Medical Countermeasures to Weapons of Mass Destruction: The NATO Advantage

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INTRODUCTION

Until recently, research into the development of medical countermeasures to weapons of mass destruction was confined almost exclusively to the defence community. This had advantages, particularly insofar as access to the WMD agents was concerned, but there were disadvantages as well, notably the relative dearth of personnel when compared to similar civilian (*eg* university) resources. Additionally, many of the research targets are either Classified or involve applications that have (or had) limited civilian use. The establishment in the early seventies of RSG-3 (subsequently TG004) went a long way toward addressing these problems and resulted in very significant advances in the treatment of nerve agent poisoning and its associated sequelae (neuropathology, for example). Similar success in development of medical countermeasures to other WMDs, for example sulfur mustard, was less successful but nonetheless significant advances in the medical treatment and prophylaxis of chemical and biological warfare agents were discovered by this group of scientists. There were also significant theoretical advances regarding, for example, the structure of the enzyme acetylcholinesterase that will not be discussed in this paper but contributed greatly to our understanding of fundamental mechanisms of neural transmission and the role of this enzyme in the cholinergic nervous system. And recent attention has been directed toward the "emerging" threats such as some of the exquisitely lethal toxins.

As mentioned above, generally speaking, the defence research community is much smaller that its civilian counterpart and the research had to be focused on areas of immediate concern to the military sponsors. Even so, the personnel resources that could be brought to bear on a problem were still relatively small. Cooperation and collaboration in the defence research community was therefore essential. This paper will discuss the success achieved within the NATO community through the establishment of the TG004 panel and its predecessor, RSG-3.

NERVE AGENTS

Certainly, finding effective countermeasures to nerve agents has been a top priority in the defence community since the discovery of tabun in the early forties. Early work for the military was conducted by civilian scientists with funding and assistance from departments of defence (for example the effectiveness of atropine and an oxime [2-PAM] against poisoning by sarin and VX (Wilson and Ginsberg, 1955). This research was eventually moved to secure government laboratories to facilitate experimentation with nerve agents in protected laboratories and prevent their widespread distribution as well as to protect the scientific results. The

Hamilton, M.G. (2007) Medical Countermeasures to Weapons of Mass Destruction: The NATO Advantage. In *Defence against the Effects of Chemical Hazards: Toxicology, Diagnosis and Medical Countermeasures* (pp. KN2-1 – KN2-6). Meeting Proceedings RTO-MP-HFM-149, Keynote 2. Neuilly-sur-Seine, France: RTO. Available from: http://www.rto.nato.int.

sharing of Classified information among several country's civilian scientists would have presented a substantial obstacle. By using the umbrella of NATO security clearance this was prevented.

Building on the success of the early oxime therapy (remembering that atropine is needed for oximes to be effective therapeutically) several countries have had very active oxime synthesis programs. The U.S. developed a number of oximes in Dr. Hackley's laboratory related to TMB-4 and MMB-4. From Germany and Dr Hagedorn's laboratory, came the bis-pyridinium, or Hagedorn (H), oxime series. One of these, obidoxime (toxogonin), replaced pralidoxime as the oxime adjunct to atropine in the autoinjectors of some countries, including Canada (see Table 1). Others of these H-series oximes, namely HS-6 and HI-6, were shared with other countries through the NATO research panel, RSG-3. As you all know, this led to a very long and difficult research program, culminating in the submission of an Investigational New Drug (IND) submission for HI-6 to Health and Welfare Canada in the late 80's. It has subsequently undergone a series of clinical tolerance safety and pharmacokinetic (distribution) studies and is awaiting licensure under a cooperative agreement among Canada the U.K. and the Netherlands. This is a classic example of the cooperation (collaboration) evident at this NATO panel. There was a very significant unintended consequence to this HI-6 research. Because HI-6 is unstable in aqueous solution (pharmaceutical (t_{90}) stability of about 3 months) there was a requirement to "invent" some type of an autoinjector that would meet the minimum military requirement of 5 years shelf life. Several generations of such wet/dry autoinjectors have been developed and fielded since the late 1980s in order for HI-6 to be incorporated for use by the military in Canada (and some other NATO and non-NATO countries).

A major development in the emergency medical management of nerve agent poisoning was the use of pyridostigmine bromide (PBr) as a pretreatment in the event of a threat of nerve agent release. (PBr is sometimes called a "prophylaxis", but this is a misnomer because PBr requires the post-exposure administration of atropine/oxime to be effective.) PBr is a carbamate (reversible) inhibitor of acetylcholoinesterase (AChE) that is used clinically to treat myasthenia gravis (an autoimmune disease characterized by a failure of cholinergic transmission because of a reduction of acetylcholine receptor density). The administration of PBr at a dose sufficient to produce 25 to 45% inhibition of red blood cell AChE, results in a very significant increase in nerve agent survivability especially against certain agents such as soman which do not respond very well to conventional medical countermeasures. Table 2 shows the effect of PBr (combined with atropine, P2S and diazepam) on survivability after 20(!) LD_{50} challenges with nerve agents.

Another significant development comes from the use of scavenger enzymes like butyrylcholinesterase (BChE) , as a pre-treatment for nerve agent poisoning. The majority of the original work in this area was done at Walter Reed Army Institute of Research and the Institute for Chemical Defense at Aberdeen. The original work in this area used animal derived BChE and as shown in Table 3, this enzyme was very effective in protecting animals against large challenge doses of soman. BChE was initially extracted from human plasma, but the yield was quite low (about 6 g BChE from 120 kg plasma). A subsequent collaboration among ICD/DRDC-Suffield/Nexia transfected the human BChE gene into Breed Early/Lactate Early goats to produce kg (2 g/L of milk) quantities of human BChE in the milk of these animals (Langermann, 2006). The use of BChE as a true prophylactic, that is requiring no post-exposure treatment, obviously offers a tremendous advantage to military or civilian personnel entering a known contaminated environment.

The advances in pre-treatment and therapy of nerve agents required moving research into the realm of managing survivors, particularly with respect to nerve agent induced convulsions and the resulting neuropathology. Much of the pioneering work in this area was done at the Centre de Recherche du Service de Santé des Armées (CRSSA) in France and by Dr. Lipp at DRDC-Suffiled (then DRES), and more recently in Dr. M^{c}Donough's lab at ICD. The use of benzodiazepines to control convulsions and therefore mitigate

neuropathological lesions after nerve agent challenges is now an accepted adjunct in the medical countermeasures armamentarium. However, work in several laboratories has shown that if the convulsions are not controlled very early on, say within 10 minutes of onset, there is a progression of the neuropathology that is not arrested even though the overt convulsions are controlled. Recent work from CRSSA and follow-on from the University of Saskatchewan has shown that NMDA receptor antagonists have a much longer window of action and may in the future be used as neuroprotectants at times as much as 6 hours after the initial nerve agent induced convulsions (de Groot *et al.,* 2001, and see Lipton, 2006).

OTHER CHEMICAL AGENTS

In contrast to the tremendous success that has been achieved in the prevention/treatment of nerve agent poisoning, less success has been realized with respect to developing specific remedies to other chemical warfare agents, like sulfur mustard, chlorine and phosgene to name a few. This is not because of a lack of effort because these agents occupy an important area of research presented at the RSG-3/TG004 meetings but more because of the inability to define a specific mechanism of action for the toxic effects of these compounds. Nonetheless, research ideas exchanged at these NATO meetings have generated several hypotheses regarding the mechanism(s) of sulfur mustard (and Lewisite and phosgene as well) but these have met with limited success in identifying effective therapeutic agents. The research continues and some other promising avenues of research (for example, see Lundy *et al*, 1998) with respect to sulfur mustard, are under investigation, and in fact at least one new approach is being presented at this conference. Presently, however, treatment is supportive and long term sequelae can be expected (Falahati *et al*, 2004). Research in this area no doubt will continue and perhaps expand due to the potential terrorist threat(s) and the fact that some of these agents are toxic industrial chemicals (TICs; chlorine and phosgene especially). The bulk of the research will probably continue in defence laboratories, however, because of the toxicity of these agents and the need for special equipment and authorizations.

OTHER RESEARCH DEVELOPMENTS

Personal and personnel decontamination is another area where the advances have been substantial. In response to trial results presented to RSG-3 showing that contaminated Fuller's Earth defeated collective protection (behaving much like a dusty agent) research into other means of personnel protection were initiated in several countries. Two separate research philosophies were undertaken: barrier creams and reactive decontaminants. The barrier concept is best represented by SERPACWA (skin exposure reduction paste against chemical warfare agents) which was approved for human use by the FDA in 2000. It is a viscous white paste containing a 50:50 mixture of a perfluoroalkylpolyether (PFPE) and a polytetrafluoroethylene (PTFE) that is applied at closure points of the protective suits worn by soldiers—neck, wrists and ankles and also goes on sweat-prone areas. The UK is investigating barrier creams as well and a research visit by a British scientist to DRDC-Suffield to assess the effectiveness of some specific formulations was arranged through RSG-3.

Reactive replacements for diatomaceous earth based decontaminats are exemplified by M-291 and Reactive Skin Decontaminant Lotion (RSDL). M-291 is a resin based charcoal system. It is effective in removing CWAs from skin and clothing, but off-gassing can occur. RSDL is a polyethylene glycol-based product developed at DRDC-Suffield that not only immediately sequesters CW agents, and in many cases BW agents, it destroys these agents after sequestration. RSDL is non-toxic and has been shown not to cause adverse histological effects even when administered in skin wounds and remaining there for several hours. RSDL was evaluated by both the U.S. and the Netherlands for efficacy and ease of use and found to be very effective and superior to other decontaminants. This product has received FDA approval for external use in the U.S.A.

BIOLOGICAL AGENTS

Biological agents were not a part of the scope for RSG-3/TG004, but the panel did consider and report on toxins, because in reality despite being considered biological agents by some they are, in fact, chemical agents.

This really is an area of orphan drug research. Agents in this category, sometimes called mid-spectrum agents, mostly include toxins. Although some toxins can cause serious public and/or animal health concerns (*eg* botulinum toxin, saxitoxin) this is an increasingly rare event. Therefore, the search for medical countermeasures, as opposed to pre-exposure vaccination or administration of anti-toxins, is not a high priority for civilian research. However, advances in peptide chemistry, solid phase synthesis, gene transfection and extraction techniques render some of these toxins, heretofore difficult to produce in significant quantities, potential military and terrorist weapons (see Hamilton, 1996). For example, American Cyanamid has submitted proposals to the U.S. Environmental Protection Agency to field-test a viral/toxin combination that kills beet armyworm. A lethal scorpion toxin is inserted into a baculovirus, a type of virus that specifically infects larval stages of lepidoptera. The expression of the scorpion toxin after infection of the lepidopteran hosts, the beet armyworm, increases the toxicity and halves the onset time by factors of 2 to 3. Interestingly, the specificity of this insect control is at two levels: the virus only infects lepidoptera and the scorpion toxin elaborated by the introduced gene only kills insects. It does not require a great leap of imagination to think of a "vector" that will infect susceptible organisms or people.

Strategies to develop new medical countermeasures range from the traditional organic synthesis of “variations on a theme” (for example zinc inhibitors for the treatment of botulinum toxin poisoning) to combinatorial chemistry and hinge-peptide libraries. Screening such libraries offers a rapid process for identifying potential therapies to toxins. The use of “hinge peptide” libraries, which depend on conformational diversity rather than traditional molecular diversity, reduce the need for huge numbers of syntheses and screening steps and therefore accelerates the discovery of potentially active molecules (Moore *et al.,* 2005).

In summary, the contribution of defence research to both military and more recently, civilian defence against CWAs is significantly greater than the size of the defence research community. The tremendous contribution of the NATO panels to this research lies in the focus of the coutries and the scientists involved, but most importantly in the willingness of all the members to share their results. Importantly this information was shared at very early stages of the research, before formal publication of the results. In all areas of classical defence against, and medical countermeasures to, CWAs including nerve agents, vesicants, choking and emerging threats, the lead has been taken by defence scientists in the NATO community. The scope of the advances developed by this relatively small group of scientists far outweighs their size in numbers. The growing concerns over terrorist use of CWAs and other WMDs hopefully will spur an ever increasing collaboration among the defence scientific communities of NATO.

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Table 1
Relative effectiveness of several oximes against nerve agent poisoning

	Obidoxime	**2-PAM (P_2S)**	**HI-6**	**HLö-7**
Sarin	++++	++++	++++	++++
VX	++++	++++	++++	++++
VR	++	++	++++	++++
Tabun	++++	+	+++	++++
GF	+	+	++++	++++
Soman	-	+	+++	+++

The +/−values in the table represent relative effectiveness of the named oximes estimated across all animal species and protocols. A 4-plus value represents an increase in the protection ratio (LD50 with treatment/LD50 without treatment) of 5 or greater; 3 plus a PR of 3–5; 2 plus a PR of 2–3; 1 plus a PR <1.5.

Table 2
Efficacy of Pyridostigmine in Primates

Challenge Agent	7 Day Mortality*
Soman	1/6
Sarin	0/6
Tabun	0/6
VX	0/6

*Conditions: Pyridostigmine (pre), P_2S + atropine + diazepam (post). Nerve agent challenge 20 LD_{50}s each (from Leadbeater *et al.* 1985)

Table 3
Protection of Primates from Nerve Agent Poisoning by Scavenger Enzymes

Source	Challenge	Observations
Fetal Bovine Serum AChE[1]	2.7 LD_{50}s Soman	No clinical signs No cognitive deficits
Equine BChE[2]	2 LD_{50}s Soman 3-4 LD_{50}s Soman	No clinical signs Complete protection with atropine
Fetal Bovine Serum AChE[3]	5 LD_{50}s Soman	No clinical signs No performance decrements
Human BChE (Cohn Fraction IV)[4]	5.5 LD_{50}s Soman	4/6 survive (1 died, 1 eutanized @ 48hr). Rest showed no adverse effects
Recombinant Pegylated human BChE (transgenic goats: Pharmathene)[5]	5.5 LD_{50}s Soman 5.5 LD_{50}s VX	No Signs No Signs

1. Maxwell et al Tox Appl Pharmacol 1992
2. Broomfield et al J. Pharmacol Expt Ther 1991
3. Wolfe et al Tox Appl Pharmacol 1992
4. Lenz et al Chem Biol Interact. 2005
5 Langermann, Chem/Biol Med. Treatment Symposium VI, Spiez, May 2006