, glucose, and base excess. An important military goal of metabolic monitoring would thus be identifying the most critical factors heralding decompensation in battlefield casualties. In addition to providing the ability to assess the stage or severity of hemorrhagic shock, such monitoring may establish prognosis and/or determine the adequacy of therapeutic interventions.

The relationship between serum concentrations—which are commonly measured experimentally and in controlled clinical settings—and the corresponding concentrations in the tissue interstitium has not been explored systematically. This has important consequences for metabolic monitoring because it may be faster and more practical on the battlefield to sample from a transcutaneous or intramuscular sensor than to attempt intravascular sampling. Additionally, the interstitial level may be more representative of the end-organ milieu—and thus a better indicator of underlying pathophysiology. Further information is thus needed about the correlation (or lack thereof) between interstitial and intravascular concentration with regards to monitoring for decompensation.

It is possible that these substances serve not just as indicators, but also as actual mediators of the pathogenesis of decompensation. Specifically, muscle interstitial potassium has been shown to rise out of proportion to intravascular levels during hemorrhage (2;3). These changes are consistent with an effective loss of Na⁺-K⁺ ATPase (NKA) activity. Hyperkalemia may be a mechanism for vascular smooth muscle hyper-polarization and vasodilatation observed at the decompensatory stage (4;5), which can be mediated by the inwardly rectifying potassium Kir channels or by NKA (6;7).

Our objectives were to revisit the issue of interstitial changes in potassium and other metabolic substances (glucose, lactate, pyruvate, and glycerol) in the pathogenesis of hemorrhagic shock. In addition, we wished to examine how tissue-specific interstitial concentrations correlated with intravascular values. Our hypothesis was that interstitial hyperkalemia in skeletal muscle may herald the onset of decompensation.

2.0 METHODS

2.1 Animals

Male Wistar rats (n = 14, Charles River Laboratories, Wilmington, MA) were quarantined for ten days in a temperature- and light-controlled environment. Animals had ad libitum access to rodent chow (Nestlé Purina, St. Louis, MO) and water. After quarantine, animals were weighed for five to seven days prior to use in order to document continued weight gain (5 g/day). Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals, and experiments involving animals adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council.
2.2 Surgical Preparation

On the day of experimentation, the rats were initially anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. Once a surgical plane of anesthesia had been attained, the rats were shaved and the skin cleaned. A tracheostomy was performed using PE 240 tubing (Clay Adams, Inc., Parsippany, NJ). Under aseptic conditions, catheters (PE50, Clay-Adams) were placed in the following locations: left femoral artery, left carotid artery, left femoral vein, and caudal right quadrant of the peritoneum. The left femoral artery catheter was attached to a continuous blood pressure monitor (BPA, Micromed, Louisville, KY). The left carotid artery catheter was attached to a computer controlled peristaltic pump (Model 720, Instech Laboratories, Inc., Plymouth Meeting, MA) that emptied into a heparinized reservoir placed on a balance (PB303S with RS232 port, Mettler, Inc., Toledo, OH). The left femoral vein catheter was used for venous access as needed.

The peritoneal catheter was used to provide a continuous infusion of sodium pentobarbital (10% in normal saline at 0.06 µl/min/g) administered to maintain the anesthetic plane. Additional pentobarbital was administered as a 0.05-0.1 ml intraperitoneal bolus as needed to maintain loss of digital and corneal reflexes.

Microdialysis probes (CMA/20, CMA/Microdialysis, North Chelmsford, MA) were placed in a branch of the right femoral vein, in the quadriceps major of the right leg, and in the liver through an abdominal incision.

A rectal thermistor temperature probe was inserted and the core temperature was maintained at 37 °C by a homeothermic blanket (Harvard Apparatus, South Natick, MA) and heating lamp. Each animal was anticoagulated with porcine heparin (1 IU/g i.v. to a maximum 350 IU) prior to the start of the stabilization period plus an additional 100 IU i.v. 60 min later.

2.3 Hemorrhage Protocol

The WRAIR Hemorrhagic Shock Data Acquisition (HSDAQ) Program, an interactive program written in LabVIEW (National Instruments, Austin, TX) controlled the hemorrhage. This program monitored arterial blood pressure and the weight of the shed blood volume (SBV) removed from the animal. It controlled the peristaltic pump to maintain blood pressure at the desired level. Arterial pressures (systolic, diastolic, and mean), heart rate, and shed blood volume were monitored continuously and recorded every 5 seconds by the program.

Controlled hemorrhage was performed following our established protocol. The HSDAQ program was started and the animal was monitored initially for a 20-minute stabilization period after completion of the surgery. After the control period, the program commenced hemorrhage by withdrawing blood from the carotid artery into the reservoir. Mean arterial pressure (MAP) was linearly dropped to 40 mm Hg over a 15 minute period, then maintained at that value for the duration of the experiment by the additional withdrawal or return of shed blood to the animal (with program providing feedback control). In the initial (compensatory) phase of hemorrhage, blood had to be continuously withdrawn from the animal to maintain the desired MAP. After a period, however, the shed blood had to be returned to the animal from the reservoir to maintain this pressure. The point where this transition occurs is the start of decompensation, and thereafter the phase of shock is designated by the amount of blood that has been returned to the animal, expressed as a percentage of the peak SBV (8). The experiment continued until return of 50% of the peak SBV, at which time the animal was euthanized.

Control animals underwent the same surgical preparation as the bled animals but the peristaltic pump was never activated. The duration of the control experiments were matched to that of the preceding hemorrhage experiment.
2.4 Sample Collection and Analysis

Arterial blood samples were collected from the left femoral line at $t = -17$ min from the start of the bleed (during the stabilization period), at $t = 43$ min, and subsequently at thirty-minute intervals. Microhematocrit was measured in a centrifuge (Model TRIAC, Clay Adams). Arterial blood gases and potassium were measured using an i-STAT portable clinical analyzer (i-STAT Corp., East Windsor, NJ).

Microdialysis was performed continuously during hemorrhage by perfusing the implanted probes with normal saline containing 4.5 mM RbCl. The perfusate rate was 1 µl/min and it was collected in 15-µl fractions by a fractional collector (CMA/142, CMA Microdialysis).

Potassium concentrations in the microdialysis samples were measured using the internal standard technique (9) to correct for lack of 100% recovery in the probes: the fractional loss of rubidium from the perfusate was assumed equal to the fractional uptake of potassium into the perfusate from the surrounding tissue. 10-µl aliquots of the collected fractions were diluted 1:1000 in 10 ml of 2% HNO$_3$. Potassium and rubidium concentrations were then measured using inductively-coupled plasma mass spectrometry (Elan 6000, Perkin-Elmer, Norwalk, CT).

Concentrations of glucose, lactate, pyruvate, and glycerol were measured in the remainder of the microdialysis samples using a commercial analyzer (CMA 600). Internal standards were not used for these analytes, thus the concentrations obtained are underestimates.

2.5 Statistical Analysis

Statistical significance between groups was determined using two-tailed Student's test for comparing means of independent measurements. Significance was defined as a $p$ value < 0.05.

3.0 RESULTS

Table 1 shows the baseline data for the animals. There was no significant difference in animal weight or starting MAP between groups. There were no statistical differences between groups for any of the baseline analyte values in any of the tissues. The mean peak SBV for the hemorrhage group was $5.6 \pm 0.6$ ml and the mean time to peak SBV was $42.6 \pm 3.1$ min.

Figure 1 shows hemodynamic data (mean arterial pressure and shed blood volume) versus time from start of hemorrhage. The very small error bars for the hemorrhage MAP vs. time demonstrate how the HSDAQ program reproducibly maintains the hemorrhaged animals at the desired target pressure.

Shown in Figures 2-3 are arterial and microdialysis results for $[K^+]$ and various other metabolic parameters.. To emphasize the changes with time, all values are shown as ratios to baseline.
Changes in Interstitial Metabolic Parameters during Hemorrhagic Shock

### Table 1: Baseline values for animals. All values shown ± one S.E.M. (Left) MAP = mean arterial pressure, HR = heart rate, BE = base excess, Hct = hematocrit, PSBV = peak shed blood volume, L/P = lactate/pyruvate ratio. (Right) Baseline measurements of metabolic parameters from arterial samples and microdialysis (µD) probes, performed at t = −17 min from start of bleed. p > 0.05 for all values (C vs. H).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Hemorrhage (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>422±18</td>
<td>423±20</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86.4±6.3</td>
<td>88.5±4.8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>388±12</td>
<td>373±10</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.01</td>
<td>7.42±0.01</td>
</tr>
<tr>
<td>BE</td>
<td>3.0±0.5</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>Hct</td>
<td>52.6±0.6</td>
<td>51.6±1.1</td>
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<tr>
<td>PSBV (ml)</td>
<td></td>
<td>5.6±0.6</td>
</tr>
<tr>
<td>Time to PSBV (min)</td>
<td></td>
<td>42.6±3.3</td>
</tr>
</tbody>
</table>

### Potassium (Figure 2, top panels): Interstitial [K+] was higher in muscle at peak SBV than controls (ratio = 2.33 vs. 1.24 times baseline, p < 0.05); this was not reflected in vein or liver. These results are consistent with previous studies (2;3). After peak SBV, average muscle [K+] declines versus time due to dropout of animals reaching experimental end.

### Glucose (Figure 2, middle and bottom panels): Arterial glucose ratios were higher (2.68 vs. 1.06, p < 0.05) at peak SBV than controls, and then decreased. This hyperglycemia during hemorrhagic shock and subsequent fall in glucose levels during decompensation has been previously described (10). There were similar trends in venous and muscle samples, but they did not reach significance. In liver, no trend versus time for either group was appreciated.

### Lactate (Figure 3, top panel): Values in all tissues were higher in hemorrhaged animals, but only reached significance in venous samples at t = 28 (ratio = 3.05 vs. 1.42) and 43 (6.91 vs. 1.67) min. In muscle similar differences were seen (ratios = 2.8 vs. 0.8 at 28 min and 4.9 vs. 0.8 at 43 min) but hemorrhage vs. control differences were not significant due to small n = 4 from analytical problems. In liver the difference at t = 28 min just fell short of statistical significance (2.74 vs. 1.32, p = 0.068). The smaller relative increase in muscle and liver is consistent with previous results and has been used to argue that these tissues are lactate consumers in hemorrhage (11).

### Pyruvate (Figure 3, second panels): Venous samples tended to increase with time in both hemorrhage and control groups without statistically significant difference. Samples from muscle and liver in both groups showed no clear change with time.

### L/P ratio (Figure 3, third panels): Trended upward with time in control liver samples and all hemorrhaged samples without significant statistical difference.

### Glycerol (Figure 3, bottom panels): Levels in all tissues tended to increase in both hemorrhage and control animals, without significant difference between groups.
4.0 SUMMARY/CONCLUSIONS

Interstitial [K\+] in skeletal muscle during hemorrhage appears to correlate with the onset of decompensation, while intravascular [K\+] does not. Maximal hyperglycemia also correlates with peak SBV. Muscle and liver glucose may be similarly correlated, although the magnitude of the change appears to be less. The rise in venous lactate levels from microdialysis also corresponded with peak SBV. Changes in tissue lactate had similar trends but did not reach statistical significance due to small numbers. Interstitial measures of potassium, lactate, and/or glucose may prove to be of diagnostic and prognostic significance in hemorrhagic shock.

Interstitial hyperkalemia during hemorrhage has previously been demonstrated with ion sensitive electrodes (2) and ion sensitive field effect transistors (3). Furthermore, x-ray microanalysis studies have shown that intracellularly, potassium decreases and sodium increases during hemorrhage (12). These changes are consistent with a derangement of Na\textsuperscript{+}-K\textsuperscript{+} ATPase (NKA) activity. The nature of this derangement is unknown. Possible mechanisms include loss of available NKA or substrate, presence of an in vivo NKA inhibitor, or an uncoupling of NKA activity to potassium transport. Given the possible significance of hyperkalemia as an etiology for decompensation, future study in this area is warranted.

This material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3), AR 360.5. Funded by the U.S. Army Medical Research and Materiel Command.
Changes in Interstitial Metabolic Parameters during Hemorrhagic Shock

Figure 2: Relative changes in potassium and glucose. Error bars designate ± one S.E.M. Vertical lines in hemorrhage plots delineate average time to peak shed blood volume. Top panels: Potassium in arterial and microdialysis (vein, muscle, liver) samples. Center panel: Arterial glucose. Bottom panels: Microdialysis glucose from tissues. *p < 0.05 for hemorrhage vs. control.
Figure 3: Relative changes in lactate, pyruvate, L/P ratio, and glycerol. Error bars designate ± one S.E.M. Vertical lines in hemorrhage plots delineate average time to peak shed blood volume. *p < 0.05 for hemorrhage vs. control.


Targeting Complement in Treatment of Intestinal Ischemia/Reperfusion-Induced Injury

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ABSTRACT

Complement activation occurs during tissue injury and inappropriate or excessive activation contributes to the expression of pathology becoming a double-edged sword. Understanding the role of complement and its natural regulatory molecules will enable the development of therapeutic interventions to prevent excessive damage during mesenteric ischemia/reperfusion (IR). In this chapter, we will briefly review the mechanism of complement activation during intestinal ischemia/reperfusion and discuss results and significance of mesenteric ischemia/reperfusion induced injury in animal models with altered complement activation. Finally, we will discuss development of current inhibitors of complement activation and those that may be used in the future.

1.0 ISCHEMIA/REPERFUSION INTESTINAL DAMAGE

Despite advances in medical care, the mortality rate for acute mesenteric ischemia remains unchanged from the 1980’s at 81%. The lack of decreased fatalities is due to the fact that it is a rare and difficult diagnosis with rapid progression from local intestinal injury to systemic release of inflammatory mediators leading to distant organ injury. Intestinal ischemia is associated with multiple trauma conditions, such as hemorrhagic shock, burns, myocardial infarction and multiple organ failure [Trunage 1994]. These conditions lead to a reduction of blood volume that is believed to result in splanchnic vasoconstriction and functional if not true ischemia of the gut [Williams 1983; Austen 1999; Dong 1999; Eror 1999; Kilgore 1999; Rehrig 2001]. The splanchnic circulation is a large vascular bed that receives as much as 25-30% of the total blood flow bringing oxygen and nutrients to the intestine. As blood flow to the intestine decreases, the flow to the various circuits is not decreased equally with more flow shunted to the mucosa than to other networks. The rapid turnover of the mucosa makes it extremely sensitive to hypoxia. Therefore, loss of blood flow to a tissue for a limited amount of time, as little as 20 min, results in damage to the mucosal surfaces with villi disruption. However, reperfusion induces pathological changes to the tissue that are greatly enhanced compared to that of ischemia alone. These alterations of reperfusion injury after mesenteric ischemia cause additional local inflammation characterized by complement activation and deposition, neutrophil infiltration and eicosanoid generation that coincides with mucosal injury [Eror 1999; Rehrig 2001; Conner 1999]. The role of complement in mediating this injury and the possibilities to inhibit complement activation is the focus of current research.

2.0 COMPLEMENT ACTIVATION IN INTESTINAL DAMAGE

Complement is a complex cascade of over 30 proteins that are activated in an orderly manner. The cascade has 3 initiating arms: that is the classical, lectin and alternative pathways that each produces enzymatic complexes, C3 and C5 convertases (Fig. 1). The cascade continues with the cleavage of C3 and C5 and all 3 pathways culminate in a common terminal pathway. The terminating complex, the membrane attack complex, is a lytic complex that inserts into the membrane forming a pore in the cell. Since the complement pathway is capable of extreme cell and tissue damage, complement regulatory molecules that control the rate of its activation are essential and occur naturally at multiple points within the cascade. However, in many clinical conditions, unregulated complement activation and subsequent tissue damage occurs during intestinal ischemia, blunt trauma and hemorrhagic shock.

Many of the clinical conditions associated with inappropriate complement activation also reduce blood volume and lead to subsequent mesenteric vasoconstriction resulting in functional intestinal ischemia [Kilgore 1999]. When organs such as the intestine are subjected to severe vascular ischemia, followed by reperfusion of blood into the site, local as well as remote tissue inflammation and injury ensues. Intestinal damage as a result of ischemia and subsequent reperfusion varies by the region of the intestine. However, the extremes of the IR-induced injury apply to the entire intestine. In other words, the intestine can tolerate short periods of ischemia without severe injury but long periods of mesenteric ischemia followed by reperfusion results in death. Mesenteric ischemia triggers an inflammatory reaction, characterized by neutrophil infiltration,
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activation and local mucosal injury, in which complement activation plays a pivotal role. Reperfusion of the ischemic gut is believed to lead to another surge of complement activation and further mucosal injury [D’Ambrosio 2001].

Using rodent models, the mechanism of IR-induced intestinal damage has been shown to involve complement activation. The exact mechanism of complement activation during intestinal IR remains unclear as there is evidence that more than one complement pathway (classical, alternative or lectin) may be activated and enhancing tissue injury [Fleming 2000; Stahl 2003; Williams 1999]. Further evidence that complement activation is directly involved in the effector phases of intestinal IR injury has been provided by studies showing that inhibition of the complement pathway at the point of C3 or C5 activation can either prevent or substantially attenuate intestinal injury [Austen 1999; Eror 1999; Rehrig 2001; Williams 1999; Hill 1992; Fleim 2003a]. In addition, inflammatory mediators generated during complement pathway activation, such as the anaphylatoxin C5a and the membrane attack complex (MAC), are known to be able to directly cause cellular activation and injury too [Fleming 2003a; Ward 2000; Kohl 2001]. The specifics of each complement initiating pathway, the terminal complex and the anaphylotoxins will be discussed below.

![Figure 2. Complement depletion inhibits IR-induced intestinal injury. Balb/c mice were treated with CVF for 24 hr prior to subjecting animals to either sham treatment or IR. After 2 hr reperfusion, intestinal sections were collected and immediately formalin fixed. Geimsa stained intestinal sections from each treatment group were scored for mucosal injury (0-6). Each bar is the average ± SEM with 6-8 animals /group. Using ANOVA with Neuman Keuls post-hoc test, * indicates significant difference from sham group, p<0.05.](image-url)
3.0 INDICATIONS OF COMPLEMENT INVOLVEMENT

The essential role of complement in mesenteric ischemia/reperfusion induced tissue injury has been shown in numerous animal models. We have established a mesenteric IR model in mice and confirmed the role of complement in intestinal IR using cobra venom factor (CVF) to deplete complement factor 3 (C3). The intestinal mucosa of the sham-operated animals remained normal as indicated by an injury score of 0.68 (Fig. 2). Mice subjected to IR had significant intestinal injury as indicated macroscopically by swollen and edematous with areas of red streaks. Microscopically, the injury ranged from shortened and vacuolated villi to complete destruction of normal mucosal architecture with frank hemorrhage. In contrast, mice subjected to sham procedure or IR after treatment with CVF had no significant intestinal mucosal injury (Fig. 1). Thus, similar to the rat model, complement has a role in the local injury induced by IR in mice.

Activation of complement 5 (C5) leads to the production of a potent anaphylotoxin, C5a, along with C5b, the initiator of the membrane attack complex. The use of C5 deficient mice showed that either the membrane attack complex, the anaphylatoxin, C5a or a combination of both could prevent or substantially attenuate intestinal injury [Austen 1999; Wada 2001]. In additional studies, anti-C5 monoclonal antibodies have been administered to mice to prevent C5 activation and subsequent local and remote tissue damage [Fleming 2003a and 2003b; Wada 2001]. Inhibition of C5 activation by an anti-C5 antibody administered to wild type mice subjected to mesenteric IR, prevents C5a generation, PMN infiltration and deposition of the terminal complement complex on the damaged issues in a manner similar to that observed in C5 deficient mice [Wada 2001; Wang 1995; Vakeva 1998a and 1998b; Rinder 1995; Fleming 2002]. These studies however, do not distinguish between the actions of C5a and C5b-9 terminal complex or address the initiating pathways.

4.0 EVIDENCE FOR THE INITIATING PATHWAY

It is known that complement is activated immediately after injury and the severity of the trauma is directly proportional to the level of complement activation [Fodde 1998]. The complement cascade can be activated by contact with microbes but during short periods of intestinal IR, the alternative and classical pathways are both over-activated in the absence of microbial infection. It is well known that the clotting cascade activates complement. Some possible alternative complement activators include: reactive oxygen or nitrogen metabolites, exposed collagen, mitochondrial membranes and extracellular ATP [Goris 2000; Gallinaro 1992; Mollnes 1999]. In addition, in vitro data shows that damage to the endothelium activates the alternative pathway and recent data show that the absence of Factors D or B protects mice from IR-induced damage [Stahl 2003; Fruchterman 1998]. It is possible that multiple pathways are involved in the complex mechanisms of mesenteric ischemia reperfusion induced damage. Although the exact method of complement activation may differ with the traumatic insult, the down-stream events of excessive complement activation results in an inflammatory reaction.

The ability to design logical therapeutics to prevent complement action depends on our understanding of the complement pathways that are involved and the initiating factors for the specific pathway(s). The availability of a mouse model and mice engineered to be genetically deficient in a specific complement factor has aided our understanding of the role of complement components in mesenteric injury. These studies are summarized in Fig. 3 and detailed below.
4.1 Role of Classical Complement Pathway

Three observations have strongly implicated the classical pathway in the process. The first is that intestinal IR injury is significantly decreased in \textit{RAG-1-/-} mice, and reconstitution of these Ig deficient mice with purified IgM natural antibody to normal levels [Williams 1999] restores IR-induced injury. The second is that mice with normal levels of natural antibody, but in which the gene encoding complement C4 is inactivated (\textit{C4-/-}), are protected from injury [Williams 1999]. The importance of natural IgM antibody and the classical complement pathway in mediating IR injury of skeletal muscle has also been shown using a similar experimental strategy with C3, C4 and Ig deficient mice [Weiser 1997]. From these and other findings, it has been proposed that natural antibodies bind to antigen(s) revealed on the surface membrane of cells subjected to ischemia and subsequently activate complement by recruiting C1 and then cleaving C4 [Williams 1999]. This is followed by the generation of complement C3 and C5 activation fragments with ensuing increases in adhesion molecule expression and release of a cascade of inflammatory mediators, including leukotriene B4 and others [Eror 1999; Rehrig 2001; Fleming 2000]. Finally, mice deficient in complement receptors 1 and 2 (CR2-/-) are also resistant to local IR induced damage. In addition, injection of IgM and IgG from wild type mice restored all measured parameters of IR-induced injury indicating that role of antibody and the classical complement pathway are important in initiating complement activation.

4.2 Role of Lectin Pathway

The studies discussed above using C3 and C4 deficient mice, suggest that either the classical or the lectin pathways have a role in local IR-induced injury. There is a lack of intestinal ischemia studies using lectin
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pathway deficient mice or lectin specific inhibitors. However, Stahl’s group has shown deposition of MBL on hypoxic endothelial cells in vitro [Collard 1999]. In additional studies, anti-mannose binding lectin antibodies were used to determine the role of MBL in myocardial IR-induced injury [Wetsel 2000]. These studies indicate that the lectin pathway is activated during the oxidative stress associated with ischemia. Further studies will be needed to show the pathway’s role in intestinal IR-induced injury.

4.3 Role of Alternative Pathway

There is recent data indicating that the alternative complement pathway is also involved in intestinal IR injury. Factor D, of the alternative pathway, forms the alternative pathway C3 convertase by cleaving Factor B. Factor D deficient mice, lacking the alternative C3 convertase, are resistant to IR-induced intestinal and pulmonary damage. Mesenteric IR-induced complement deposition is prevented in both the intestine and the lungs. Additionally, administration of Factor D restored IR-induced decrease in intestinal lactate dehydrogenase. This restoration was prevented by treatment with anti-Factor D antibodies. Thus, it appears that the alternative complement pathway is actively involved in the complex mechanism of mesenteric IR injury.

5.0 ROLE OF THE LYTIC COMPLEX OR ANAPHYLOTOXINS

C5a, a small, glycosylated peptide, is a potent chemoattractant for PMN, monocytes and T cells (reviewed in [kohl 2001; Jordan 2001]. In vitro studies have shown that C5a induces degranulation, respiratory burst, increases adhesion molecule expression and delays apoptosis in PMN [Perianayagam 2002; Tyagi 2000; Binder 1999]. In addition, in vivo studies using anti-C5a antibodies have indicated that C5a alters vascular permeability and neutrophil activation during cardiopulmonary bypass [Tofukuji 2000], hind limb IR [Bless 1999], sepsis [Huber-Lang 2001a and 2001b; Reidemann 2002 and 2003] and inflammatory lung injury [Mulligan 1996]. C5a receptors (C5aR) are expressed on the surface of intestinal cells under inflammatory conditions, as well as on bronchial epithelial cells [Rothermel 2000]. Blockade of these receptors using C5aR antagonists (C5aRa) indicates a role for C5a in systemic activation of neutrophils in multiple animal models [Pellas 1998; Heller 1999; Haynes 2000; Arymugam 2002]. To distinguish the role of C5a from that of C5b-9 on local and remote tissue injury, we inhibited the actions of C5a during mesenteric IR by treating wild type mice with a cyclic hexapeptide C5a receptor antagonist (C5aRa) and we administered C5a to C5 deficient (C5−/−) mice subjected to mesenteric IR. These experiments showed that during IR, C5a is sufficient to induce limited local damage and eicosanoid production but not systemic PMN activation. In addition, systemic C5a administered during IR can induce VCAM-1 expression on remote organs such as the lung without inducing increased vascular permeability.

This inflammation involves anaphylatoxin recruitment and subsequent activation of granulocytes as well as upregulation of endothelial adhesion molecules, the local release of other inflammatory mediators and cytokines. Together these potent mediators may result in local damage or may activate the inflammatory response (and complement) systemically. Extensive systemic complement activation can lead to a whole body inflammatory reaction such as adult respiratory distress syndrome, systemic inflammatory response syndrome, and multiple organ failure.
6.0 COMPLEMENT INHIBITION OF INTESTINAL DAMAGE

Because this cascade of proteins results in cell lysis and tissue destruction, the complement system includes multiple regulatory proteins that prevent non-specific complement activation. Some of these regulatory proteins are cellular receptors for the breakdown products of the components of the system. The understanding of natural regulatory proteins and receptors and their involvement in protection of mesenteric damage has allowed the design and development of therapeutic interventions that inhibit complement activation and prevent inflammatory tissue damage. Complement inhibitors are currently being studied to determine their ability to inhibit tissue damage as a result of mesenteric IR (summarized in Fig.4). Using a rat model of intestinal IR, several groups showed that administration of sCR1, a regulator of both classical and alternative pathways, significantly reduced rat local and systemic injury, PMN infiltration, and leukotriene B\textsubscript{4} (LTB\textsubscript{4}) production [Eror 1999; Hill 1992].

In mice, complement receptor 1-related gene/protein y (Crry) is a membrane regulatory protein altering the activity of both the classical and alternative complement pathways. Using a recombinant soluble form of Crry fused to the hinge, CH2, and CH3 domains of mouse IgG\textsubscript{1} (Crry-Ig), mice were pretreated either 5 min prior to, or 30 min after, the initiation of the reperfusion phase of mesenteric IR. IR-induced injury was reduced after Crry-Ig was administered. Pre-treatment with Crry-Ig reduced the local intestinal mucosal injury and decreased generation of LTB\textsubscript{4}. When given 30 min after the beginning of the reperfusion phase, Crry-Ig resulted in a decrease in IR-induced intestinal mucosal injury comparable to when it was given 5 min prior to initiation of the reperfusion phase. Despite the presence of substantial number of neutrophils, Crry-Ig administered 30 min after the initiation of the reperfusion prevented the IR-induced tissue injury damage. This indicates that although neutrophils may have a role in the damage, complement inhibition is beneficial.
C1 inhibitor (C1 Inh) inhibits the earliest steps of the classical and the mannose binding lectin pathways. When C1 Inh was administered to mice prior to mesenteric IR, mucosal injury and was effectively inhibited in a dose dependent manner. These findings emphasize the importance of complement activation in ischemia/reperfusion and highlight the potential therapeutic use of C1 Inh in limiting and/or preventing damage caused by ischemia/reperfusion.

Because the local damage itself is not believed to be life threatening, other groups have focused on C5a as a cause of the excessive systemic inflammatory response. Using a small peptide C5a receptor antagonist that binds the human C5a receptor, it has been shown that serum markers of systemic inflammation, neutrophil activation and remote organ injury can be prevented even when the peptide is given during the ischemic period, prior to beginning reperfusion [Fleming 2003a and 2003b; Arumugam 2002].

Recently, IVIg (high-doses of immunoglobulins modified for intravenous use [Basta 1996] have successfully blocked complement mediated tissue injury in a rat model of mesenteric ischemia/reperfusion [Anderson 2001a, 2001b, and 2002]. Therefore, although the exact mechanism of complement activation has not been elucidated, it is apparent that complement plays a substantial role in both local and systemic tissue injury during ischemia of multiple organs.

7.0 DEVELOPMENT OF THERAPEUTICS TO PREVENT COMPLEMENT-MEDIATED INTESTINAL INJURY

As indicated above, complement activation is part of the pathogenic process in mesenteric IR. The complement activation process appears to involve each of the three initiation channels as well as the common terminal pathway and anaphylotoxin in the damage process. As discussed above, animal models of mesenteric IR have clearly shown, that inhibition of complement activation can, prevent, improve or reverse the disease pathology. Naturally occurring inhibitors control the amount of injury induced by the cascade of complement activation and may become useful therapeutics. However prior to the therapeutic use of these inhibitors, a number of important questions must be answered. First, is complement central in pathology of mesenteric IR? Second, which pathway is the primary initiator of the activation? Third, is general complement inhibition associated with side effects such as suppression of the innate immunity and the appearance of overwhelming infections?

Complement inhibitors for therapeutic use in mesenteric IR are being designed in a logical fashion. First, using molecular engineering, monoclonal antibodies that block activation of central complement factors can be humanized and used in the treatment of disease. An anti-C5 antibody is in human trials for use in other diseases. Second, the half-life of natural complement activation inhibitors such as DAF, CD59, CR1 can be extended by genetically fusing with the Fc portion of IgG. Third, fusing of multiple complement inhibitors that act at different stages of the activation cascade may act at different phases resulting in more effective complement inhibition. Fourth, recently, peptide inhibitors blocking the action of convertases have emerged as a new promising approach. Compstatin represents such an example as it inhibits complement activation by blocking C3 convertase-mediated cleavage of C3 [Sahu 2003]. Fifth, and lastly, in response to the consideration that the use of complement inhibitors may cause systematic inhibition and unwanted side effects, from the complete lack of complement, such as overwhelming infection, investigators have considered the fusion of complement inhibitors to molecules that will direct it to the site of inflammation. For example, complement inhibitors can be conjugated to selectin ligands that will direct them to sites of increased selectin
expression, i.e., inflammation or delivered via targeted liposomes to a specific location where the inhibitor is released in a concentrated region.

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Targeting Complement in Treatment of Intestinal Ischemia/Reperfusion-Induced Injury


Opioid Peptides Increase Blood Pressure and Enhance Survival of Rats Undergoing Hemorrhagic Shock without Fluid Resuscitation

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ABSTRACT

Rats weighing 300-350 g had catheters placed in the femoral artery (for hemorrhage), tail artery for blood pressure (BP) measurements and the tail vein (for administration of opioids) controls received saline or opioids without hemorrhage. For the moderate hemorrhage studies (5.5 ml hemorrhage volume) animals received saline or Deltorphin-D (Delt-D) a delta specific opioid receptor agonist prior to hemorrhage without fluid resuscitation and post-treated animals received saline or Delt-D 1 mg/kg or Delt-D 2 mg/kg following hemorrhage without fluid resuscitation. BP, blood loss and rectal temp, at beginning and end of hemorrhage were determined. The effect of Delt-D infusions on the expression of Ubiquitin B and C (UBB and UBC) was determined. Heat Shock Protein (HSP-70), and inducible Nitric Oxide Synthase (iNOS) mRNA transcripts in heart, leg and brain were determined after 2 hr. Preinfusions of Delt-D did not significantly effect BP while 2 mg/kg post-hemorrhage infusions without resuscitation fluid significantly increased BP compared to controls and decreased core temp by 4.5°F compared to controls. Delt-D infusions increased iNOS and HSP70 mRNA in heart and leg in non-hemorrhaged controls and UBB in brain of non-hemorrhaged controls. Pre-treated Delt-D animals had elevated brain iNOS and HSP70 mRNA, and post-hemorrhage Delt-D treated animals had elevated UBC mRNA in heart and brain and HSP70 mRNA in leg tissue. For the severe hemorrhage protocol (9.0 - 11.0 ml hemorrhage volume representing 53-61% of total blood volume), rats were infused with either 3.0 mg/kg of a highly specific mu opioid, (ZGI-06) or a Delt-D variant (ZGI-07) and ischemic tolerance (ie BP and 6 hr survival) was monitored. Controls were infused with l.0 ml PBS. Six hr survival was 33% for controls, 60% for ZGI-06 and 72% for ZGI-07, BP increased within 30-45 seconds after infusion of ZGI-06 by 29.5±13.0 mmHg vs. controls –1.5±19.4 mmHg while ZGI-07 increased BP by 38.8±18.5 mmHg vs. control.

INTRODUCTION

The role of delta-specific opioids in providing multiorgan, myocardial and cerebral ischemia protection has been elucidated over the past 12 years. Evidence has accumulated that delta opioid
agonists composed of two subtypes specific for \textit{delta}_1 and \textit{delta}_2 opioid receptor subtypes can confer myocardial ischemic preconditioning (IPC) and pharmacological (delayed) ischemic preconditioning (PPC) in dog multiorgan autoperfusion bloc\textsuperscript{1}, in isolated rat\textsuperscript{2,4}, rabbit\textsuperscript{5,7} and pig heart models\textsuperscript{8} as well as in intact ischemic rat\textsuperscript{9,10} and pig heart models\textsuperscript{11}, a mouse hypoxic model\textsuperscript{12,13} and even in myocardial cell culture model\textsuperscript{14}. It is also now known that universal opiate antagonists, naltrexone as well as specific \textit{delta}_1 and \textit{delta}_2 antagonists can block or retard both classical IPC and PPC in a dose dependent manner\textsuperscript{14}. The predominance of \textit{delta} opioid receptor mRNA transcripts in human myocardium has been recently in documented\textsuperscript{15}. It has also been demonstrated that the IPC occurring in patients following two sequential angioplasty balloon inflations could be abolished following infusions of the universal opiate antagonist, naloxone\textsuperscript{16}. Similarly infusions of the non-specific delta opioid, DADLE, into organ baths containing human trabeculae obtained during bypass surgery provided IPC as evidenced by their enhanced contractile force following ischemia\textsuperscript{17}. The intermediary role of mitochondrial and sarcolemmal Delt-D), a highly specific \textit{delta}_2 opioid agonist, could provide PPC and reduce left ventricular infarct size in an intact ischemic pig heart model\textsuperscript{8} when infused 45 min prior to ischemia. Delt-D is a 17 amino K\textsubscript{ATP} channels in classical IPC and PPC has been documented in intact ischemic\textsuperscript{10} and isolated\textsuperscript{15} rat heart models and in myocardial cell cultures\textsuperscript{18}.

We have recently documented that infusions of DPDPE (D-Pen\textsuperscript{2-5}, Enkephalin), a highly specific \textit{delta}_1 opioid agonist, and Deltorphin-D (acid peptide originally isolated from skin secretions of the Brazilian frog \textit{Phyllomedusa burmeisterie}\textsuperscript{19}. The focus of the present study was (1) to determine in a moderate hemorrhage protocol (ie, 5.5 ml total hemorrhage volume) if infusions of a Delt-D (Delt-D) without any fluid resuscitation will enhance ischemic tolerance, blood pressure (BP), and alter the expression of mRNA transcripts of Ubiquitin B and C, Heat Shock Protein (HSP-70) and Inducible Nitric Oxide Synthase (iNOS) and (2) to determine in severe hemorrhage protocol (ie 9.5 to 11.5 ml) total hemorrhage volume if infusions of a ZGI-06 on a ZGI-07 without concomitant fluid resuscitation enhance ischemia tolerance (ie increase BP and 6 hr survival) compared to controls receiving PBS infusions.

**MATERIALS AND METHODS**

**HEMORRHAGIC SHOCK MODEL**

The hemorrhagic rat model was that of Summers et al\textsuperscript{21}, where we used male Sprague Dawely rats weighing 300 to 350 mg. Catheters were placed into the femoral artery (for bleeding), femoral vein (for opiate injections) and tail artery (for BP measurements), and were brought underneath the skin to an incision at the back of the neck where they exited the body. Rats were hemorrhaged the day after catheterization, and during hemorrhage BP was allowed to drop to 40-50 mmHg.

**Moderate Hemorrhage Protocol**

Saline 0.5 ml or Delt-D at a concentration of either 1 or 2 mg/kg was administered at the end of the hemorrhage protocol (lasting about 15 min. and representing about 5.5 ml per rat blood loss which is approximately 30% of total blood volume). Rats were killed at 2 hr following UHS and tissues
(brain, heart and leg muscle) were collected for mRNA isolation and northern blot analysis for stress proteins (Ubiquitin, HSP-70 iNOS). Core temperature was monitored at the beginning and end of the experiment. The data collected included BP and mRNA data for the heart, leg and brain tissue using Ubiquitin (UBB and UBC), HSP70, and iNOS probes. Rats were randomly divided into 1 of 7 groups. The groups included: control – no hemorrhage saline (n=5), control no hemorrhage Delt-D 1 mg/kg (n=4), hemorrhage pretreatment saline (n=5), hemorrhage pretreatment Delt-D 1 mg/kg (n=6), hemorrhage post-treatment saline (n=6), hemorrhage post-treatment Delt-D 1 mg/kg (n=4) and hemorrhage Post-treatment Delt-D 2 mg/kg (n=5).

Severe Hemorrhage Protocol
Rats were hemorrhaged 9.5 to 11.0 ml representing 51-60% of total blood volume. A highly specific mu opioid, ZGI-06 or ZGI-07, or PBS were infused (dissolved 1.0 ml PBS pH 7.4) into the femoral vein over a 20-30 second interval when blood pressure declined to between 40-60 mmHg. Ischemic tolerance was measured (increased BP and 6 hr survival).

RESULTS

Moderate Hemorrhage Protocol

Pretreatment – Blood Pressure

![Graph showing blood pressure changes](image)

**Figure 1: Pre-treatment BP values. No significant difference in BP noted between slopes of Saline Control and Deltorphin-D (Delt-D) groups.**

Delt-D pretreatment had no significant effect on BP prior to hemorrhage (Fig. 1).
Post-treatment - Blood Pressure

Analysis of post-treatment 5 min slope data indicated significant differences between the 3 hemorrhage treatment group at the beginning of the recovery period (0-5 min), (11-15 min), (16-20 min) and near the end (46-50 min) in rats injected with Delt-D at a conc. of 2.0 mg/kg compared to controls but not at a conc of 1.0 mg/kg. (Fig. 2). In the heart tissue, UBC mRNA transcripts was significantly elevated in Delt D2 treated animals in comparison to controls, and iNOS and HSP70 mRNA in the heart of Delt-D – controls were significantly higher when compared to all other groups (Fig. 3). The leg tissue was similar to the heart tissue in that animals receiving only Delt D1 (control) showed significant increases in iNOS and HSP70 compared to all other groups and the post-treatment Delt-D1 and Delt-D2 animals showed elevated HSP70 levels compared to all groups (control-no hemorrhage Delt D1) as seen in Fig. 4. In brain UBC mRNA transcripts were elevated in Delt-D treated animals and iNOS was elevated in pretreated saline and HSP70 and iNOS were elevated in the Delt-D pretreated group as seen in Fig. 5.

![Graph showing blood pressure values](image)

**Figure 2:** Post-treatment BP values, 5 min intervals. Significant differences in slopes between 3 groups observed in first 5 min (p≥0.05), at 11-15 min (p=0.04), 16-20 min (p=0.05) and 46-50 min (p≥0.05)
Figure 3: Heart mRNA. UBC was upregulated in Post-Delt-D2 animals compared to controls (p = 0.04). In Delt-D Control no hemorrhage group iNOS and HSP70 significantly increased (p ≥ 0.05).

Figure 4: Leg mRNA. UBB but not UBC upregulated in controls compared to post-treatment Delt-D2 (p=0.04). Delt-D control no hemorrhage had significantly increased iNOS and HSP70 compared to all other groups (p≥ 0.05). Post-treatment Delt-D1 and Delt-D2 had significantly elevated HSP70 compared to all groups except Delt-D control (p≥ 0.05).
Severe Hemorrhage Protocol

Hemorrhage (9.0-11.0 ml representing 51-60% of total blood volume) resulted in the following: BP increased by 29.5 ± 13.0 mmHg for ZGI-06 infused rats (n=11) within 30-45 sec following infusion while controls (n=6) decreased by 1.5 ± 19.5 mmHg (p=0.01). ZGI-07 infused rats (n=11) increased BP by 38.8 ± 18.5 mmHg vs controls (p=0.002) as seen in Figure 6. Six hour survival for controls was 33% (n=2), 54% (n=6) for ZGI-06 and 72% (n=8) for ZGI-07.
CONCLUSIONS

Moderate Hemorrhage Protocol

1) Pre-hemorrhage Delt-D treatment does not significantly alter BP compared to saline controls.

2) Delt-D at 2 mg/kg increases BP vs saline controls during the 1st hour following hemorrhage without fluid resuscitation.

3) Following hemorrhage Delt-D treated animals had 4.5°F decrease in temperature compared to saline treated controls.

4) In Heart, UBC mRNA levels were significantly elevated in Delt-D treated animals, following hemorrhage.

5) In the brain, Delt-D pretreated animals had up regulated levels of UBC and HSP70, UBC and HSP70 which are thought to be involved in cyto protection during times of stress (i.e. hemorrhage). Also endogenous opioid system may be involved in modulating peripheral nervous system during hemorrhage which would directly effect BP and heart rate (Molina, Clin. Exp Pharm Physiol 29(3) 248, 2002).
6) In the heart and brain, post treated Delt-D animals had enhanced levels of UBC mRNA compared to controls.

7) In both heart and skeletal muscle, iNOS mRNA levels were lower compared to controls. iNOS is over produced in tissues during extreme stress and has been implicated in tissue damage and death.

8) In skeletal muscle, HSP70 mRNA was elevated in post-hemorrhage treated animals. Previous studies have shown that HSP70 regenerates denatured protein in skeletal muscle.

Severe Hemorrhage Protocol

9) Six-hour survival was 33% for controls 54% for ZGI-06 and 72% for ZGI-07.

10) ZGI-06 increased BP by $29.5 \pm 13$ mmHg vs control $-1.5 \pm 19.5$ mmHg ($p=0.01$) and ZGI-07 increased BP by $38.8 \pm 18.5$ mmHg vs control within 30-45 seconds after infusion.

11) *Delta* and *mu* specific opioids increase BP and 6hr survival in severely hemorrhaged rats without concomitant fluid resuscitation.

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Heat Shock Protein-70 Inducers and iNOS Inhibitors as Therapeutics to Ameliorate Hemorrhagic Shock

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ABSTRACT

Hemorrhagic shock is the principal cause of death of soldiers in the battlefield. Although the underlying mechanisms are still not fully understood, it has been shown that nitric oxide (NO) overproduction and inducible nitric oxide synthase (iNOS) overexpression play important roles in producing injury caused by hemorrhagic shock. In addition, polymorphonuclear neutrophils (PMN) infiltrate injured tissues and leukotriene B4 (LTB4) generation increases. In a hemorrhage/resuscitation-induced injury model, iNOS, cyclooxygenase-2, and CD14 are all upregulated. The early response genes such as iNOS and cyclooxygenase-2 then promote the inflammatory response by the rapid and excessive production of NO and prostaglandins, respectively. It has been evident that either upregulation of inducible heat shock protein 70 kDa (HSP-72) or downregulation of iNOS can limit tissue injury caused by ischemia/reperfusion or hemorrhage/resuscitation. In our laboratory, geldanamycin, a member of ansamycin family, induces HSP-70i overexpression, to inhibit iNOS expression, to reduce cellular caspase-3 activity, and to preserve cellular ATP levels. With future combat operations expecting longer evacuation times and limited availability of medical supplies far-forward, significant improvements in fluid resuscitation will be required if potentially salvageable injured are to be saved. The action of adjunct therapy agents in the resuscitation fluid that limit tissue injury will further reduce the hemorrhage-induced injury. Since HSP-70i overexpression and iNOS inhibition exhibit cytoprotection, a compound such as geldanamycin as a HSP-70i inducer as well as an iNOS inhibitor will represent a best additive to resuscitation fluids that may reduce hemorrhage-induced injury.

1.0 INTRODUCTION

Hemorrhagic shock is the leading cause of death and complications in combat casualties and civilian trauma. It has been shown to cause systemic inflammatory response syndrome, multiple organ dysfunction, and multiple organ failure [Baue 1998]. Analysis of the historical data (Table 1) demonstrates that the mortality rates from World War I, World War II, the Korean War, and the Vietnam Conflict are very similar and also show no improvement over this period. Moreover, the rates of soldiers who died of wounds after reaching a treatment facility during the Vietnam Conflict did not improve in spite of the rapid evacuation times. The published data also show that approximately half of those killed in action died of hemorrhagic shock. Hemorrhagic shock is an old problem and to find useful remedies that are capable of reducing casualty caused by hemorrhagic shock is a most important task in combat medicine.

In this paper, we will discuss effects of hemorrhagic shock, resuscitation fluids used for hemorrhagic shock, and amelioration of additives to resuscitation fluids. The additives will be focused on inducers of heat shock protein 72 kDa (HSP-72, an inducible form of the HSP-70 family) and inhibitors of inducible nitric oxide synthase (iNOS).

### 2.0 EFFECTS OF HEMORRHAGIC SHOCK

Hemorrhage leads to systemic inflammatory response syndrome, multiple organ dysfunction, and multiple organ failure [Baue 1998]. A variety of biomolecules is known to be involved in this response. In rodents, increases in inducible heat shock protein 70 kDa (HSP-72) stimulated by heat stress limit injury to tissues caused by ischemia/reperfusion [Stojadinovic 1995] or by hemorrhage [Mizushima 2000]. Likewise, inhibition of nitric oxide (NO) production results in significant reduction of local tissue damage, polymorphonuclear neutrophil (PMN) infiltration, and leukotriene B4 (LTB4) generation caused by ischemia/reperfusion [Charier 1999]. Mice deficient in inducible NO synthase (iNOS) also demonstrate limited hemorrhage/resuscitation-induced injury [Hierholzer 1998; Moore 1994]. Therefore, remedies that induce HSP-70i and/or inhibit iNOS might prove very useful for reducing hemorrhage/resuscitation-induced injury in man.

The above observations are consistent with the idea that the low oxygen supply resulting from conditions such as ischemia or hemorrhage affects the expression of iNOS, which then influences the expression of other proteins that alter cell viability. It has been shown that hypoxia results in alteration of iNOS, Bcl-2, and P53 mRNA expression in cultured human intestinal epithelial T84 cells and Jurkat T cells, alterations that can be modulated by treatment with a NOS inhibitor [Kiang 2003b]. It is also found that hypoxia increases the activity of caspase-3, an aspartate-specific cysteinyl protease involved in apoptosis, an activity that is blocked by NOS inhibitors [Kiang 2003b].

A full time-course study of the effect of hemorrhage on a series of stress-related proteins such as c-JUN, Kruppel-like factor 6 (KLF6), iNOS, HSP-70i, and hypoxia inducible factor 1α (HIF-1α) has been reported in a hemorrhage mouse model [Kiang 2004b]. Based on Western blot data obtained from mouse lung, jejunum, heart, kidney, liver, and brain, c-JUN, KLF6, iNOS, HSP-70i, and HIF-1α are upregulated. In jejunum, c-JUN protein was overexpressed within 1 h, but levels returned to baseline values 3 h later. KLF6 began to increase significantly 6 h later, reached the maximum at 24 h, and remained at that level at 48 h after hemorrhage. iNOS increased at 6 h, reached a maximum at 12 - 24 h, and returned to baseline values at 48 h. HSP-70i increased at 12 h and remained at elevated levels at 48 h. HIF-1α also increased at 12 h and continued to increase at 48 h. The sequence of protein appearance was c-JUN, KLF6, iNOS, HSP-70i, and HIF-1α. KLF4 (a repressor to iNOS, [Warke, 2003]) and c-FOS (an AP-1 protein) were not detected, and NF-
κB 65 kDa was detected but not affected by hemorrhage. A similar time-course observation on these stress-related proteins was also obtained in lung, kidney, liver, and heart. Sham-treated mouse organs displayed no changes in the basal levels of c-JUN, KLF6, and iNOS. In addition to changes in this series of stress-related proteins listed above, hemorrhage also increases cellular caspase-3 activity [Kiang 2004a] and reduces cellular ATP levels [Chaudry 1973, Chang 2000; Paxian 2003].

2.1 NO and NOS

Like ischemia and reperfusion or hypoxia [Kiang 2003b], hemorrhage increases NO production and NOS overexpression [Kiang 2004b]. The substrate of NO is L-arginine with O₂ and the enzymes involved in this process are either constitutive NOS (cNOS) or iNOS. The chemical biology of NO shows its direct effects and indirect effects [Thomas 2003]. The direct effects are NO reacts with heme-containing proteins, named the primary mode of NO action. These reactions are generally rapid, require low concentrations of NO, and are the genesis of most of the physiological effects of NO. In contrast, the indirect effects, namely the secondary mode of NO action, include formation of N₂O₃, ONOO⁻, NO₂, and HNO that react with cellular targets and may result in a major configuration change in critical molecules. It has been shown that indirect effects require much higher concentrations of NO than direct effects. It appears that NO produced at low concentrations for short periods primarily mediates direct effects, whereas high local NO concentrations sustained over prolonged periods mediate indirect reactions.

**Table 2: Types of NOS and Their Characteristics**

<table>
<thead>
<tr>
<th>NOS</th>
<th>Sizes(kDa)</th>
<th>Chromosome</th>
<th>Features</th>
<th>Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS (NOS1)</td>
<td>160</td>
<td>12</td>
<td>Ca²⁺-dependent NADPH, FAD, FMN, Heme Calmodulin PKC phosphorylation Gene has no TATA box</td>
<td>Neurons</td>
</tr>
<tr>
<td>eNOS (NOS3)</td>
<td>135</td>
<td>7</td>
<td>Ca²⁺-dependent NADPH, FAD, FMN, Heme Calmodulin PKC phosphorylation Myristoylation and palmitoylation Gene has no TATA box</td>
<td>Endothelium</td>
</tr>
<tr>
<td>iNOS (NOS2)</td>
<td>130</td>
<td>17</td>
<td>Ca²⁺-independent NADPH, FAD, FMN, Heme Calmodulin PKC phosphorylation Gene has a TATA box</td>
<td>Macrophages</td>
</tr>
</tbody>
</table>

NO production is mediated by NOS. NOS can be divided into two major categories: constitutive form (cNOS) and inducible form (iNOS) [Griffith 1995; Nathan 1994].
Heat Shock Protein-70 Inducers and iNOS Inhibitors as Therapeutics to Ameliorate Hemorrhagic Shock

cNOS includes nNOS and eNOS and it is Ca$^{2+}$-dependent, whereas iNOS is not. Both cNOS and iNOS contain a reductase domain and an oxidase domain. However, eNOS has myristoylation and palmitoylation sites, whereas nNOS and iNOS do not. The iNOS promoter has a TATA site and differs in that from the cNOS promoters which are TATA-less promoters. The cNOS promoters are GC rich and are regulated primarily by Sp1 and other members of the Sp1-like family. In contrast, NF-κB plays a crucial role in the regulation of iNOS. In the murine iNOS promoter, the downstream NF-κB binding site (-76 to -85 bp) seems the most important one [Xie 1994], however, the upstream NF-κB site (-974 to -960 bp) also seems to have some functionality and cooperativeness with the downstream site [Spink 1995]. For the human iNOS promoter, the NF-κB motif at -5.8 kb is most critical for cytokine-induced promoter activity, whereas the sites at -5.2, -5.5, -6.1, and -8.2 kb have a cooperative effect [Taylor 1998; Marks-Konczalik 1998].

cNOS is thought to generate low levels of NO at submicromolar range for short durations. iNOS generates NO for prolonged periods and at local concentrations as high as 1-5 µM. Since the chemistry and biological outcome depend on the concentration of NO, the proximity of a biological target to the NO source becomes critical. For example, cells or tissues close to macrophages that produce high levels of NO will be subjected to direct and indirect effects due to the primary and the secondary modes of NO actions. In contrast, if they are far from the NO source, they will experience only direct effects as the primary mode of NO action.

2.2 Downregualtion of iNOS

A series of NOS inhibitors have been designed and extensively studied [Salemo 2002]. Among them, L-NNA (N-nitro-L-arginine), L-NMA (N-methyl-L-arginine), L-NIL (N-(1-iminoethyl)-L-lysine, and L-NIO (N-(1-iminoethyl)-L-ornithine) are shown to effectively inhibit iNOS gene expression, NO production, and caspase-3 activity [Moore 1994; Kiang 2003b].

In addition to the use of NOS inhibitors, iNOS deficiency can be caused by deletion of the iNOS gene. This has been shown to reduce or prevent hemorrhage-induced injury and death [Hierholzer 1998]. This result further confirms that iNOS is responsible for the hemorrhage-induced injury. Therefore, downregulation of iNOS by any means will be beneficial to patients suffering from hemorrhage.

Other than iNOS inhibitors and iNOS gene deletion, compounds such as ansamycin can inhibit iNOS activity and gene expression. Both geldanamycin and 17-allylamino-17-demethoxygeldanamycin, members of the ansamycin family, have been demonstrated to reduce NO production, iNOS mRNA, and cytokine-induced activation of the iNOS gene promoter in cultured cells [Murphy 2002], in rats [Pittet 2001], and in mice [Kiang 2004b].

3.0 HEAT SHOCK PROTEINS

Heat shock proteins (HSPs) are present in most cells. They represent multigene families that range in molecular size from 10 to 174 kDa. Based on their molecular weights, they are divided into HSP-10, HSP-20, HSP-40, HSP-60, HSP-70, HSP-90, and HSP-110. Table 3 lists the members of each family, their locations, and functions in the cell [Kiang 1998].
Table 3: Members of HSPs and Their Locations and Functions [Kiang 1998]

<table>
<thead>
<tr>
<th>HSP</th>
<th>Members</th>
<th>Locations</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP-110</td>
<td>HSP-110/104</td>
<td>Cytosol/nucleus</td>
<td>Cytoprotection</td>
</tr>
<tr>
<td>HSP-90</td>
<td>HSP-90(\alpha)</td>
<td>Cytosol/nucleus</td>
<td>Endogenous steroid receptor antagonist</td>
</tr>
<tr>
<td></td>
<td>HSP-90(\beta)</td>
<td>Cytosol/nucleus</td>
<td>Cytoprotection</td>
</tr>
<tr>
<td></td>
<td>GRP-94</td>
<td>Endoplasmatic reticulum</td>
<td>Chaperone</td>
</tr>
<tr>
<td>HSP-70</td>
<td>GRP-78 (Bip)</td>
<td>Endoplasmatic reticulum</td>
<td>Chaperone</td>
</tr>
<tr>
<td></td>
<td>HSP-75 (GRP-75)</td>
<td>Mitochondria</td>
<td>Chaperone</td>
</tr>
<tr>
<td></td>
<td>HSP-73</td>
<td>Cytosol/nucleus</td>
<td>Chaperone</td>
</tr>
<tr>
<td></td>
<td>HSP-72</td>
<td>Cytosol/nucleus</td>
<td>Cytoprotection</td>
</tr>
<tr>
<td>HSP-60</td>
<td>HSP-60</td>
<td>Mitochondria</td>
<td>Cohort to HSP-75</td>
</tr>
<tr>
<td></td>
<td>HSP-56</td>
<td>Cytosol</td>
<td>Binds to steroid receptors and FK506</td>
</tr>
<tr>
<td>HSP-40</td>
<td>HSP-47</td>
<td>Endoplasmatic reticulum</td>
<td>Collagen chaperone</td>
</tr>
<tr>
<td>HSP-20</td>
<td>HSP-27</td>
<td>Cytosol/nucleus</td>
<td>Chaperone; Cytoprotection</td>
</tr>
<tr>
<td>HSP-10</td>
<td>HSP-10</td>
<td>Mitochondria</td>
<td>Cohort to HSP-60</td>
</tr>
</tbody>
</table>

3.1 Structure of HSP-72 and Its Functions

The molecular structure of HSP-72 (an inducible form) is very similar to that of HSP-73 (a constitutive form). They both contain a globular unit linked to a \(\beta\)-sheet and an \(\alpha\)-helical tube. The globular unit is the ATPase domain (1-386 a.a., 44 kDa); the \(\beta\)-sheet and the \(\alpha\)-helix tube (384-543 a.a., 18 kDa) are the peptide binding domain; and the tail (542-646 a.a., 11 kDa) of HSP-73 has the signal peptide of EEVD, but not the tail (542-640 a.a., 10 kDa) of HSP-72. Proteins in the cytoplasm recognize the EEVD of HSP-73 as a chaperone protein, whereas HSP-72 has no such EEVD as a chaperone but instead acts as a cytoprotectant.

3.2 HSP-72 Gene Regulation

HSP-72 gene regulation involves phosphorylation, heat shock transcriptional factor, heat shock elements, and ions.

3.2.1 Heat Shock Elements

Genomic footprinting of the human HSP-70 promoter has revealed that heat stress induces a quick binding of heat shock transcriptional factors (HSFs) to a region encompassing five nGAAn sequences named heat shock elements (HSEs), three of them are GAA (also called perfect HSEs) and other two are GAC and GGG (also called imperfect HSEs). The sequences of HSEs at site 3 and site 4 are dyad symmetrical, and HSFs preferably bind to HSEs at sites 3 and 4 [Kiang 1998]. In mammalian cells, HSEs are usually bound by HSF4 to keep other HSFs away.

3.2.2 Heat Shock Transcriptional Factors

Four different HSFs have been identified in vertebrate: HSF1, HSF2, HSF3, and HSF4 [Morimoto 1996; Nakai 1997]. HSF2 has two isoforms due to splicing: HSF2A and HSF2B [He 2003]. HSFs have a binding
domain, a helical trimerization surface, and a short conserved element. The binding domain is to bind protein and HSEs; the trimerization surface has leucine zipper coiled-coil motifs for trimer formation. There are two genes in mice and humans encoding HSFs. The sizes for human HSF1, HSF2, HSF4, and chicken HSF3 are 529 a.a., 510 a.a., 463 a.a., and 467 a.a., respectively. Human HSF1 can be activated by various stimuli, human HSF2 only by hemin, chicken HSF3 only by heat, while human HSF4 is not activated.

It has been reported that mouse HSF1, chicken HSF3, and mouse HSF4 bind to all 5 HSEs, whereas mouse HSF2 interacts with only 4 HSEs. Activation of HSF1, 2, or 3 upregulates HSP-72 [Kiang 1998]. In contrast, HSF4 is responsible for downregulation of HSP-72 [Nakai 1997].

3.2.3 HSP-72 Autoregulation

Normally, HSFs that reside in the cytosol of mammalian cells are bound to HSPs under unstressed conditions. Under stress conditions such as hemorrhage, HSFs are separated from HSPs. Then, these HSFs are available for phosphorylation by protein kinase C or other serine/threonine kinases. They form homotrimers [Kroeger 1993] or heterotrimers [He 2003]. The trimers enter the nucleus, bind to HSEs located on the promoter region of HSP genes, and become further phosphorylated by HSF kinases. Transcription is then initiated and translation is resulted. Therefore, new HSP-72 is synthesized in the cytosol of cells. Soon, the elevated HSP-72 is bound by cytosolic HSFs. The complex of HSP-72 and HSFs turns off the further increase in HSP-72. The steps of HSFs phosphorylation, trimerization, and its translocation from the cytosol to the nucleus are Ca²⁺-dependent [Kiang 1994].

3.3 HSP-72 Protein Regulation

HSP-70 family is present in every cell type and tissue under both unstressed and stressed conditions. The inducible form of HSP-70, HSP-72, is induced by physiological stressors, pathological stressors, and environmental stressors. The degree of induction depends on the level and duration of exposure to stressors. The increase usually is transient, but how long it persists is different in various cell types, ranging from hours, days, or weeks. It can be regulated by hormones, intracellular pH, cellular cAMP levels, intracellular concentrations of Ca²⁺, and activation of protein kinase C, protein kinase A [Kiang 1998; 2003a] and protein tyrosine kinase [Kiang 2004b]. Aging is also known to result in overexpression of HSP-72 that in turn desensitizes Ca²⁺ machinery and turns off new synthesis of HSP-72 [Kiang 1996]. Bioflavonoid such as quercetin prevents HSF1 binding to HSEs, thereby leading to attenuation of HSP-72 gene expression [Murphy 2002].

4.0 INTERACTION BETWEEN HSP-72 AND iNOS

Using immunoprecipitation and immunoblotting analysis, it has been found that HSP-72 forms a complex with iNOS after hemorrhage [Kiang 2004b]. No complex formation is detected between HSP-72 and p53 or Bcl-2 proteins, suggesting that HSP-72 specifically couples to iNOS. Treatment with geldanamycin increases HSP-72 expression and decreases the hemorrhage-induced increase in iNOS expression. The complex formation between HSP-72 and iNOS is still observed. It is possible that the complex formed between HSP-72 and iNOS might decrease the enzymatic activity of iNOS and decrease NO production. As a result of a low level of NO, the injury caused by hemorrhagic shock is markedly diminished.
5.0 RESUSCITATION FLUIDS

Currently, the major cause of death in potentially salvageable battlefield casualties is hemorrhage [Bellamay 1984]. About 20% of these deaths are preventable if the bleeding can be quickly controlled or minimized [Bellamay 1987; Bellamay 1995] and sufficient resuscitation fluid is administered in time to maintain critical tissue perfusion. However, it has been recognized that resuscitation fluids are not innocuous and that they may actually potentiate the cellular injury caused by hemorrhagic shock [Committee on fluid resuscitation for combat casualties 1999]. It has been proposed that some resuscitation fluids may contribute to delayed multiple organ dysfunction [Baue 1998].

The optimal resuscitation fluid is still a matter of debate. In particular questions have been raised about the use of lactated Ringers (LR). LR has been shown to increase neutrophil activation. Hemorrhage elevates mRNA levels of iNOS, KLF6, p-38 MAPK, p53, Bcl-2, and caspase-3 genes in rat lung and ileum. The LR resuscitation does not block their increases. Instead it increases some of them further [Kiang 2004c]. Alam et al. [2002] also reported that 51 genes were altered in rats treated with hemorrhage plus LR resuscitation. The dissection of the role of each of these changes poses a significant challenge.

6.0 ADJUNCT THERAPY AGENTS IN THE RESUSCITATION FLUID

As mentioned above, resuscitation may actually potentiate the cellular injury caused by hemorrhagic shock [Committee on fluid resuscitation for combat casualties 1999] since delayed multiple organ dysfunction and failure occur and mortality results after resuscitation [Baue 1998]. Because iNOS is known to be responsible for the hemorrhage-induced injury and HSP-72 is shown to offer cytoprotection, it is likely that agents or remedies that can reduce iNOS expression and/or increase HSP-72 will be beneficial in conjunction with administration of resuscitation. iNOS inhibitors such as L-NIL, and L-NIO can be potentially useful for this purpose. Likewise, HSP-72 inducers such as herbimycin A, heat stress, ethanol, and ansamycin are also in the list for consideration. To be more precise, ansamycin actually is a HSP-72 inducer as well as an iNOS inhibitor. Therefore, ansamycin and its derivatives should be evaluated for their potential to decrease systemic inflammatory response syndrome, multiple organ dysfunction, and multiple organ failure.

7.0 CONCLUSIONS

Hemorrhage/reperfusion-associated pathophysiology is complicated and poorly understood. Many adverse effects of hemorrhage/resuscitation are common to ischemia/reperfusion and hypoxia/reoxygenation. Resuscitation eventually does not reverse the hemorrhage-induced changes, but instead worsens the changes. The complexity of the cellular response to hemorrhage/resuscitation complicates efforts to design approaches to treat or prevent injury resulting from resuscitation. Nevertheless, an additive in resuscitation fluids, which blocks iNOS, induces HSP-72, and restores ATP depletion, may be potentially therapeutic to salvageable patients.

8.0 PERSPECTIVE

Hemorrhage induces overexpression of iNOS and HSP-72 proteins, increases in cellular caspase-3 activity [Kiang 2004a], and reduction in ATP [Chaudry 1973; Chang 2000; Paxian 2003]. iNOS overexpression appears early and it leads to the NO production and its direct and indirect effects. HSP-72 overexpression appears 12 h after occurrence of hemorrhage. Because of its late appearance, it is possible that HSP-72 is not serving its usual protective role in hemorrhage and resuscitation, but that it is rather facilitating tissue repair...
Heat Shock Protein-70 Inducers and iNOS Inhibitors as Therapeutics to Ameliorate Hemorrhagic Shock

for salvaging the damage caused by hemorrhage and resuscitation. Treatment with ansamycin is known to increase HSP-72 and reduce iNOS, cellular caspase-3 activity and ATP loss. Taking together the data obtained from ansamycin or agents such as glutamine or crocetin [Van Way 2003], adjunct therapy with novel resuscitation fluid such as ATP-MgCl₂ (Nalos 2003) or ethyl pyruvate (Fink 2004) may be a novel approach to address the problems raised from hemorrhagic shock.

Despite the data in the literature, there is little known about the proper time course that these agents should be added to resuscitation fluids after hemorrhage in order to prevent tissue injury. Their pharmacodynamics and pharmacokinetics are still not characterized. More studies are needed to advance the understanding of hemorrhagic shock in order to form a useful therapeutic protocol or a drug formula that can be followed and practiced.

9.0 ACKNOWLEDGMENTS

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10.0 REFERENCES


Heat Shock Protein-70 Inducers and iNOS Inhibitors as Therapeutics to Ameliorate Hemorrhagic Shock


Heat Shock Protein-70 Inducers and iNOS Inhibitors as Therapeutics to Ameliorate Hemorrhagic Shock


Application of Gene Expression Analysis with Microarrays and Proteomics to the Problem of Hemorrhagic Shock and Resuscitation

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ABSTRACT

Hemorrhage is the principal cause of death of soldiers on the battlefield. With dispersed troops and future combat operations expecting longer evacuation times and limited availability of medical supplies far-forward, significant improvements in fluid resuscitation will be required if casualties are to be saved. While it is known that a drop in blood pressure below 40 mm Hg or loss of more than 50% of the blood volume is fatal, most cells in the body, with the exception of brain cells, can survive for several hours with minimal oxygen or nutrients. Hence, morbidity from blood loss involves factors beside lack of oxygen and nutrients. Little more is known, however, about how the body responds to loss of blood or which organs are most affected. An understanding of the temporal responses of tissues to hemorrhage will lead to improved strategies of intervention before irreversible deterioration occurs.

We are using gene expression analysis with microarrays to assess the responses of various organs to severe hemorrhage in rodents to uncover the prominent metabolic pathways involved. Until recently, traditional molecular techniques allowed analysis of only one gene at a time. Throughput was very limited and an accurate picture of the molecules that orchestrate the regulation of health and the dysfunction that occurs during disease or injury has not been possible. The microarray, which allows analysis of changes in expression of thousands of genes, promises to help clarify the molecular and genetic basis of health and disease and speed drug discovery. This information will guide the rational development of new resuscitation fluids with appropriate drug additives.

1.0 BACKGROUND

Although hemorrhage is the principal cause of death of soldiers on the battlefield and is an important component of injury in civilian trauma, we possess only a rudimentary understanding of the cellular basis for the physiological alterations that occur following severe blood loss in any mammalian species. Consequently, designing therapies to meet cellular needs following the global ischemia of severe hemorrhage has been primarily through trial and error. Utilizing recent advances in genomics and proteomics, the present endeavor attempts to better understand the response of the mammalian organism to hemorrhage and to begin to

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1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

systematically investigate cellular responses to the global ischemia that occurs during severe blood loss in order to provide new strategies for saving the lives of trauma patients who will otherwise die from their wounds.

1.1 Microarrays

Although each cell in the body contains a complete set of instructions (the genome) for specifying all the functions of the body, only a limited amount of this genetic material is active, and the portions of the genome that are active are specific for each cell type. The repertoire of the thousands of genes that are expressed in each cell type is termed the transcriptome. Until recently, traditional molecular techniques allowed analysis of only one gene at a time. Microarrays, so-called because many thousands of fragments of genes can be packed into an area of several square centimeters, are also known as DNA chips or gene chips, and they represent the first widely used application that builds upon the information provided by sequencing genome projects to the study of biological questions.

Knowledge about DNA sequences allows definition of genes by a unique, relatively small piece of the gene. The technique for making gene chips by synthesizing short oligonucleotides onto a glass substrate by a photolithographic process, was first introduced by the biotechnology company Affymetrix (Santa Clara, CA) [2, 3]. Most laboratories produce their own chips by spotting preformed complementary DNA (cDNA) or oligonucleotides by a technique developed by Patrick Brown's laboratory at Stanford University [4-6]. During the course of a study, one can collect samples of blood or tissues at various times. Then the RNA from each sample is isolated and a copy made with an enzyme that can generate cDNA. This cDNA is combined with the complement attached to the chip and after removal of unbound material, it is scanned by a fluorescent scanner to detect sites of molecular hybridization to determine if that gene was being expressed by the cell or tissue under investigation at the time the messenger RNA (mRNA) was isolated. The chemical conditions necessary for allowing this specific, one-to-one combination, known as molecular hybridization, are very well defined. This application of microarrays is termed gene expression analysis. If one wants to know the affect of a drug or disease on the activity of many genes, gene expression analysis is one of the least expensive and most robust techniques currently available. By combining this technology with computers that can track and record the activity of genes, thousands can be followed simultaneously.

1.1.1 Subarrays

Figure 1 shows a subarray of 480 nucleic acid fragments produced at the US Army Institute of Surgical Research. Each spot is about 120 microns (0.12 mm) in diameter, and was deposited by a stainless pin in a special computer-controlled robot. The exact order of each spot is tracked by appropriate software on the computer. About 80,000 spots can be produced on a standard microscope slide. Although there is still controversy about the exact number of genes in mammalian genomes, (estimates are currently about 40,000), representative fragments of the entire genome can in theory be placed on microarrays and all genes analysed simultaneously. RNAs, the immediate products of genes, are the effectors of the transcriptome. The RNAs are isolated and complementary copies (cDNA) made that incorporate a fluorescent dye. When hybridized to the array, each cDNA finds its appropriate complementary sequence on the array, roughly in proportion to its concentration in the cell. By quantifying the fluorescence in a laser-activated scanner, the quantity of RNA present in the original mixture can be determined. In practice, an appropriate control from organs or cells that have not been perturbed is labeled with one color fluorescent dye while the experimental sample is labeled with a different colored dye. The figure below shows an example of a subarray from a microarray used for quantifying gene expression from rat tissues.
2.0 MICROARRAY APPLICATION

One example of the use of microarray technology applied to the problem of hemorrhagic shock and resuscitation at the US Army Institute of Surgical Research is examining the genetic responses to 40% hemorrhage in rat and mouse models as a function of time (1, 3, 6, 12, 24, and 48 hours). Analysis of these results in the lung indicates that biochemical pathways for biogenic amines, eicosanoids, inflammation, and steroid metabolism were prominently affected (p<0.05). By performing similar analysis in other organs (liver, kidney, and intestine) and following up these results with proteomic analysis, it is hypothesized that a set of common metabolic pathways will be identified and confirmed that is affected by severe blood loss. This information will guide the rational development of new resuscitation fluids with appropriate drug additives. While we plan to examine several organs and tissues in these animals we are focusing first on lung as it is the predominant organ to fail in humans after severe trauma [7]. In animal models, hemorrhage produces lung injury despite locations of the primary insult elsewhere [8-11].

2.1 Analysis of Relative Gene Expression with Two Color Microarrays

Figure 2 illustrates the steps in performing gene expression analysis with microarrays. Tissues from animal intestine, liver, lung, kidney, spleen, heart, skeletal muscle (gastrocnemius) skin, and brain were removed and placed in RNALater™ (Ambion, Austin, TX): Total RNA was isolated from each tissue from each animal and its quality analyzed by gel electrophoresis. A reference preparation consisting of equal amounts of RNA pooled from 10 organs (liver, lung, kidney, spleen, heart, skeletal muscle, skin, jejunum, and brain) of untreated control animals was used as reference RNA. Five-µg samples exhibiting undegraded RNA from each rat lung were reverse-transcribed in the presence of a C-6 amine modified random hexamer and aminoallodeoxyxyuridine to produce fluorescent labeled cDNA. Following reverse transcription, RNA was degraded. After separation from unbound dye, the samples were again lyophilized and then reconstituted in...
hybridization solution. Following hybridization on a microarray (one per rat) and washing to remove unhybridized cDNA and scanning with an Axon 4000B (Axon Instruments, Union City, CA) at 10 micron resolution, the resulting 16-bit TIFF images were analyzed with GenePix 4.1 software (Axon Instruments) for calculation of Cy5 and Cy3 fluorescence intensities at each spot.
2.1 Cluster Analysis

The tools for handling the large data sets generated by microarray technology are in development and constantly improving and as are statistical tools. The principal tool in use currently is known as cluster analysis, which organizes data on the basis of similar patterns of expression. Figure 3 illustrates the results of alterations in gene expression in the lung of mice after a 40% reduction in blood volume as a function of time. The cluster analysis program associated genes whose expression was altered together as a function of time after hemorrhage. By performing gene expression analysis with groups of at least 3 animals, each with its own microarray, animal variation in gene expression can be ascertained. The clustered genes seen in Figure 3 all were analyzed by ANOVA and were significant at the p< 0.01 account for 1,146 genes out of 17,249 spotted on the microarray. Cluster analysis shows that most of the genes that were altered up scored as upregulated. Many of the genes altered in expression were upregulated at multiple times.
Application of Gene Expression Analysis with Microarrays and Proteomics to the Problem of Hemorrhagic Shock and Resuscitation

Figure 3
Figure 4 shows an inset of genes upregulated in Figure 3. Many of the genes altered in mouse lung are termed expressed sequence tags ESTs. It is highly likely that these are genes but the specific functions are as yet unknown. By combining these results with functional genomics and proteomics we expect to be able to determine the role of the genes in the global response to ischemia that occurs following hemorrhage. The goal of using gene expression analysis in developing resuscitation fluids will be to use this genetic information to determine if a particular resuscitation fluid is either reducing the shock response or accelerating the early return to the preshock state.
3.0 SUMMARY

By using microarray technology applied to the study of the genetic response to hemorrhagic shock and resuscitation in animal model organs we hope to better understand the cellular response of tissue to hemorrhage. This information will guide the development of new resuscitation fluids. We predict that as we develop better resuscitation fluids, we will see decreases in the magnitude of the genetic responses of organs to hemorrhage that will translate into an increase in survival and a reduction in the time required to recover from hemorrhage.

4.0 REFERENCES


Research on Tourniquet Related Injury for Combat Casualty Care

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ABSTRACT

The tourniquet has been used for over 300 years for effective hemorrhage control during surgery and trauma. However, tourniquets are far from benign, causing a host of complications collectively known as tourniquet injury. A tremendous body of clinical experience and scientific research has resulted in principles of safe use and advances in tourniquet design, minimizing tourniquet injury under clinical conditions. Unfortunately, battlefield conditions preclude adherence to these safe principles and the use of surgical tourniquets. The United States Army Institute of Surgical Research (USAISR) has developed an integrated program designed to address the unique nature of tourniquet use under combat conditions with the goal of increasing the rate of limb salvage and saving lives.

1.0 INTRODUCTION

Since the tourniquet was introduced in 1674 on the battlefield by the French military surgeon, Moral, it has been routinely used to control bleeding during surgery or following extremity trauma involving severe vascular damage. While properly applied tourniquets are extremely effective in controlling hemorrhage, their use is far from benign. Tourniquet-related injury consists of compression injury to the underlying skin, nerve, and muscle, as well as ischemia/reperfusion injury (I/R) to the underlying and distal muscle and nerve [3, 4]. When tourniquets applied for long durations are removed, a severe systemic inflammatory response leading to damage to remote organs can take place, in some cases resulting in fatality [5]. This understanding has led to clinical practices and advances in tourniquet design that have minimized the risk of these complications during surgery. Specifically, minimizing tourniquet application duration to less than 2 hours and the use of wide, pneumatic tourniquets that minimize tissue compression, have led to safe and practically complication-free use [3].

Unfortunately, the circumstances that dictate the use of tourniquets on the battlefield typically exclude compliance with safety principles and tactical constraints often violate the 2-hour safe period. The duration of trauma tourniquet application is usually controlled by the length of time it takes to evacuate the soldier to a far-forward medical treatment facility for definitive vascular repair, a delay that often exceeds 2 hours. While it is well recognized that extended tourniquet application often results in the loss of muscle function or limb amputation, it has been generally accepted that the priority is “life over limb.”

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Design is another major distinction between surgical and trauma tourniquets. The wide, pneumatic tourniquets popular in surgery today are not practical on the battlefield. Specifically, they are too large to carry. Because life-threatening extremity arterial wounds are often near the groin or auxiliary regions, the width of a surgical tourniquet may preclude effective placement for these wounds. Finally, concerns over the inherent propensity of pneumatic bladders to leak have led to their dismissal as impractical on the battlefield. As a result, the military has seen virtually no advancement in reducing tourniquet injury. Even the newly fielded one-handed tourniquet [9], while effective for hemorrhage control, does not resolve the tourniquet-related injury observed on the battlefield over 300 years ago.

It is the goal of our program to advance tourniquet design and to optimize limb salvage by integrating the relevant scientific, clinical, and military medical literature supported by our own laboratory studies to produce: 1) tourniquet guidelines; 2) medical treatments; and 3) new tourniquet designs to optimize limb salvage.

2.0 MILITARY TOURNIQUET EXPERIENCE

Although the scientific literature contains little research on the consequences of tourniquets during trauma, the pre-Vietnam military medical literature contains a wealth of relevant information based on thousands of cases involving tourniquet application. The majority of these reports document cases during WWII [10]. This information constitutes a resource virtually unknown to modern day military medical personnel, unavailable in medical reference databases. Currently, a major effort is underway to unearth this literature for future publication in the form of a review article.

What is an appropriate combat tourniquet? When is it appropriate to use a tourniquet? When and by whom should a tourniquet be removed? Under what conditions should a tourniquet not be released or removed? What are the most effective ways to increase limb salvage while using a tourniquet? These questions and concerns among soldiers, medics, and military medical officers were addressed by a panel of experts who convened at the 2003 Advanced Technology Applications for Combat Casualty Care Conference. Recommendations were made and published [8].

3.0 SMALL ANIMAL STUDIES

3.1 Characterizing Tourniquet Injury In An Animal Model.

Research to characterize tourniquet injury in an animal model at the United States Army of Surgical Research (USAISR) was initiated three years ago with the development of a rat model of tourniquet injury. Using a pneumatic tourniquet system, we have explored a range of durations of tourniquet application for which we have assessed animals at 2 hr, 2 d, or 2 wk following tourniquet release. These time points were chosen as they characterize the acute injury (2 hr), the peak of the injury process (2 d), and the intermediate stage of muscle recovery and regeneration (2 wk). Muscle injury is determined primarily by examining muscle function using an in situ preparation, as well as standard histology and vital staining. Muscle edema and atrophy are determined using wet weights and wet-to-dry weight ratios.
The most conspicuous response to tourniquet release is profound edema (Fig. 1). The affected limb is paralyzed, with no response to painful stimuli for at least 2 days. In-situ, muscles do not respond to electrical stimulation of the motor nerve. However, direct stimulation of the muscle does elicit force production, although it is well below that produced by the corresponding contralateral muscle. Taken together these observations indicate both nerve and muscle injury (Fig. 2). At day 14, peak force production is similar regardless of whether the muscle is stimulated directly or via the motor. However, force production is significantly reduced compared with the contralateral control muscle [7].

The magnitude of the injury depends on the muscle examined. The plantaris, a predominantly fast-twitch (type II) muscle is significantly more vulnerable to tourniquet injury than the predominantly slow-twitch (type I) soleus muscle. (Fig. 3)[6, 7]. A hallmark of aerobic training is a shift in the metabolic profile to that characterized by type I muscle fibers, e.g., high mitochondrial content and capillary density [2]. Thus, fitness level prior to injury may be an important mediator in determining the extent of tourniquet injury. Regardless, a better understanding of the specific reasons for these differences should help in the development of treatments that can reduce the magnitude of the injury and hasten recovery.
Figure 2: Force traces. Stimulation of the motor nerve resulted in little force production (A). Direct stimulation of the muscle resulted in greater force (B), demonstrating injury to motor nerve. Regardless, force is significantly reduced compared to the contralateral control (C). This pattern occurred in all animals tested on day 2 independent of muscle (soleus or plantaris) or tourniquet duration (2 or 4 hr).
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3.2 Effect of Hemorrhage Induced Hypotension on Tourniquet Injury

Clinically, tourniquets are used to create a bloodless surgical field. In contrast, a trauma tourniquet is preceded by severe hemorrhage. We are currently addressing the question of whether hemorrhage-induced hypotension impacts the extent of tourniquet injury. In these studies animals undergo a hemorrhage of approximately 30-35% of their total blood volume, followed immediately by tourniquet application for 4 hours. These animals are then compared to appropriate groups that have undergone a sham hemorrhage. These studies are currently in progress and will be of importance in determining the injury pattern from tourniquet use after trauma-induced blood loss.

3.3 Remote Injury

Tourniquets are known to cause injury to remote organs [1, 5]. The extent of the injury is related to the duration of tourniquet application. This is of minimal clinical concern in civilian surgery, however, the extended period of time between tourniquet application on the battlefield and removal of the tourniquet at far-forward medical treatment, make it a significant concern for combat casualty care. We have done preliminary studies of the damage to all major organ systems following 3 hr of tourniquet application. A biochemical

Figure 3: A) Ratio of tetanic tension of treatment/contralateral control muscles. Significant reductions took place for all muscles and treatments with the exception of the soleus at 2 hr tourniquet (TK). B) Ratio of normalized (muscle wt.) tetanic tension of treatment/contralateral control muscles. Significant reductions took place only in the plantaris, indicating an increase in non-contractile elements, probably fibrotic tissue. In contrast the reduction in force for the soleus reflects atrophy and/or fiber loss. *Different from contralateral control (p < 0.05).
maker of cellular stress, 3-nitrotyrosine (3-NT), was significantly elevated in the lung and liver following 3 hr of tourniquet application in our rat model (Fig. 4). These responses were rather modest. However, this was not unexpected as the magnitude of systemic responses may be significantly affected by the mass of the directly injured tissue, i.e., the rather modest responses observed may be a function of the relatively small muscle mass involved within the rat model. Furthermore, while military applications dictate tourniquet use for hemorrhage control; hemorrhage per se has been associated with increases in nitrosative stress. We are therefore currently investigating the effects of both the mass of the injured muscle, as well as tourniquet application in combination with hemorrhage, on systemic nitrosative stress. In addition to nitrosative stress, ongoing studies are examining a number of indicators to quantify the systemic inflammatory response.

3.4 Gene Expression Profiles
The reduction of tourniquet injury through the development of pharmacological interventions requires an understanding of the response of muscle to both the ischemic and reperfusion phases of injury. The most efficient method for assessing the response of a cell or tissue to injury or a drug is with gene expression analysis with cDNA microarrays. The gene expression profile of skeletal muscle in response to I/R is currently unknown so we are using this technique to characterize I/R injury in skeletal muscle using our rat
tourniquet model. An understanding of the genetic response to both ischemia and reperfusion may lead to pharmacological interventions and therapies that can address both components of this injury, leading to greater tissue salvage and ultimately saving limbs.

4.0 HUMAN STUDIES

4.1 Reducing Duration of Tourniquet Use

Many of the injurious effects of tourniquets cannot be avoided. Regardless of advances in tourniquet engineering and treatments to reduce the injury process, biophysical limits will always exist. However, recent development and fielded hemostatic agents and polymeric wound dressings can be used in conjunction with a tourniquet to reduce the required duration of tourniquet application. This scenario involves initial prompt application of a tourniquet to a severely bleeding extremity by the injured soldier or a buddy. When the tactical situation allows, a medic would then apply an appropriate wound dressing (convert), release (not remove) the tourniquet and then observe for effectiveness of the wound treatment. If hemorrhage is not adequately controlled, the tourniquet would again be tightened. Successful control of hemorrhage by a wound dressing would obviously reduce tourniquet injury, which results from physical compression. Additionally, it would reduce I/R by allowing reperfusion of the limb by the remaining patent collateral circulation. Together these factors would greatly increase the chances of limb salvage.

The preceding scenario requires a tourniquet that can be rapidly applied and easily released, and easily re-applied if required. Our lab is currently screening a number available trauma tourniquets in human subjects to determine which tourniquets best meet these requirements. This effort is composed of both laboratory and field testing. The purpose of laboratory testing is primarily to confirm that a candidate tourniquet is effective, i.e., it is capable of occluding arterial blood flow. This is assessed using Doppler auscultation. All candidates that are determined to be effective will then be field tested by combat medics. Ultimately, a multifactor selection matrix will be used to determine the best tourniquet for fielding.

5.0 PRODUCT DEVELOPMENT

5.1 Advanced Tourniquet Design

Dr. Jan Gooch, a National Research Council Senior Fellow at the USAISR, has focused on designing and engineering the initial models of the next generation of military trauma tourniquets. Ongoing interaction with Special Operation Force medics assures the design parameters meet the requisite flexibility and cube constraints required for the battlefield. As discussed above, concerns regarding inherent leaking and the bulky nature of pneumatic orthopedic tourniquets have kept these devices from being fielded for combat use. However, the appreciation of the superiority of a pneumatic design was recognized by far-forward military personal over 60 years ago [1]. Considering all of these concerns, Dr. Gooch’s current prototype is a self-contained narrower version of an orthopedic tourniquet. By employing a self-inflating system equipped with a servo system to monitor and maintain a prescribed pressure, the system averts the problem of leaking. Additionally, in the event of a catastrophic leak, the system can be used in the manner of a traditional strap-and-buckle tourniquet. Formal testing of these systems on phantoms, followed by human subjects is planned for the next year.
6.0 CONCLUDING REMARKS

We have presented a description our program that takes an integrated approach to reducing tourniquet-related injury. Scientific research and military experience will produce new treatments, procedures, guidelines and devices aimed at a single goal, to change the axiom “saving life over limb” to “saving life and limb”.

7.0 REFERENCES


1.0 INTRODUCTION

On the battlefield, hemorrhage from wounds remains the leading cause of mortality, accounting for 50% of all deaths [1]. Hemorrhage is also the second leading cause of mortality among injured civilians, accounting for 39% of civilian trauma deaths [2-4]. The primary field-ready methods for control of hemorrhage—tourniquets, direct pressure, bandages, and clamping—have not changed greatly in several centuries [5]. These interventions, even in the hands of experts, are not always effective [6]. In Vietnam, 50% of combat deaths resulted from wounds with uncontrollable bleeding. Of these wounds, about 11% were inflicted in sites accessible for first aid treatment without need for surgical intervention [1,7]. More effective hemostatic methods could have been prevented up to one third of the deaths from exsanguination during the Vietnam War [1, 8]. This background information strongly illustrates the need to develop a better hemostatic method to improve the immediate care and survival of casualties in the field.

For the past nine years, the United States Army has worked closely with the American Red Cross (ARC) to develop a field-ready hemostatic dressing that can effectively stop arterial bleeding from major wounds. The ARC has an active program to develop fibrin sealant hemostatic agents, the focus of one of our research projects. The organization also controls 50% of collected human plasma, the current source of fibrinogen and thrombin proteins, which are the main components of fibrin sealant dressing. This article briefly reviews the history and development of fibrin sealant dressing as well as other hemostatic products (e.g., chitosan dressing) and the important role that the US Army Institute of Surgical Research (ISR) has played in developing these products.

2.0 HISTORICAL BACKGROUND

The first experiments to control bleeding with fibrin date back to 1909, when Bergel reported the hemostatic properties of fibrin powder in the operative field [9]. The first attempt to make a form of dry fibrin hemostatic product for use by trauma surgeons was during World War I, when Grey [10] and Harvey [11] produced prepolymerized fibrin tampons and thin plaques to control bleeding in parenchymal organs. Although these
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Materials are passive hemostatic agents, incapable of polymerizing and cross-linking directly with the tissue, they worked relatively well. The combination of fibrinogen and thrombin was first used in 1944 by Cronkite et al. [12] and Tedrick et al. [13] for anchoring skin grafts, but because of poor adhesion, it was not widely accepted. During World War II, fibrin glue, fibrin sheet foam, and fibrin powder were mass-produced from pooled plasma, but were withdrawn in 1946 because they transmitted hepatitis. Subsequently, in 1977, all pooled human plasma fibrinogen products, including a commercial liquid FS preparation that was licensed in Europe, were recalled by the FDA because of the high risk of hepatitis transmission. Today, advances in viral removal (nanofiltration) and inactivation technologies (solvent-detergent, pasteurization, and dry heat treatment), combined with sophisticated donor screening, have reduced the risks of viral transmission from plasma products to extremely low levels. This decreased risk, coupled with strong clinical interest, has led to a resurgence in the development of FS products in the United States.

3.0 FIBRIN SEALANT (FS)

FS is composed of purified, virally inactivated human fibrinogen and human thrombin that combine to form a fibrin clot. FS may be used for control of bleeding, tissue gluing, and as a delivery vehicle for drugs and biologics. The hemostatic and adhesive properties of FS are important for certain types of surgical procedures and appear to be useful in treating severe trauma injuries [14].

The hemostatic function of FS mimics the final stages of the blood coagulation cascade. Once the protein components are dissolved in a fluid (e.g., saline or blood), the enzymatic activity of thrombin converts fibrinogen to fibrin monomers by cleaving small peptides (fibrinopeptides A and B) from the molecules. The fibrin monomers rapidly assemble into fibrils and fiber strands, thereby forming a three-dimensional gel network. Thrombin also converts inactive Factor XIII (FXIII), present with fibrinogen, into its active form (FXIIIA) in the presence of calcium chloride. The FXIIIA transforms the soluble fibrin gel into a dense, insoluble fibrin clot at the bleeding site [15,16]. The fibrin clot binds to the tissue by different modes (covalent, non-covalent and mechanical bonding) and physically stops the bleeding. In contrast to passive hemostatic agents (collagen, cellulose, etc.) that promote the patient’s own blood clotting mechanism, FS coagulates independently from patient blood and is therefore useful even in patients with severe coagulopathy. The product is ideally suited for treating traumatic and surgical bleeding in hemophiliac patients [17].

3.1 Liquid Fibrin Sealant

Commercial liquid preparations of fibrin sealant are highly effective when they are used in a conventional setting (e.g., a pre-scheduled operation); however, they do suffer from several limitations if they are considered for trauma application. For example, liquid FS is difficult and time-consuming to prepare because it involves hydrating two lyophilized products, which requires warming and prolonged agitation. The liquid mixture can successfully control a majority of surgical oozing that is of low volume and pressure, but it cannot treat high volume venous or high-pressure arterial hemorrhages, as it is diluted and washed away by the high volume of blood flowing out of lacerated large vessels. In order to address these limitations and broaden the spectrum of the injuries that can be treated with FS, a new physical form of fibrin sealant in the shape of a dry dressing was developed.

3.2 Dry Fibrin Sealant Dressing (DFSD)

DFSD technology was developed for both field medical (pre-hospital) and conventional surgical applications. The dressings are simple to use and designed for self-application, buddy-application or administration by an
emergency medical technician. The victim or caregiver only needs to open the packaging, remove the product, and press the dressing onto the bleeding wound for approximately two to three minutes to treat superficial or extremity injuries that may result in significant blood loss. DFSD may also be used in a hospital setting to control severe parenchymal hemorrhages that either cannot be controlled by conventional methods or require a meticulous operating procedure with the risk of prolonged ischemic time and organ failure.

3.2.1 Early Development of DFSD

The development of DFSD involved several formulation and structural design changes before the final product was ready for manufacturing and distribution. The first prototype of DFSD applicable to trauma surgery was developed at the Letterman Army Institute of Research in the early 1990s [18]. The aim was to deliver a large amount of fibrinogen and thrombin to a wound, producing a fibrin clot with greater density and strength than forms naturally, binding tightly to underlying tissues. The first in vivo test of the prototype dressing, which was composed of powdered fibrinogen and thrombin spread on gauze, was performed at the Letterman Army Institute of Research in 1993. The results, published in 1995, convincingly demonstrated that application of DFSD on an arterial laceration (pig femoral artery) could minimize the bleeding and maintain arterial blood pressure at a normal level [14].

The original ARC dressing (1st generation) produced with advice from the Letterman team in 1995 had a similar composition; i.e., a mixture of dry powdered fibrinogen and thrombin was pressed on a silicon backing material. The backing was intended for handling and application of DFSD onto the wound until hemostasis was achieved, after which it would be removed, leaving behind only the reabsorbable fibrin clot. This prototype was tested in vivo in two severe hemorrhage models including a defined femoral arteriotomy [19] and a complex ballistic injury [20] in the extremity of large animals. The dressings produced hemostasis after application and reduced the overall bleeding by 83% (123 ± 48 ml) and 77% (124 ± 64 ml) in both conditions, as compared to the control treatments (standard gauze or placebo dressing) with blood loss of 734 ± 134 ml and 377 ± 64 ml, respectively. The mean arterial pressure was at least 25 mmHg higher in the dressings treated animals than those in the controls. Hemostasis was achieved in the arteriotomy model without compromising the arterial blood flow to the hind leg of the animals [19].

The first generation of DFSD was later abandoned because of the instability of the powdered materials, which frequently fell off, and the non-absorbable nature of the cotton and silicon backings. An alternative dressing with absorbable backing was developed (2nd generation) that had additional patient benefits. It could be placed in the wound safely and be reabsorbed entirely by the body without the need for removing the backing sheet and disturbing the hemostatic clot. The other necessary change in these dressings was a new method for incorporating fibrinogen and thrombin into the dressing. In the new product, fibrinogen and thrombin were layered (one layer of fibrinogen over one layer of thrombin) on top of an absorbable backing material (Vicryl™ or Dexon™ mesh) and freeze-dried into one sheet. The underlying protein layer was attached to the supporting material by the addition of a thin layer of sucrose that embedded the absorbable mesh. However, the ex vivo laboratory testing, which measured the adhesiveness of the dressing to vascular tissue, revealed very poor adhesive strength of these dressings regardless of which component was the top layer [21]. The reason appeared to be that as soon as saline was added to dissolve the dressing, a thin layer of fibrin clot was formed at the interface of the two proteins and prevented the proper mixing of fibrinogen and thrombin. The result was the formation of a non-homogeneous clot with low adhesive properties and poor hemostatic efficacy. The structural design of the dressings therefore had to be modified.

In the new design (3rd generation), the thrombin layer was placed between two layers of fibrinogen in a “sandwich” configuration. The new design allowed better mixing of active components and complete
polymerization of the fibrinogen into a uniform fibrin clot that showed superior adhesive properties [21]. The concentration of fibrinogen, which is the critical factor in determining the adhesive strength of the dressing, was optimized (15 mg/cm²) based on the results of a study using large animals that was conducted at William Beaumont Army Medical Center, in El Paso, TX [22].

The final laboratory-produced DFSD (Fig. 1) was tested extensively in a variety of innovative hemorrhage models developed by ISR scientists to determine the efficacy and potential benefit in military operations. For example, a model of severe liver injury was developed in large animals (pigs) using a custom-designed clamp with two 4.5 cm sharpened blades that lacerated major hepatic veins and liver tissue in a manner similar to a gunshot injury [23].

Hemorrhage in this model is so severe that it often results in exsanguination of the animal if not treated (Fig. 2). The same injury was also produced in animals in which their natural blood clotting capacity was severely diminished by blood dilution and hypothermia (coagulopathy syndrome), similar to conditions that develop in trauma patients or in battlefield casualties. In these circumstances, the bleeding is more persistent, harder to control and more likely to be fatal if not treated promptly. DFSD controlled these life-threatening hemorrhages in both models and offered a simple and effective method of hemostasis that was superior to standard care (gauze packing) practiced in hospitals [24]. The long-term effects of the dressings were also evaluated in specialized urological operations performed in large animals. For example, application of DFSD dressing on the prostatic bed following prostatectomy (removal of the prostate gland) reduced blood loss by 25 to 30% and shortened the operation time to half that required for other groups [25]. Similarly, when DFSD
was used to treat bleeding from the kidney after a partial nephrectomy operation [26] or a stab wound injury [27], the hemorrhage was stopped more rapidly (less blood loss) and the surgery was completed in a shorter time resulting in less ischemic injury compared with standard surgical techniques. The secondary bleeding and leakage of urine from the injured portion of the kidney were also prevented during the six-week post-operative recovery period, during which time the organ healed properly. These long-term studies were conducted at Brook Army Medical Center in San Antonio, TX.

Figure 2. Photographs of the severe liver injury and profuse venous bleeding model in swine. A. The liver tissue and underlying major veins of the two medial lobes are lacerated twice using a custom-designed clamp with sharp blades (X shape). B. The result appears as a large stellate wound (approx. 10 X 8 X 4 cm) similar to a gunshot injury. C. The wound is packed with three dressings and held for four minutes (or shorter if hemostasis is achieved), after which the animal is closed, resuscitated and monitored for one hour. D. Treatment with DFSD produced hemostasis during the four-minute compression period and bleeding is completely stopped.

4.0 SELECTION OF BEST HEMOSTATIC DRESSING FOR MILITARY USE

In addition to DFSD, ISR investigated a number of other hemostatic dressing products with potential benefit to soldier care. Some of these agents were already approved (e.g., Surgicel, Avitene) and some were under development to be licensed by the FDA and used in a variety of clinical settings. The U.S. Army wants to select the most effective hemostatic dressing that meet the far-forward needs of military use. The ideal hemostatic dressing, as defined by the US Special Operation Command, should meet the following conditions:

1. Able to stop large-vessel arterial and venous bleedings within 2 minutes after application on the wound.
2. Ready to use; no mixing or special preparation.
3. Simple to apply by the wounded personnel, his buddy or medics without any training.
4. Stable at room temperature for at least two years and in extreme temperatures (between 40 and \(-10^\circ C\)) for several weeks or longer.
5. Safe to use and pose no risk of bacteria or viral transmission.

Companies and organizations were solicited to submit their products for vigorous testing to see which would meet the above criteria. A total of nine hemostatic dressings, including two fibrin sealant dressings (one made by the ARC and the other by Nycomed in Austria), were submitted for evaluation. Except for the fibrin sealant dressings, the remaining dressings are considered passive hemostats, promoting the clotting mechanisms of the patient’s own blood as a means to stop the bleeding. In general, passive hemostats act by enhancing platelet aggregation, accelerating intrinsic and extrinsic pathways of clot formation and protecting the blood clot from degradation. In some cases, they were claimed to cause a local vasoconstriction (e.g., the Marine algae polymer dressing) and thereby reduce bleeding from the vessels. The reduced efficacy of passive hemostats in trauma patients with decreased blood clotting capacity (coagulopathic patient) has been acknowledged.

The dressing candidates were subjected to two severe hemorrhage tests in large animals. In the first study [28], the dressings were applied to Grade V liver injury in swine, which represents high volume venous bleeding. The efficacy of each dressing was compared with a control treatment in which cotton gauze was used to stop the hemorrhage. The ARC fibrin sealant was the only product that significantly increased hemostasis and reduced bleeding when compared with control treatments. In the second study [29], the dressings were tested in an even more challenging model that involved severe high-pressure arterial bleeding that produced 100% mortality within 10 minutes after injury. The hemorrhage was produced by making a 4.4 mm diameter hole in the abdominal aorta of the pig, which was allowed to bleed freely for 6 seconds. While bleeding continued, dressings were placed through a pool of blood over the injury site and pressure held for 4 minutes. Hemostasis was determined following removal of manual compression. For controls, either the Army standard gauze dressing was used (negative control) or the vessel was clamped and properly sutured (positive control). The only dressing that effectively sealed the vascular injury and prevented further blood loss and the death of the animals was the fibrin sealant dressing made by ARC (Fig. 3). The efficacy of this dressing during a 1-hour observation period (short-term) equalled that of the standard suturing technique. Animals treated with other hemostatic dressings exsanguinated during the observation period.

5.0 MANUFACTURING OF DFSD

Although none of the nine dressings tested met all of the desired criteria for military use, the ARC DFSD dressing met the more important requirements including those for high efficacy, ready-to-use, stability at room temperature, and biological safety of the product. The promising outcomes of the animal studies, along with a well-planned proposal submitted by the ARC and a manufacturing facility (CSL Bioplasma, Victoria, Australia) prompted substantial financial support by the US Army Medical Research and Material Command to advance the production of this dressing from the laboratory bench to a large-scaled manufacturing facility in 2001.

One of the shortcomings of the ARC DFSD, noticed during testing, was the fragile nature of the fibrinogen sheets, which break down easily and slough off when the dressings were handled. One reason for this problem was the incorporation of thrombin as a thin layer between the two fibrinogen sheets in a sandwich configuration. Because of the differences in protein and buffer composition between fibrinogen and thrombin, the crystallized thrombin layer acted as a weak interface between the dry fibrinogen sheets and reduced the firm attachment of the fibrinogen layers. As a result, the upper layer of fibrinogen was frequently delaminated
and easily flicked off during handling or rough transportation. To minimize this problem, two modifications were made at the manufacturing level. First, the dressings were made, stored, and transported in rigid plastic containers so that they would be protected from some inevitable hits and damage during transportation. Second, the design for incorporating thrombin into the dressing was changed. This modification required further experimentation to prove the equivalency of the scaled-up production dressing with the laboratory-made dressings that were tested earlier.

In the newly designed dressing, thrombin was placed as minute aliquots (∼100 dots, 1 cm apart), spread evenly over the first layer of fibrinogen and covered with the second layer of the fibrinogen. This “polka dot” arrangement allowed better attachment of fibrinogen layers, particularly on the area void of thrombin, and easier way toward total automation of dressing production and robotic application of the components. Initial in vitro tests performed at the manufacturing facility did not show any significant difference between the new dressing design and the prototype in which thrombin was sprayed as a continuous layer. This result, however, had to be confirmed in a challenging in vivo study to ensure equal efficacy of the dressings.

The equivalency study was performed at the ISR and the dressings (prototype vs. first polka dot design) were tested in the liver hemorrhage model (described earlier) in normal swine [30]. Hemostasis achieved with each dressing was compared with that of a control group in which the liver injury was treated with standard gauze. Although the polka dot design reduced the hemorrhaging, it appeared to be less effective in reducing blood loss (31% larger blood loss than the prototype design), and achievement of hemostasis was not substantially better than with the gauze. This result clearly indicated that, despite the favorable in vitro test results by the
manufacturer, the in vivo efficacy of the newly designed dressings was not equal to the prototype. The lower efficacy of the polka dot design appeared to be due to inadequate mixing of thrombin and fibrinogen and therefore incomplete polymerization of fibrinogen across the dressing once it was dissolved in blood.

A new pattern with better distribution of thrombin throughout the dressing seemed to be a logical approach to improve the mixing and consequently the hemostatic function of the product. This was achieved by dividing the same amount of thrombin into smaller aliquots and evenly distributing them between the fibrinogen layers. This design retained the advantage of preventing the delamination of fibrinogen layers but allowed more direct contact of the thrombin with fibrinogen and better mixing upon dissolution. The new polka dot designed dressings, along with the prototypes, were subjected to the same in vivo testing by the ISR staff, using the severe liver hemorrhage model in swine [30]. The results showed significant improvement in hemostatic function of the new product. The efficacy of scaled-up production was equal to the prototype dressings produced in the laboratory setting. Last year, this dressing received preliminary approval by FDA in the military arena for treating external injuries and is currently distributed among Special Operations Forces under an Investigational New Drug (IND) protocol.

6.0 CHITOSAN HEMOSTATIC DRESSING

Recently, a new hemostatic dressing has been developed by the Oregon Medical Laser Center with potential utility to control severe extremity hemorrhages (figure 1). The dressings are made of chitosan, a derivative of a natural polysaccharide known as chitin found abundantly in shellfish (e.g., shrimps). Various forms of chitin and chitosan have been shown to enhance hemostasis in experimental studies. The chitosan dressings offer several advantages over DFSD with regard to the durability and ease of application, particularly in the far-forward military arena. Application of these dressings over wounds does not require any special precautions or use of non-adhesive gloves, which may be necessary for DFSD. They also seem to maintain their adhesive function even if they become wet before being placed on the wounds. Because of their chemical structure (very similar to cellulose), these dressings are more stable and tolerant to prolonged exposure to extreme temperatures (-50° to +140° F). The chitosan dressing was not available when the hemostatic efficacies of the other nine dry dressings were compared; however, the encouraging preliminary data provided by the manufacturer prompted evaluation of a chitosan dressing (prototype) in the standard liver hemorrhage model in swine [31]. The results of this study at the ISR showed that chitosan dressing treatment could significantly reduce blood loss, mortality rate, and enhance hemostasis, when compared with a control treatment (cotton gauze).

More recently, the manufacturing company (HemCon, Inc) received FDA approval for the use of the chitosan dressing to treat external bleeding. This led to large-scale manufacturing and production of hundreds of dressings ready for shipment to the battlefield. The limited in vitro and in vivo tests carried out by the company showed no significant differences between the scaled-up production and the prototype versions previously tested successfully at ISR. However, the experience with the DFSD development obligated ISR and the Army to thoroughly test the product before recommending its shipment and distribution among the troops.

Samples of production dressings were subjected to a standardized model of liver hemorrhage in swine. Data were compared with results obtained using the prototype chitosan dressing under identical study conditions. The production version of the dressing did not improve initial hemostasis, nor change the overall bleeding or survival rate, compared with gauze used as control dressings. These results clearly indicated that the efficacy of the mass-produced dressings was inferior to the prototype version and not suitable for release in the military arena.
This conclusion led to rapid improvements in the manufacturing process of the chitosan dressings by HemCon, Inc and production of a new version that was more flexible and absorbant with better adhesive properties than the previous product. The new dressings were expeditiously (in one day) tested in the standard hemorrhage model with participation of nearly the entire ISR staff. Once the hemostatic efficacy of these dressings was confirmed in the large animal study, shipment of the final product was recommended for possible treatment of combat casualties in Iraq and Afghanistan.

7.0 SUMMARY

The results of nearly a decade of laboratory and animal research are the development and production of two highly effective hemostatic dressings with potentially life-saving properties. These dressings are presently distributed among the soldiers in far-forward military operations overseas. Each product has its own unique advantages and may be more suitable for use in special circumstances. The chitosan dressings are more stable, durable, easy to use and less expensive. They are more likely to be utilized in the first aid stage for temporary control of bleeding. On the other hand, DFSD’s are more flexible (after contact with blood) and better able to conform and adhere to a complex injury, with proven efficacy against the most aggressive and life-threatening hemorrhages. The required careful application process of the DFSD is a potential limiting factor for widespread use of this product.

This report summarizes the important role that the United State Army has played in the development of hemostatic products relevant to military needs and treatment of combat casualties. The invention and employment of challenging hemorrhage models in large animals has been essential in identifying the most promising hemostatic dressings and guiding these products through manufacturing and optimization processes to the extent that they are now available for use in our Armed Forces.

7.0 REFERENCES


Bone and Soft Tissue Trauma Research at the USAISR

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ABSTRACT

Since its establishment in 1943, the United States Army Institute of Surgical Research (USAISR) has conducted research focused on improving the surgical care given to soldiers. Just as our predecessors addressed the unacceptably high impact of thermal injury on combat casualties, the Bone and Soft Tissue Research Team focuses research on combat casualties by examining the epidemiology of combat wounds to identify needed improvements in combat casualty care. This paper provides an overview of the bone and soft tissue trauma research currently being conducted at the USAISR and introduces the available combat casualty data from recent conflicts that were used to identify areas where research can achieve maximum impact on reducing the morbidity and mortality rates of combat casualties.

1.0 COMBAT CASUALTY STATISTICS

Because the Vietnam War ended over a quarter century ago, we sought data from more recent conflicts on which to base our research efforts. While we have been fortunate to have suffered relatively few combat casualties at the end of the 20th century, Operation Just Cause (Panama), Operation Desert Storm (Kuwait and Iraq) and the Battle of the Black Sea (Somalia) offered the opportunity to investigate more recent patterns of combat injury. Fortunately, the patterns of injury were documented both during and after each of these conflicts, as had been done in Vietnam by the Wound Data and Munitions Effectiveness Team (WEDMET).

Despite the great disparity in missions, environments, enemy, weaponry, and the units engaged, the distribution of wounds was surprisingly consistent in these three conflicts (Table 1 and Figure 1).

In each conflict, extremity wounds predominated, constituting between 70% to 75% of all wounds. Another interesting finding from the data collected following Operation Just Cause is the preponderance of minor to moderate wounds in combat casualties as indicated by injury severity scores (Figure 2), a finding consistent with a similar analysis of casualties from Vietnam. With this new appreciation of the importance of extremity wounds in general, and wounds of mild to moderate severity in particular, we set out to identify medical interventions which target these types of wounds.

1 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Table 1: Anatomical distribution of injury as a percentage of total number of wounds in observed in soldiers wounded in action during Operation Just Cause (OJC), Operation Desert Storm (ODS) and the Battle of the Black Sea (Somalia).

<table>
<thead>
<tr>
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<th>ODS</th>
<th>Somalia</th>
<th>Weighted Average</th>
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<tr>
<td>Extremity</td>
<td>70%</td>
<td>71%</td>
<td>75%</td>
<td>71%</td>
</tr>
<tr>
<td>Thorax</td>
<td>9%</td>
<td>4%</td>
<td>7%</td>
<td>6%</td>
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<tr>
<td>Head/Neck</td>
<td>9%</td>
<td>15%</td>
<td>14%</td>
<td>13%</td>
</tr>
<tr>
<td>Pelvis</td>
<td>4%</td>
<td>2%</td>
<td>1%</td>
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<td>Eye</td>
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<td>2%</td>
</tr>
<tr>
<td>Spine</td>
<td>1%</td>
<td>1%</td>
<td>0%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Figure 1: Anatomical distribution of wounds which were recorded for WIA in Operation Just Cause, Operation Desert Storm, and the Somalia. Data were compiled from McBride, et al [3] (Operation Just Cause), Uhorchak et al. [4] (Operation Desert Storm) and Mabry, et al. [5] (Somalia). The patterns of anatomical distribution of injury are highly consistent, and highlight that extremity wounds cause a greater number of casualties than all other wounds combined.
2.0 BATTLEFIELD TREATMENT OF FRACTURES

Of the ¾ of combat wounds that are to the extremities, a large fraction of these injuries include trauma to bone. In fact, fractures constituted 28% of the combined 941 injuries documented for Desert Storm, Just Cause, and Somalia. Not only are these an extremely common injury, fractures in combat casualties result in unusually high morbidity due to high rates of bone loss, mal-union, and osteomyelitis. To reduce the negative impact of fractures, we are developing products that start at the buddy/medic level (non-invasive pelvic band for fracture stabilization and improved splints and casts) and that continue through all echelons of treatment to definitive care (antimicrobial coated external fixator pins and antimicrobial bone graft substitute).

2.1 Pelvic Fracture Stabilizer

While pelvic fractures constitute a relatively small proportion of the fractures on the battlefield, they do occur. These fractures are caused by falls during airborne and fast-rope insertion, as well as miscellaneous accidents.
In order to improve our ability to stabilize these casualties during evacuation and the initial phases of diagnosis and treatment in echelon II medical treatment facilities, we have conducted an analysis of commercially available pelvic compression bands. Cadaveric studies conducted at the USAISR and by academia have documented the utility of these devices in stabilizing the pelvic ring, and reducing the volume of the pelvic cavity, which may aid in hemostasis. In addition, clinical experience suggests that improved stability during transport and manipulation of the patient for medical evaluation significantly reduces pain. These devices are lightweight and inexpensive, and much easier to use than improvised devices and invasive fixators. As a result, we anticipate medical evacuation vehicles will carry pelvic bands in the future.

2.2 Improved Splint/Cast

Although casts are highly effective treatment for fractures, plaster of paris requires water and is high in both volume and weight, making it a less than ideal substance in far-forward medical treatment facilities. Given the great advances in materials science in recent decades, we hypothesized that a polymer could be identified and engineered that would replicate the mechanical properties of plaster and gauze casts while greatly reducing weight. These materials are currently under development through several partnerships with both academia and industry. One promising system, a composite Kevlar mesh/polyurethane epoxy, achieves high strength and rigidity, and is extremely light weight. This composite system can be formed to an extremity as it is pliable before the epoxy completes curing then rapidly stiffens and provides mechanical stability to a fractured limb. Although the impetus for the development of this product is the burdensome weight of plaster casts, we also anticipate that these improved devices may serve as splints, particularly in Special Operations units which operate without easy access to evacuation to an echelon II facility.

Another promising line of research seeks to develop a splint that can off-load the lower extremities. Figure 3 illustrates the impact of minor extremity wounds on units in combat. In this photograph from the recent war in Iraq, a single wound to a lower extremity required three uninjured personnel to aid in evacuation. A device that would allow a soldier to remain ambulatory after injury could have a significant impact on unit effectiveness. We do not anticipate returning the wounded soldier to full function without further care, however, merely freeing other soldiers from the need to carry the casualty would significantly impact unit combat effectiveness. In addition, the casualty may be able to continue to contribute to mission success by performing limited duties such as manning a defensive position.

2.3 Antimicrobial Coated External Fixator Pins

Once a casualty with an open fracture reaches a treatment facility with surgical capability, external fixation is often used to provide mechanical stability while the soft tissue wound heals. Due to the combined effects of higher inoculum and longer delays between injury and initial surgical wound care (irrigation and debridement), external fixators are often used for extended periods. One possible outcome of this extended period of external fixation is pin tract infection, which can lead to pin loosening (loss of structural stability), osteomyelitis, and delays in conversion to internal fixation [1, 2]. In order to combat this problem, the USAISR has developed and tested several prototype antimicrobial coated external fixator pins. Of those tested, standard stainless steel or titanium pins coated with a combination of hydroxyapatite (the calcium-containing mineral found in bone) and lipid stabilized chlorhexidine (a potent antimicrobial) (Fig. 4) proved the most effective, with an 80% reduction in the rate of pin infection in a large animal model of intentionally contaminated pins [6, 7]. These coated pins have been nominated for advanced development, and investigators at the USAISR are currently working with USAMMDA and industry partners to speed the fielding of this promising product.
Figure 3: Wounds to the lower extremities have significant impact on combat units. As shown in the above photo (taken in the early days of Operation Iraqi Freedom), 3 marines were removed from fight in order to aid in the evacuation of an injured solider unable to ambulate. ©Joe Raedle/Getty Images, reproduced with permission.

Figure 4: Antimicrobial coated external fixator pins. The pins shown in panel A are commercially available stainless steel (1) and titanium (2) self tapping external fixator pins that have been coated with lipid stabilized chlorhexidine and hydroxyapatite in order to improve the stability of the interface between the bone and the pin and reduce the incidence of pin tract infection related complications. Panel B shows sections of coated pin placed onto a Petri dish seeded with *Staphylococcus aureus* bacteria. The clear region surrounding each pin section indicates that bacteria were un-able to grow around these pins and is indicative of the effective local antibacterial action of the coated pins.
2.4 Antimicrobial Bone Graft Substitute

Grossly contaminated open fractures are commonly treated with prophylactic local antibiotics. Local delivery of antibiotics involves the implantation of antibiotic impregnated cement beads that are fabricated in the operating room by combining polymethylmethacrylate and an antibiotic, and hand forming this paste into small spheres. These beads elute antibiotic for a period of 2 to 6 weeks, then become nothing more than a foreign body, which requires surgical removal. Thus the currently available treatment for a grossly contaminated open fracture requires multiple surgeries. Furthermore, these cement beads are not FDA approved, do not enhance healing, may in fact negatively impact healing, and delay autologous bone graft placement. The repeated implantation and removal of antimicrobial polymethylmethacrylate beads require multiple surgeries resulting in extended hospital stays and convalescence. The USAISR is currently conducting research to test a single product that can serve both as the antimicrobial implant to sanitize the wound as well as an osteoinductive matrix to reduce the number of surgeries to a single trip to the operating room, and thereby speed wound healing. Our research program to develop and test antimicrobial bone graft substitutes was the subject of an article by Beardmore et al.[8]. We believe that improved splints, non-invasive pelvic stabilizers, and antimicrobial external fixator pins can improve combat casualty care beginning at the level of self and buddy aid, through combat medic care and stabilization, to repair in surgery capable treatment facilities.

3.0 SOFT TISSUE TRAUMA CARE

Soft tissue trauma that occurs as the result of combat wounds is a diverse category which includes the entire spectrum of injuries from minor to severe. While it is tempting to ignore the need to treat minor wounds in favor of focusing on wounds that are life-threatening, the statistics cited above highlight the need to address the entire spectrum of wounds. Although minor wounds have less impact on the individual soldier, they are by far the most common, and even moderate decrements in soldier health can have severe impacts on unit capability when multiplied by their high incidence rate. A minor wound that may require only minimal care is at risk for infection. Data from Somalia [5] as well as from British casualties in the Falkland Islands Campaign [9] show that approximately 15% to 20% of combat wounds result in infection. Infected wounds result in increased morbidity and mortality when compared to similar wounds that are not infected, and lead to longer hospitalization. USAISR has focused soft tissue trauma research on preventing infectious complications in mild, moderate and severe wounds, improving wound care for soft tissue wounds which require treatment in a forward medical treatment facility, and reducing the rate of limb loss due to tourniquet application.

3.1 Prevention and Treatment of Wound Infection

In order to reduce the rate of both soft and hard tissue infection, the USAISR initiated a program to push antibiotic therapy into the pre-hospital phase of medical care. For conventional forces, antibiotics are currently unavailable in the field. Although some Special Force medics carry and administer antibiotics, current drugs require intravenous access, which can be problematic in the far-forward environment. A review of the relevant literature showed that the pharmaceutical industry has developed promising oral antibiotics with low toxicity, excellent bioavailability, and long half-lives. In collaboration with the US Special Operations Command, the USAISR convened a panel of military and civilian subject matter experts, representing Military Medicine, Infectious Disease, Surgery, Pharmacology, and Microbiology to review currently available, FDA approved antibiotics for self-aid. The consensus of the panel was that gatifloxacin (Tequin®) is the most appropriate drug for self-administration any time a combat wound results in a break in the skin. This recommendation has been made to the Doctrine Developers (AMEDD Center and School Directorate of Doctrine and Combat Development) and is currently being implemented.
3.2 Advanced Wound Dressings

The gauze field first-aid dressing and recently developed and fielded hemostatic dressings provide wounded soldiers with excellent tools for the control of hemorrhage. However, these dressings are not practical for the protection of mild to moderate wounds [10]. Interviews with combat medics indicate that the standard field dressings will often slide distally if a casualty resumes normal activities. The failure to properly protect mild to moderate wounds from both bacterial contamination and further mechanical damage can be the source of high wound infection rates and an unacceptable degradation of individual performance. In order to provide a dressing for these wounds, we have initiated research on dressings that can be sprayed, painted, or dusted on to soft tissue wounds to provide a barrier to contamination and abrasion. Work to date has identified an extremely promising polymer product developed by an industry partner, and modified to meet the need for a combat wound dressing. In order to assess the efficacy of this dressing, we have conducted tests utilizing an animal model of a contaminated soft tissue wound. This product out-performed other candidate products, and has the potential to serve as paint on protection that will reduce pain and wound infection, while speeding healing and maintaining maximum individual performance, thus reducing the need for evacuation. A clinical trial is currently in progress as an initial step toward transition of this product to advanced development and fielding.

3.3 Wound Irrigation and Tissue Viability

For a typical traumatic wound, devitalized tissue is debrided and wounds are irrigated with what is usually described as “copious” or “adequate” volumes of sterile saline. While inexpensive in a fixed treatment facility, sterile saline can be an extreme logistical load for the deployable treatment facility, especially if current dogma is followed and 8 to 12 liters of saline are used to irrigate each wound. In order to minimize the weight and volume of fluid consumed for initial wound care, investigators at the USAISR are progressing on two fronts.

First, we are attempting to reduce the volume of fluid required for debridement using improved delivery devices and irrigation fluids. Because combat casualties have evacuation times that are longer than the typical civilian trauma patient, we are currently developing an animal model of combined bone and soft tissue damage and contamination. This model will include a delay after contamination and before irrigation to more closely mirror extended evacuation times. This model will serve as the test-bed for innovations that may reduce logistical loads such as use of potable water for irrigation, irrigation fluid additives (detergents or antimicrobials), pulsatile pressure delivery, and parallel flow delivery. In particular, we will investigate the ability of each of these technologies to meet or exceed the reduction in contamination provided by 10 liters of sterile saline.

Second, we are working to minimize the loss of salvageable tissue through the development of technologies to visualize tissue viability. After conducting an extensive survey of emerging technologies for non-invasively interrogating tissue, Optical Coherence Tomography was identified as the most promising. As there is already extensive industry and academic investment in the development of the core technology, the USAISR entered into a partnership with the Beckman Laser Institute (BLI, University of California, Irvine) to adapt this emerging technology to address the military medicine need. While the technical details of this technology are beyond the scope of this paper, devices in development at BLI have already shown promise in producing images of epidermis and endothelium with sub-cellular resolution. Research conducted at the USAISR will attempt to determine if this imaging modality will be similarly successful in imaging other tissues, and whether these images will be useful as a diagnostic for tissue viability. Our technological goal is the development of a non-invasive, near real time, optical biopsy device which can be used to delineate margins of salvageable tissue. While this work is in the very early stages, a prototype device has been constructed and is currently under initial testing and validation.
3.4 Advanced Tourniquets

Though long out of favor, tourniquets for hemorrhage control when tactical or logistic restraints prevent immediate access to surgical intervention have been returned to use as self or buddy aid (for information regarding the recently fielded one-handed tourniquet, interested readers are referred to the article by Ryan, et al., this issue). While tourniquets do provide a potentially life-saving capability to the soldier, their use is not without risk. When discussing the use of tourniquets for far-forward use, the phrase “life over limb” is often used to describe the trade-off between life-saving hemostasis and the potential for subsequent amputation of a limb after tourniquet use. Our goal is to minimize the negative impact of tourniquet application by conducting research on several fronts, including investigating the pathophysiology of tourniquet injury, developing guidelines for nerve and muscle preserving tourniquet use guidelines, developing novel tissue salvage drug therapies, and designing a second generation tourniquet that is both hemostatic and soft tissue friendly. Readers interested in current research efforts on the effect of tourniquet injury and hemorrhage, and the development of protective use guidelines and therapies are referred to the article by Walters, et al. in this publication.

4.0 CONCLUSION

Though combat wounds cover virtually the entire spectrum of trauma, analysis of available statistics permits us to predict where soldier-focused research and development can have the greatest impact. Additionally, nascent trends in military doctrine such as the development of the Objective Force and an increased reliance on Special Operations units to shape the battlefield highlight the need for innovation in field medicine. The reduction of logistic loads to improve unit deployability and increased dispersion on the future battlefield will demand reduced reliance on medical evacuation and an increased effort to deliver initial medical care as far forward as possible. By providing our soldiers with improved trauma care we seek to reduce the need for immediate medical evacuation from the battlefield while simultaneously mitigating the impact of extended evacuation. This in turn will reduce the adverse effects of injury, reduce workload and logistical requirements for theater medical assets, and increase the speed and number of soldiers returned to duty following wounding.

While current efforts are focused on those problems where we can achieve maximum impact in the near term, continued improvements in combat casualty care require us to advance our understanding of the pathophysiology of trauma. In addition to the research outlined above, investigators in the Bone and Soft Tissue program are involved in longer range research programs with the goal of improving wound healing after initial stabilization and repair of trauma. These efforts include characterizing factors which facilitate regrowth of bone following fracture, improving muscle regeneration to maximize recovery of strength, modulating nerve regeneration after injury and accelerating re-epithelialization of skin defects. We foresee that these lines of research will continue to advance the art and science of caring for combat casualties in order to minimize the impact of injury on our soldiers and the units that depend on them.
5.0 REFERENCES


### Summary

Papers presented all had direct short or long-term bearing on combat casualty care and included outstanding science on haemostasis, shock research and resuscitation, as well as technologies and monitoring with rationale solutions for existing problems.

### Keywords/Descriptors

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### Abstract

Papers presented all had direct short or long-term bearing on combat casualty care and included outstanding science on haemostasis, shock research and resuscitation, as well as technologies and monitoring with rationale solutions for existing problems.
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