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TITLE: Intraosseous Erythropoietin for Acute Tissue Protection in Battlefield Casualties Suffering Hypovolemic Shock

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14. ABSTRACT- The project investigated whether intraosseous administration of erythropoietin (EPO) during hemorrhagic shock could ameliorate organ injury and facilitate initial resuscitation and subsequent survival. Three series of 24 pigs each were completed, with the pigs randomized 1:1 to receive EPO or NaCl intraosseously at the onset of hemorrhagic shock. In the first series, 50% of the blood volume (BV) was removed triggering an adaptive response that maintained O2 consumption close to baseline levels yielding a survival rate of 83% at 72 hours. In the second series, 65% of the BV was removed exhausting the adaptive response and markedly reducing resuscitability to 25%. In the third series, also removing 65% of the BV, vasopressin was given during hemorrhagic shock as initial bolus (0.04 U/kg) followed by infusion (0.04 U/kg·min-1), dramatically increasing initial resuscitability to 92% and subsequent 72-hour survival to 83%. EPO attenuated increases in arterial blood lactate and favored higher mean aortic pressure but without effects on post-resuscitation organ function or survival. Further analysis of the vasopressin effect using the second series as control showed that in addition to increasing mean aortic pressure, vasopressin concomitantly increased cardiac index ($p = 0.022$) with a statistically insignificant trend towards higher O2 consumption and lower arterial lactate. These effects suggest that vasopressin – in addition to its arterial vasoconstrictive effect – increased venous tone enhancing venous return and the corresponding forward blood flow by translocating blood from capacitance vessels into the effective circulating volume. Work is planned to examine the effects vasopressin (and EPO) under conditions of greater hemorrhagic shock severity.

15. SUBJECT TERMS- Shock, Hemorrhagic; Resuscitation; Erythropoietin; Vasopressin; Venous Capacitance; Swine; Polytrauma and Blast Injury; Maintenance of Tissue Viability

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INTRODUCTION

Critical loss of blood volume as a result of injury in the battlefield is responsible for a high percentage of death and disability among American combatants. Current treatments do not include interventions that can ameliorate tissue injury. Erythropoietin – a naturally occurring hormone which is best known for regulating the production of red cells – has been shown to also activate potent cell survival mechanisms and help protect organs and tissues during ischemia and reperfusion including the heart,[1-10] brain,[11-14] spinal cord,[15] kidney,[16,17] liver,[18] and skin.[19] In previous studies, we identified beneficial effects of erythropoietin when given during resuscitation from cardiac arrest in experimental models[20] and in human victims of sudden cardiac arrest.[21] These observations in the cardiac arrest setting prompted us to hypothesized a similar effect in other low flow states with sufficient severity to cause ischemia and subsequent reperfusion injury, such as hemorrhagic shock. The underlying mechanistic rationale proposed by us involved activation of non-genomic pathways expected to render mitochondria resistant to ischemia and reperfusion injury and hence maintain their bioenergetic function. We have recently garnered support for this mechanism in a rat model of cardiac arrest and resuscitation demonstrating activation of Akt and PKCε in heart tissue of rats treated with erythropoietin coincident with preservation of complex IV activity and myocardial function after resuscitation from cardiac arrest (submitted for publication). For the present studies – aimed at assessing the effects of erythropoietin during hemorrhagic shock – we developed a swine model of controlled bleeding and completed three series of 24 experiments each randomized 1:1 to receive an intraosseous bolus of erythropoietin (1,200 U/kg) or 0.9% NaCl upon removal of 10% of the blood volume. The series varied in hemorrhagic shock severity. For *series-1* we removed 50% of the blood volume, for *series-2* we removed 65% of the blood volume, and for *series-3* we (also) removed 65% of the blood volume but infused vasopressin during hemorrhagic shock to increase resuscitability and survival. *Series-3* exposed a dramatic effect of vasopressin on initial resuscitability and subsequent survival which prompted us – after consultation with our contracting officers – to redirect our research focusing on the effects of vasopressin. The experiments and findings are described below.

BODY

The studies were approved by our Institutional Animal Care and Use Committee and conducted according to institutional guidelines.

Animal Preparation

Male domestic pigs (32–48 kg) were sedated with ketamine hydrochloride (30 $mg \cdot kg^{-1}$ intramuscularly). Anesthesia was induced with propofol (2 $mg \cdot kg^{-1}$ through an ear vein) and the animals intubated with a size 8 tracheal tube initiating positive pressure ventilation with a volume controlled ventilator (840 Ventilator System, Nellcor Puritan Bennett, Boulder, CO) set to deliver a tidal volume of 10 $mL \cdot kg^{-1}$, peak flow of 60 $l \cdot min^{-1}$, and FiO_2 of 0.5. Respiratory rate was adjusted to maintain an end-expired PCO_2 ($P_{ET}CO$) between 35 and 45 mmHg, measured using an infrared mainstream capnometer (Capnogard, Novometrix Medical Systems, Wallingford, CT). Anesthesia was continued using isoflurane (1.75% to 2.75%) and a 1:1 mixture of nitrous oxide and oxygen. The electrocardiogram was recorded through defibrillator adhesive skin pads. All procedures were done using sterile technique. A 7-Fr high-fidelity micro-tip catheter or a 7-Fr micro-tip catheter transducer (both from Millar Instruments, Houston, TX) was advanced through the right femoral artery into the descending thoracic aorta for pressure measurement. A 7-Fr thermodilution balloon-tipped pulmonary artery catheter was advanced through the left internal jugular vein into the pulmonary artery for measuring core temperature and obtaining cardiac output measurements by thermodilution along with pressures in the right atrium and pulmonary artery. A high-fidelity microtip pressure transducer catheter (Millar Instruments, Houston, TX) was advanced through the surgically exposed left carotid artery into the left ventricle for pressure measurements. In *series-2* an additional 7-Fr angiographic catheter was advanced through the left cephalic vein into the coronary sinus with the aid of fluoroscopy. The catheter was then looped laterally and advanced inferiorly for a distance of approximately 5 cm into the great cardiac vein. Proper position was confirmed by fluoroscopy and demonstration of great cardiac vein blood oxygen saturation less than 40%. A 23-Fr cannula (Bio-Medicus, Medtronic, Minneapolis, MN) was advanced through the surgically exposed left external jugular vein into the right atrium and connected via tubing to a blood transfusion bag that was heparinized. Blood was withdrawn or reinfused using a roller pump controlled by custom-developed software in LabVIEW guided by continuous measurements of blood weight using an electronic scale connected with our LabVIEW based software (i.e., close-loop, computer controlled, gravimetric technique). In addition, the rate of blood withdrawal was monitored using a flow probe connected to a modular flowmeter system (T402 Console, Transonic Instruments, Ithaca, NY). Core temperature was maintained between 37.5°C and 38.5°C with a water-circulated blanket (Blanketrol II, Cincinnati SubZero, Cincinnati, OH).

Experimental Protocol

Three series of 24 experiments each were conducted. Within each series, animals were randomized 1:1 to receive a 1,200 U/Kg bolus of erythropoietin (Procrit epoetin alpha, Janssen Biotech, Horsham, PA) or 0.9% NaCl intraosseously into the left tibia upon removal of 10% of the blood volume. In *series-1*, 50% of blood volume was withdrawn over 30 minutes. In *series-2* and *series-3*, an additional 15% of blood volume was withdrawn over an ensuing 30 minutes for a total of 65% blood volume removal in 60 minutes (Figure 1). After blood removal animals were left untreated in hemorrhagic shock for 60 minutes in *series-1* and 30 minutes in *series-2* and *series-3*. Crystalloid fluid resuscitation was then commenced using 0.9% NaCl. For *series-1* and *series-2*, a conventional 3:1 rule was applied administering a total volume of 0.9% NaCl three times the amount of blood removed. One third was given over 30 minutes, one third over the subsequent 60 minutes, and the last third over 150 minutes. Crystalloid infusion was followed by reinfusion of the blood removed over 60 minutes. In *series-3*, a crystalloid fluid resuscitation protocol consistent with current protocols for hypotensive resuscitation was used concomitantly examining the effect of no fluid resuscitation. Accordingly, half of the animals received a volume of 0.9% corresponding to only 50% of the blood volume removed administered over 30 minutes. The other half did not receive fluids. For this *series-3*, to minimize early demise related to the severity of the hemorrhagic shock as observed in *series-2*, vasopressin (Pitressin, JHP Pharmaceuticals, Rochester, MI) was given during hemorrhagic shock as follows; a 0.04 U/kg bolus was injected intraosseously upon completion of 50% blood volume removal followed by a continuous infusion at a rate of 0.04 $U \cdot kg^{-1} \cdot min^{-1}$ using a pump (PHD 2000 Syringe Pump Series, Harvard Apparatus, Holliston, MA). The rate of infusion was doubled if the mean arterial pressure (MAP) decreased below 20 mmHg. The infusion continued throughout the hemorrhagic shock phase and was stopped at the beginning of blood reinfusion after which it

was continued only if the MAP decreased below 20 mmHg. Blood reinfusion was started in *series-3* at 150 minutes from the start of hemorrhagic shock.

Survival was assessed in *series-1* and *series-3*. High mortality during hemorrhagic shock in *series-2* precluded meaningful assessment of survival. Thus, at the completion of experiments in *series-1* and *series-3*, all catheters were removed, vessels ligated, and the skin stapled. The animals were then allowed to recover from anesthesia and the endotracheal tube removed after resumption of spontaneous breathing. The animals were returned to their pens and observed for 72 hours. A fentanyl dermal patch was used for analgesia throughout the 72-hour postresuscitation period. If additional analgesia was needed 2.2 mg/kg of flunixin meglumine was administered intramuscularly. The neurological status was evaluated at 24, 48, and 72 hours post-resuscitation using a neurological deficit score (0 = best; 100 = worst) and a cerebral performance category score (1 = normal; 2 = mild disability; 3 = severe disability; 4 = coma; and 5 = death).[22] In *series 3*, blood was also sampled at 24, 48 and 72 hours from the superior vena cava after the animals were sedated with ketamine hydrochloride (30 $mg \cdot kg^{-1}$ intramuscularly). Pigs were euthanized at 72-hours by intravenous injection of euthanasia solution (5ml). The heart, left lobe of the lungs, right kidney, liver and small bowel were removed for analysis. Whole left lung was weighed before and after drying in an oven at 70°C for at least 72 hours for calculations of the wet/dry ratio.

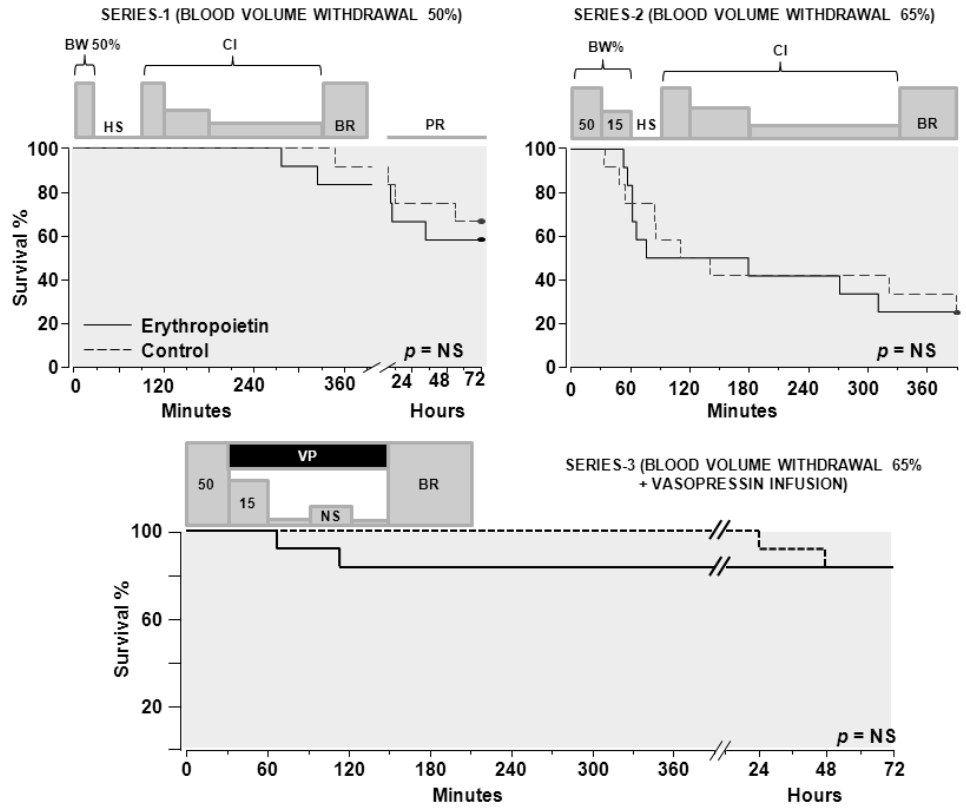


Figure 1: Experimental protocol and survival curves are shown for *series-1*, *series-2*, and *series-3*. All three series had an initial sample size of 12 pigs per group. Shown are survival curves as percentage of baseline. Survival was examined in *series 2* only for the acute phase whereas *series-1* and *series-3* included 72 hour survival. BW, blood withdrawal; NS, normal saline; BR, blood reinfusion; VP, vasopressin. The *p*-value was calculated using the Gehan-Breslow test and showed no statistically significant differences associated with erythropoietin administration in either series. However, there was a marked increase in survival by addition of vasopressin in *series-3* compared with *series-2* ($p<0.001$).

Measurements

Blood analysis: Blood samples were collected from the aorta and pulmonary artery in all three series with the addition of great cardiac vein in *series 2*. Blood samples were processed on site within minutes for pH, PO_2, PCO_2, hemoglobin, and lactate using a cartridge based device (OPTI® CCA-TS Blood Gas and Electrolyte Analyzer, OPTI Medical Systems, Roswell, GA) and for common hemoglobin types (oxy-, met-, carboxy-, and reduced-) using a co-oximeter (AVOXimeter 4000, A-VOX systems Inc., San Antonio, TX). O_2 content in aortic (CaO_2) and pulmonary artery (CvO_2) blood was calculated according to the following equation:

where 1.39 denotes ml of O_2 bound to 1 g of hemoglobin (Hufner's number), S_FO_2 the fraction of oxyhemoglobin relative to the four hemoglobin types, and 0.003 the O_2 solubility coefficient. Aortic blood samples were also taken and processed for complete blood count, chemistry (BUN, creatinine, ALT, AST, ALP, and troponin I) in a hospital laboratory (Captain James A. Lovell FHCC VA Hospital, North Chicago, IL).

Hemodynamic Measurements: Thermodilution cardiac output was measured in duplicate after bolus injection of 0.9% NaCl (5 ml) into the right atrium (HP-Philips M012AT cardiac output module, Amsterdam, The Netherlands). Cardiac

output was normalized to body surface area using the Kelly equation (body surface area [m^2] = 0.073·body-weight$^{2/3}$ [kg]).[23] Pressure signals were zeroed to midcavity level, sampled at 250 Hz, digitized using a 16-bit data acquisition board (AT-MIO-16XE-50; National Instruments, Austin, TX), and analyzed using custom developed software (Labview 6.0, National Instruments).

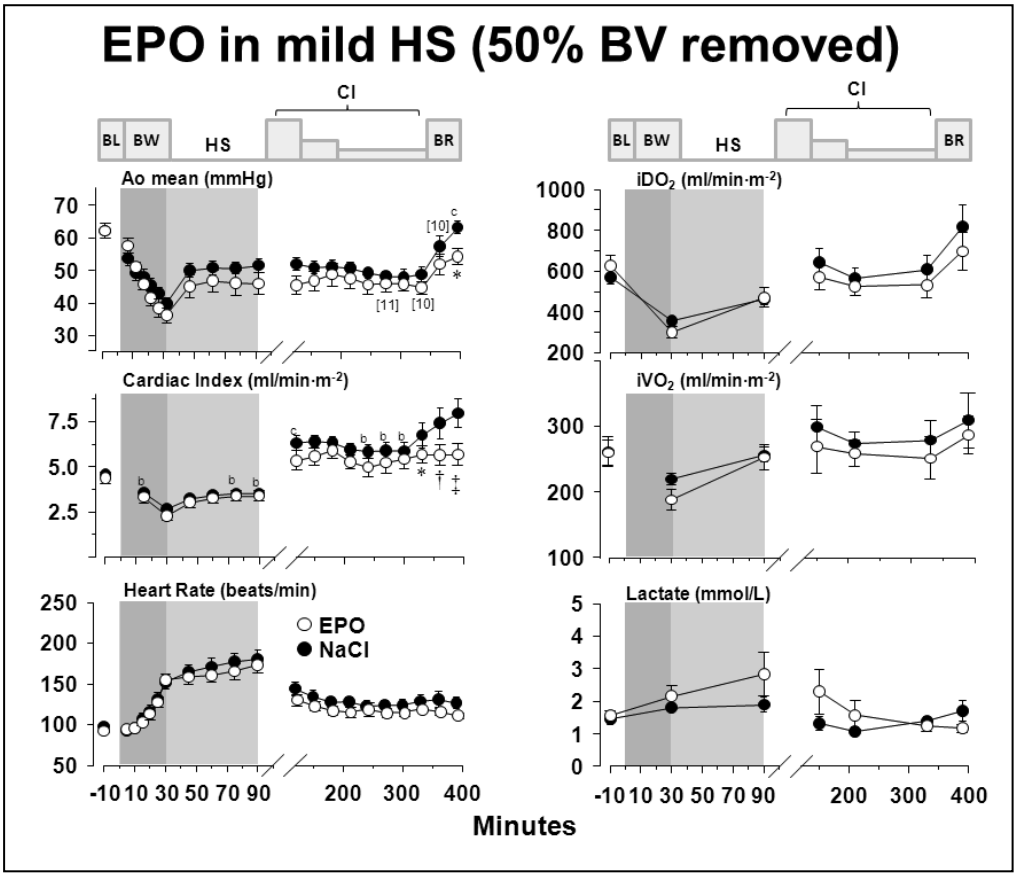
Cardiac Function: Indices of cardiac function were derived from left ventricular pressures, reporting the maximal and the minimal rate of left ventricular pressure change (dP/dt_{max} and dP/dt_{min}), the stroke volume index (SVI), and the left and right ventricular stroke work index (LVSWI and RVSWI, respectively) corresponding to the SVI times the difference between systolic and end-diastolic left ventricular pressures and between the mean pulmonary and right atrial pressures, respectively, expressed in centijoules (cJ) multiplying by 0.013332.[24]

Statistical Analysis

For all variables two-way repeated measures ANOVA was used to test for treatment effect between groups and their interactions over time identifying differences at specified time points when present (SigmaPlot 11.0, Systat Software, Point Richmond, CA). The data were presented as means ± SD unless otherwise stated. A two-tailed probability value of $p < 0.05$ was considered significant.

Results

In *series-1* the 50% reduction in blood volume was associated with 83% survival at 72 hours (Figure 1, left upper graph). The blood volume reduction triggered



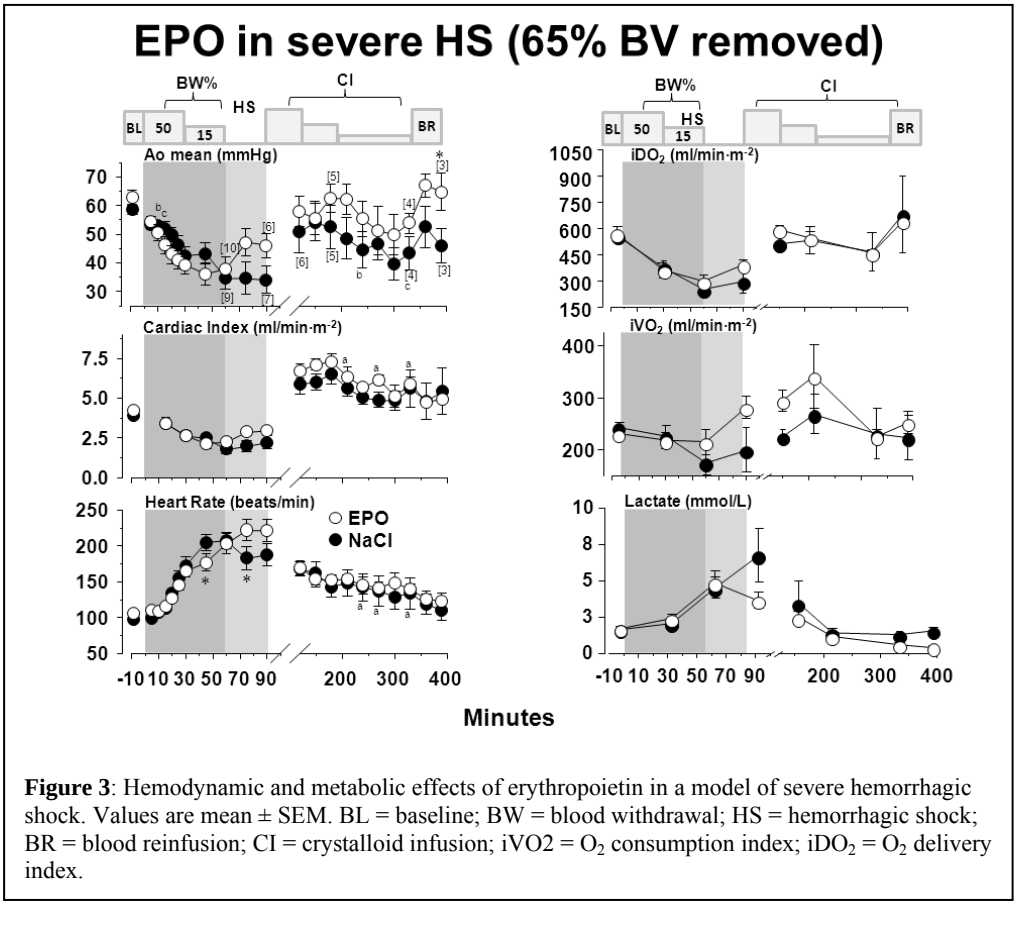


Figure 3: Hemodynamic and metabolic effects of erythropoietin in a model of severe hemorrhagic shock. Values are mean ± SEM. BL = baseline; BW = blood withdrawal; HS = hemorrhagic shock; BR = blood reinfusion; CI = crystalloid infusion; iVO2 = O_2 consumption index; iDO_2 = O_2 delivery index.

an adaptive response that encompassed a prominent increase in heart rate that served to maintain the cardiac index and therefore systemic O_2 delivery at levels moderately lower than

baseline enabling increases in systemic O_2 extraction to maintain O_2 consumption near baseline levels with a very minimal increase in arterial lactate (1.5±0.5 to 2.4±1.8 mmol/l; $p<0.01$) as shown in Figure 2. Under these conditions of mild hemorrhagic shock, no effects of erythropoietin on resuscitation or survival were observed. However, removal of an additional 15% of the blood volume for a total of 65% in *series-2* exhausted the adaptive response causing an O_2 debt leading to anaerobic metabolism with generation of lactic acid (Figure 3) and dramatically reduced resuscitability to only 25%. Most deaths occurred during removal of the additional 15% blood volume and the subsequent observation without administration of fluids. There were no survival differences between erythropoietin and controls animals (Figure 1, right upper graph). However, a trend was observed suggesting the erythropoietin could ameliorate the lactic acid increases and augment mean aortic pressure (Figure 3). However, the high mortality rate with reductions in sample size precluded statistical validation of these observations.

Series-3 was conducted seeking a protocol that could preserve the metabolic severity observed in *series-2* while allowing resuscitability in a larger percentage of animals to properly study the effects of erythropoietin under conditions of severe hemorrhagic shock as originally intended. Review of the literature suggested that administration of vasopressin could help improve resuscitability and survival.[25,26] The infusion of vasopressin as described in the Methods section had a dramatic effect increasing

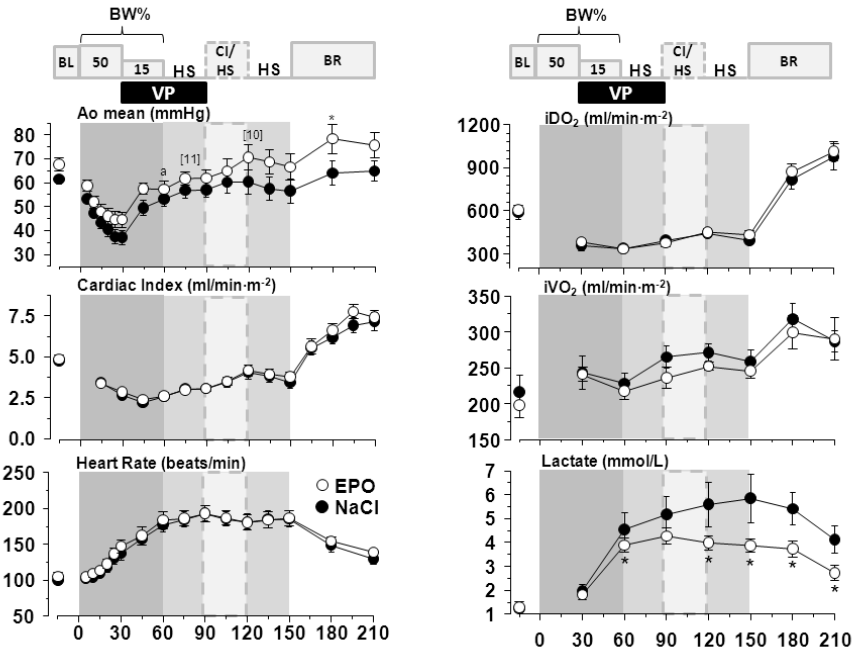


Figure 4: Hemodynamic and metabolic effects of erythropoietin in a model of severe hemorrhagic shock receiving vasopressin. Values are mean ± SEM. BL = baseline; BW = blood withdrawal; HS = hemorrhagic shock; BR = blood reinfusion; CI = crystalloid infusion; iVO2 = O_2 consumption

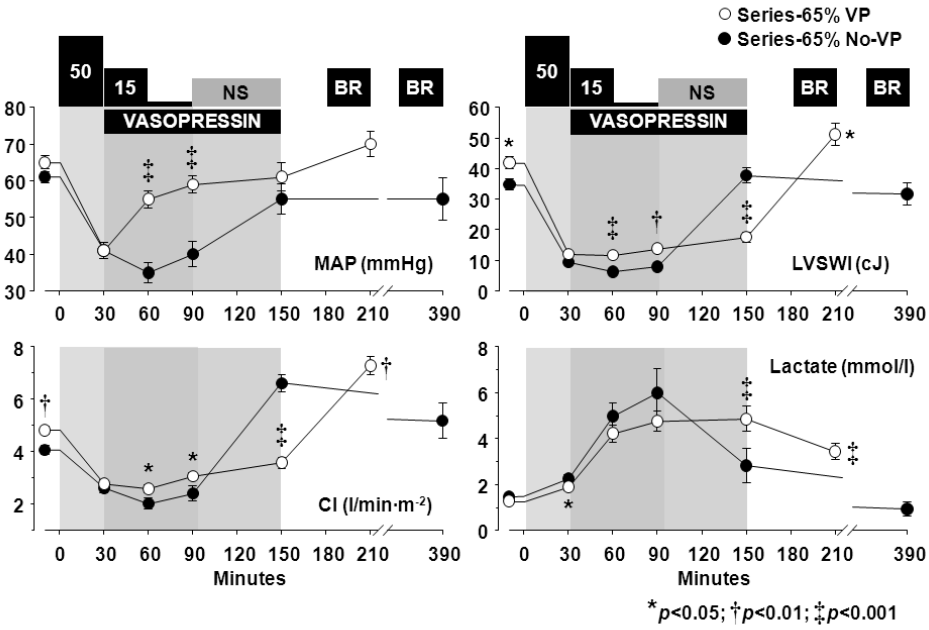


Figure 5: Comparison between hemorrhagic shock *series-2* and *series-3*. Values are mean ± SEM. BR = blood reinfusion; NS = normal saline; MAP = mean aortic pressure; LVSWI = left ventricular stroke work index; CI = cardiac index.

resuscitability from 25% in *series-2* to 92% for the same amount of blood volume removed. At 72 hours, 83% of the animals in *series-3* were alive having recovered fully within the initial 24 hours after onset of hemorrhagic shock (Figure 1, lower graph); two

survivors had to be euthanized (at 24 and 48 hours) for inability to ambulate. Accordingly, infusion of vasopressin was exceedingly effective to avert death associated with severe hemorrhagic shock produced by 65% blood removal. The difference in survival comparing series-2 and series-3 was highly statistically significant ($p<0.001$ by Gehan-Breslow test). The impressive effect of vasopressin on resuscitability occurred before administration of crystalloids demonstrating a survival effect that was independent of fluid resuscitation. In fact, as described below, half of the animal did not receive crystalloids during hemorrhagic shock without compromising resuscitability and survival.

As in the two preceding series, administration of erythropoietin had no effect on initial resuscitation or survival. However, the metabolic trend identified in *series-2* suggesting that erythropoietin could ameliorate lactic acidosis was confirmed in *series-3* showing a prominent and statistically significant attenuation in lactate increase (Figure 4). There was also evidence of an effect on MAP as suspected in *series-2*, an effect which could be related to the vasoconstrictive effect of erythropoietin.

Further analysis of the data comparing *series-2* with *series-3* revealed that the MAP (mmHg) – as intended – was increased by vasopressin at the end of the additional 15% blood volume removal (60 minutes) (35 ± 13 vs 55 ± 12; $p<0.001$) and at the end of hemorrhagic shock before fluid administration (90 minutes) (40 ± 12 vs 59 ± 17; $p<0.001$). In addition, vasopressin elicited a physiologically unanticipated hemodynamic effect on forward blood flow and on aortic lactate (Figure 5). Instead of the selective vasoconstrictive effect on the arterial circuit leading to increases in aortic pressure by reducing blood flow to "non-vital" organs and tissues, the "pressor" effect of vasopressin was associated with increases in cardiac index ($l/min \cdot m^{-2}$) at the end of the additional 15% blood volume removal (2.02 ± 0.87 vs 2.59 ± 0.67; $p<0.05$) and at the end of hemorrhagic shock (90 minutes) (2.41 ± 1.04 vs 3.06 ± 0.62; $p<0.05$) and a trend toward lesser increases in arterial lactate (mmol/l) at the end of the additional 15% blood volume removal (4.98 ± 2.60 vs 4.22 ± 1.81; $p=0.261$) and at the end of hemorrhagic shock (90 minutes) (6.00 ± 3.92 vs 4.74 ± 2.03; p=0.207). The higher blood flow, we hypothesized, could reflect increased vascular tone of capacitance vessels more effectively distributing the remaining blood volume into the effective circulating volume.

Another important aspect of *series-3* is that pigs were resuscitated and survived even without having to administer a crystalloid infusion, demonstrating a window of at least 150 minutes from the start of bleeding to the start of blood reinfusion in which "hemostasis" and vasopressin infusion was all what was needed to stabilize the animal and ensure resuscitability and survival. Despite no effect on resuscitability and survival, administration of normal saline was associated with an increase in cardiac index ($l/min \cdot m^{-2}$) (2.98±0.83 vs 4.20±0.75, $p<0.002$ at 150 minutes) without further differences after blood reinfusion (7.23±1.87 vs 7.35±1.42, *NS*) but with a trend towards a lower lactate (mmol/l) at 150 minutes (5.84±3.34 vs 3.87±0.93, $p=0.074$) that became significant after blood reinfusion (4.12±1.93 vs 2.73±0.98, $p=0.046$).

Summary and Significance

The studies show that erythropoietin given at the onset of hemorrhagic shock in a swine model of controlled hemorrhagic shock fails to improve resuscitability and survival regardless of the severity of hemorrhagic shock. However, administration of erythropoietin attenuates increases in arterial lactate. This effect could reflect an activation of mechanisms protective of mitochondrial bioenergetic function akin to those observed in our cardiac arrest models and those reported by other investigators. Further work is required to assess the clinical relevance of this effect, such as protection from development of subsequent organ failure.

Intraosseous infusion of vasopressin during severe hemorrhagic shock was highly effective for initial resuscitation and subsequent survival without organ dysfunction. Vasopressin was effective with or without concurrent fluid administration. The hemodynamic effects suggest that in addition to arterial vasoconstriction, vasopressin concomitantly augmented venous tone favoring translocation of blood from capacitance to resistance vessels.

Limiting fluid resuscitation may have beneficial effects; acutely along the concept of permissive hypotension and after stabilization by minimizing complications derived from fluid overload leading to organ edema and dysfunction. Many available pharmacological agents are known to exert potent effects on venous capacitance and might find an important clinical application for hemorrhagic shock. Logistically, deployment of a small pump loaded with a vasopressor agent that can be attached to the victim for intraosseous delivery in the battlefield upon recognition of injuries associated with severe hemorrhagic shock without the need to infuse large amounts of fluids appears feasible and attractive.

KEY RESEARCH ACCOMPLISHMENTS

Contrary to our initial hypothesis, erythropoietin given in bolus dose (1,200 U/kg) through the intraosseous route at the onset of hemorrhagic shock failed to improve resuscitability and survival regardless of the severity of hemorrhagic shock.

Erythropoietin, however, attenuated increases in arterial lactate suggesting – as original hypothesized – that erythropoietin activated mechanisms rendering mitochondria more resistant to ischemia reperfusion injury. This effect could be useful clinically to reduce the risk of subsequent multiple organ system failure.

Vasopressin – used to ensure survival under conditions of severe hemorrhagic shock – was remarkably effective for ensuring initial resuscitability and subsequent survival without organ dysfunction without the need for concurrent fluid resuscitation.

The hemodynamic effects observed during vasopressin infusion suggest that in addition to arterial vasoconstriction, vasopressin concomitantly augmented venous tone favoring translocation of blood from capacitance to resistance vessels thus increasing the circulating blood volume.

Vasopressin was effective for initial resuscitation and subsequent survival in the presence and in the absence of concomitant fluid resuscitation.

The study supports exploring the concept of venous-tone-augmentation for hemodynamic support during hemorrhagic shock and potentially other low intravascular volume states.

Intraosseous administration of vasopressin (or other agents eliciting similar hemodynamic effects) using a small portable pump without concomitant fluid resuscitation may be logistically advantageous for far-forward deployment during combat operations to improve the survivability of battlefield casualties.

REPORTABLE OUTCOMES

The data from experiments completed and described in the previous reports are being prepared for submission for peer-review publication. Part of work was accepted for presentation at the 2012 American Heart Association Scientific Sessions and their abstracts published in circulation, as listed below:

1. Whitehouse K, Borovnik-Lesjak V, Miao Y, Baetiong A, Velmurugan S, Currie B, Radhakrishnan J, Gazmuri RJ. Effects of erythropoietin during hemorrhagic shock in a swine model. *Circulation* 2012;126:A18674.
2. Borovnik-Lesjak V, Whitehouse K, Baetiong V, Currie B, Radhakrishnan J, Gazmuri RJ. Identification of critical level of blood volume reduction in a swine model of hemorrhagic shock. *Circulation* 2012;126:A12073.
3. Gazmuri RJ, Whitehouse K, Borovnik-Lesjak V, Baetiong A, Radhakrishnan J. Vasopressin infusion during severe hemorrhagic shock increases systemic blood flow and markedly improves survival in a swine model. *Circulation* 2012;126:A15905

CONCLUSION

The work completed demonstrates a metabolic effect of erythropoietin that is evidenced by reductions in lactic acidosis under conditions of severe hemorrhagic shock. This effect – thought not detectable with the current models used – could have a clinical benefit in attenuating the risk of subsequent multiple organ system failure development. Infusion of vasopressin during severe hemorrhagic shock had a dramatic effect on initial resuscitation and subsequent survival regardless of concomitant fluid resuscitation. This unexpected observation along with the effects of erythropoietin provides the rationale for refocusing the subsequent experiments, as already discussed with contracting officers and as detailed below:

The plan reflects a change in direction prompted by the remarkable survival observed in the last experimental series associated with vasopressin infusion. Specific experiments will be conducted to assess the effects of vasopressin delivered as in *series-3* (i.e., 0.04 U/kg·min^{-1}) but under conditions of greater hemorrhagic shock severity and duration. Three groups of 8 pigs each will be randomized to have 65%, 70%, or 75% of their blood volume removed over 60 minutes with the duration of hemorrhagic shock after completion of blood removal extended from 90 minutes (as in the preceding series) to 150 minutes for a total duration of 210 minutes from the beginning of blood removal (simulating initial injury) to the start of blood reinfusion (simulating treatment after arrival to a hospital). Consistent with the goal of developing an approach logistically advantageous for far-forward deployment of medical care, fluid resuscitation will not be provided. Animals will be recovered from anesthesia and observed for 72 hours to assess – as in the preceding series – effects on organ function. In the same series, animals will be block randomized 1:1 to erythropoietin or vehicle control delivered intraosseously at the onset of hemorrhagic shock (as in the preceding series) such that each group will have half of the animals treated with erythropoietin.

Survival curves will be analyzed to determine whether vasopressin infusion remains effective under conditions of greater hemorrhagic shock severity. The data will also allow analysis of the initially hypothesized effects of erythropoietin on ischemia and reperfusion injury.

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APPENDICES

N/A

SUPPORTING DATA

Included in the body of the presentation.