

Trans Sodium Crocetin: 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 crocetin (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 crocetin (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

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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. The

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 (\pm 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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