A Comparison of the Hemoglobin-Based Oxygen Carrier HBOC-201 to Other Low-Volume Resuscitation Fluids in a Model of Controlled Hemorrhagic Shock

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Background: **The ideal resuscitation fluid for military applications would be effective at low volumes, thereby reducing logistical constraints. We have previously shown that the bovine hemoglobin-based oxygen carrier HBOC-201 is an effective low-volume resuscitation fluid. The goal of this experiment was to evaluate the effectiveness of HBOC-201 in comparison with other low-volume resuscitation fluids in a swine model of controlled hemorrhagic shock.**

Methods: **Forty-two immature female Yorkshire swine (55–70 kg) were divided into seven groups of six. Animals were hemorrhaged to a mean arterial pressure of 30 mm Hg. After 45 minutes, animals were resuscitated to a mean arterial pressure of 60 mm Hg with one of the following agents: hypertonic saline 7.5% (HTS), hypertonic saline 7.5%/Dextran-70 6% (HSD), pentastarch 6%, hetastarch 6%, or HBOC-201. Lactated Ringer's (LR) solution was used as a standard resuscitation control. Another group of animals received no resuscitation. Resuscitation was continued for 4 hours. Hemodynamic variables and oxygen consumption were measured continuously. Arterial and mixed venous blood gases and serum lactate levels were measured at intervals throughout the experiment. Data were analyzed using analysis of variance with Tukey's post hoc test when appropriate. Significance was defined as $p < 0.05$.**

Results: **Five of six animals in the no-resuscitation control group, six of six in the HTS group, and one animal in the HSD group died before completion of the study. All other animals survived to completion. Animals receiving resuscitation with HBOC-201 had significantly lower cardiac output, mixed venous oxygen saturation levels, and urinary output throughout the resuscitation period; however, there were no differences with regard to lactate, base excess, or oxygen consumption. Animals receiving HBOC-201 required significantly less fluid than any other group.**

Conclusion: **In this model, hypotensive resuscitation with HBOC-201 restores tissue oxygenation and reverses anaerobic metabolism at significantly lower volumes when compared with HTS, HSD, pentastarch, or hetastarch solutions. These data suggest that HBOC-201 would be an effective primary resuscitation fluid for far-forward military or rural trauma settings where logistic constraints and prolonged transport times are common. However, when HBOC-201 is administered as a primary resuscitation fluid in hypotensive protocols, common clinical markers for determining adequacy of resuscitation may not be useful.**

Key Words: **Hemoglobin-based oxygen carrier (HBOC), Oxygen therapeutic, Resuscitation, Hypotension.**

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Hemorrhage is the leading cause of death on the battlefield[1] and contributes significantly to early deaths after penetrating and nonpenetrating civilian trauma. In addition to control of ongoing hemorrhage and expeditious evacuation, initial resuscitation attempts must be aimed at restoration of blood volume to prevent early deaths from exsanguination and to ameliorate the short- and long-term effects of hemorrhagic shock. Traditional resuscitation has involved administration of large volumes of isotonic crystalloid solutions followed by blood products as necessary.

In military operations, where medical care must often be delivered in an austere and dangerous environment, logistical constraints limit the availability of resources. Large volumes of crystalloid solutions and blood products are often not available at the location of greatest need, at or near the site where the injury has occurred. The use of blood products is further constrained by issues of availability, storage, shelf life, and safety. These difficulties have led to a search for alternatives to large-volume crystalloid and blood product resuscitation. The ideal solution would be readily available from an abundant source, easily stored at ambient temperatures with a long shelf life, easy to administer, and safe from concerns regarding contamination or antigenicity. It would be effective in small volumes to restore hemodynamic stability and it would reduce or delay the need for blood product administration. Cube and weight restrictions would be mini-

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mized and potency would be maximized to improve availability and effectiveness in the field.

Promising alternative solutions include hypertonic salt solutions, starch-based colloid solutions, and hemoglobin-based oxygen-carrier (HBOC) solutions. Hypertonic saline and hypertonic saline/dextran solutions have been shown to be safe and effective low-volume resuscitation fluids in both animal and human studies.[2–4] These data have led to interest in the use of hypertonic saline solutions in the initial treatment of hemorrhage for both military and civilian trauma. Hydroxyethyl starch-based solutions have well-established uses as plasma volume expanders.[5] They provide plasma volume expansion greater than one and one half times the volume administered, and they have been investigated for use in the treatment of hypovolemia secondary to hemorrhage.[6–8] The development of HBOC solutions and their application to hemorrhage resuscitation has been intensely studied in recent years.[9–11] The product Hemopure (HBOC-201, Biopure Corporation, Cambridge, MA) is one such solution containing polymerized bovine hemoglobin in balanced salt solution. It has been studied in a variety of animal studies and has completed phase III human clinical trials.[12–16] The rheologic properties and favorable oxygen unloading characteristics of HBOC solutions may enable improved perfusion and oxygenation of tissues relative to non–oxygen-carrying solutions when used to treat hypovolemia from hemorrhage.[17] This solution also requires no crossmatch and can be stored for periods of up to 3 years at room temperature. Previous studies of Hemopure in our laboratory have shown it to be effective at low volumes in restoring tissue perfusion and reversing anaerobic metabolism.[18]

Each of these fluids shows promise as an alternative to traditional resuscitation for use in the treatment of hemorrhagic shock in both civilian and military settings. This current study attempts to compare these fluids in a swine model of severe controlled hemorrhagic shock, with emphasis on both effectiveness of resuscitation and on volume of fluid required for resuscitation.

MATERIALS AND METHODS

Hemorrhagic Shock Model

This protocol was approved by the Wilford Hall Medical Center Animal Care and Use Committee. All animals used in this study were handled according to the *Guide for the Care and Use of Laboratory Animals*. Forty-two female Yorkshire swine weighing between 55 and 70 kg were used in this study. The animals were sedated with an intramuscular injection of ketamine (15–20 mg/kg) and atropine (0.04–0.4 mg/kg) followed by induction of anesthesia with 2.5% sodium thiopental (6.6%, 25 mg/kg) and endotracheal intubation. Anesthesia was maintained with isoflurane and end-tidal levels were monitored and adjusted to achieve absence of response to surgical stimulation without depression of heart rate or mean arterial pressure (MAP). After completion of instrumentation but before initiation of the experiment, all animals were paralyzed with intravenous pancuronium (0.05–0.07 mg/kg) and maintained under neuromuscular blockade with repeated doses as needed to prevent spontaneous ventilation. Mechanical ventilation was maintained within the following parameters: respiratory rate, 10 breaths/min; tidal volume, 7 to 10 mL/kg; F_{IO_2}, 0.50; and positive end-expiratory pressure, 3 mm Hg. Tidal volume was adjusted to achieve normocarbia before baseline measurements and maintained at that volume for the remainder of the experiment. Electrocardiogram electrodes and a rectal temperature probe were placed. Temperature was monitored throughout the procedure and normothermia was maintained through the use of a bed warmer incorporated into the operating table and through adjustment of the room temperature as needed. An 8-French introducer sheath (Baxter Healthcare, Irvine, CA) was placed percutaneously into the right external jugular vein through which a VIP thermodilution pulmonary artery catheter (Baxter) was placed. Bilateral lower extremity cutdowns were made to expose the femoral vessels, and bilateral femoral arteries and a single femoral vein were cannulated with segments of intravenous extension tubing (Medex-Furon Co., Hillard, OH). Systemic arterial pressure was continuously monitored through one arterial line, whereas hemorrhage was performed via the second arterial line. Resuscitation fluids were administered into the femoral vein. Finally, a suprapubic bladder catheter was placed using an open technique. All incisions were closed with skin staples and all tubing and catheters were secured in place. After completion of the instrumentation phase, animals were allowed to equilibrate for 15 minutes. Baseline measures of heart rate, systemic and pulmonary arterial pressures, core temperature, and cardiac output (Marquette model 7010, GE/Marquette, Milwaukee, WI) were taken and repeated at intervals throughout the experimental protocol.

After baseline measures, subjects were rapidly hemorrhaged from the femoral artery until the MAP reached 30 mm Hg. Hemorrhage was continued as needed to maintain the MAP at 30 $\pm$ 2 mm Hg for 45 minutes. No intervention was made if the MAP decreased below 30 mm Hg during the shock period. Shed blood was collected in blood bags and net weight was used to estimate volume of hemorrhage. At the conclusion of the 45-minute shock period, animals were randomized to receive either one of six resuscitation fluids or no resuscitation (control group). The following resuscitation fluids were used: lactated Ringer's (LR) solution, HBOC-201, hypertonic saline 7.5% (HTS), hypertonic saline 7.5%/Dextran-70 6% (HSD), hetastarch 6% in lactated electrolyte solution (Hextend, Abbott Laboratories, Abbott Park, IL), and pentastarch 6% in buffered electrolyte solution (PentaLyte, Biotime Inc., Berkely, CA). The HTS and HSD solutions were prepared in our laboratory from stock solution of 30% NaCl. The resuscitation fluids were given by gravity infusion into the femoral vein until the MAP increased to 60 mm Hg. Fluid administration was continued over a 4-hour period to

maintain the MAP at 60 ± 2 mm Hg; however, no intervention was made if the MAP exceeded 60 mm Hg.

The model was chosen for familiarity and simplicity to maximize our ability to consistently reproduce an injury in a large number of animals and to validate previous findings. Two control groups, isotonic crystalloid (LR) and no resuscitation, were included. The LR group was included to provide a familiar reference, and the no-resuscitation group was included to establish and quantify the severity of the injury with this model.

Blood Samples

Blood was sampled serially during the experiment at the following time points: at baseline; at initiation of the 45-minute shock period (commenced at initial decrease of MAP to 30 mm Hg); at 30 minutes of shock; at 45 minutes (completion) of shock; and at 30, 60, 90, 120, 180, and 240 minutes of resuscitation. Samples were drawn from the femoral artery and pulmonary artery for blood gas analysis and lactate measurement. Femoral arterial samples were also collected and placed into standard blood tubes for complete blood counts, serum chemistry determination, and measurement of coagulation times and fibrinogen levels. Blood gas analysis was performed on an ABL 725 (Radiometer, Copenhagen, Denmark). Complete blood counts were performed on a Baker 9120 (BioChem Immunosystems, Allentown, PA). Serum chemistry measures were made on a Roche modular system P (Hitachi, San Jose, CA), and coagulation times and fibrinogen levels were measured on an STA (American Bioproducts, Parsippany, NJ).

Pulmonary Artery Catheter/Cardiac Output Measurements

Pulmonary arterial pressures and core body temperature were measured continuously throughout the experiment. Pulmonary artery wedge pressure and cardiac output were measured at the following time points: at baseline; at initiation of the 45-minute shock period (commenced at initial decrease of MAP to 30 mm Hg); at 30 minutes of shock; at 45 minutes (completion) of shock; and at 30, 60, 90, 120, 180, and 240 minutes of resuscitation. Cardiac output and pulmonary artery wedge pressure were measured at end-expiration. Cardiac output was measured using the thermodilution method and calculated as the mean of three serial injections. Analysis and calculations were made using a Marquette 7010 monitor (GE/Marquette).

Oxygen Consumption

Indirect calorimetry was included to use oxygen consumption as a global physiologic measure and provide insight into the effectiveness of HBOC-201 to deliver oxygen at the tissue level. Oxygen consumption was continuously measured using a Vmax 29n (SensorMedics, Yorba Linda, CA) metabolic cart aligned in series with the ventilator circuit.

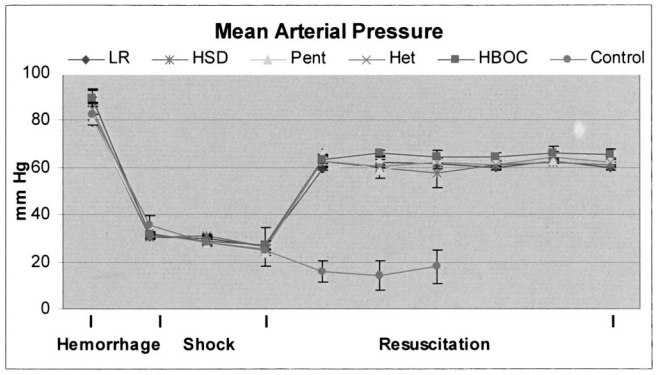

Fig. 1. *Mean MAP ± SE (mm Hg). *p < 0.05, ANOVA. Hemorrhage, shock, and resuscitation phases are shown. The HTS group did not achieve restoration of MAP to the goal of 60 mm Hg. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

Statistical Analysis

Data are reported as mean ± SE for each group. Statistical analysis was performed using SPSS for Windows version 11.0 (SPSS, Inc., Chicago, IL). Comparisons included repeated measures and one-way analysis of variance (ANOVA) followed by post hoc analysis (Tukey's test) where appropriate. Significance was defined as $p < 0.05$.

RESULTS

Survival

Five of six animals in the control group died before completion of the 4-hour resuscitation period. All animals in the no-resuscitation group remained hypotensive and progressed to cardiovascular collapse. Oxygen consumption remained markedly depressed, and severe lactic acidosis developed. Median survival was less than 60 minutes. The one animal that survived the 4-hour observation period was profoundly hypotensive and bradycardic throughout the observation period.

Six of six animals in the HTS group and one of six animals in the HSD group died before completion. Administration of HTS failed to restore MAP to 60 mm Hg at any time during the resuscitation period (Fig. 1). HSD was effective in restoring MAP; however, one animal rapidly decompensated after initial elevation of MAP to 60 mm Hg and was unresponsive to further fluid administration. All animals in the LR, HBOC-201, hetastarch, and pentastarch groups survived to completion. These results are summarized in Table 1. Because of poor survival in both the HTS and the control groups, statistical comparisons of hemodynamics, metabolic markers, and oxygenation included only the LR, HBOC-201, hetastarch, pentastarch, and HSD groups. The control group data are included in graphic representations to provide a reference for comparison.

Table 1 Survival Table: Six Animals in Each Group

Group	Survival (%)
Lactated Ringer's	100
No resuscitation	17*
HTS 7.5%	0*
HSD	83
HBOC-201	100
Hetastarch	100
Pentastarch	100

N = 6 for all groups.
* $p < 0.05$ χ^2

Fluid Volumes

All groups were equivalent with regard to weight, hemorrhage volume, and hemorrhage volume per kilogram. There were significant differences between groups with regard to volume of resuscitation and urine output (Fig. 2). Resuscitation volume was largest in the LR group (7,283 ± 633 mL) and lowest in the HBOC-201 group (486 ± 51 mL). The remaining groups required over 1.5 L (HSD, 1,508 ± 159 mL; hetastarch, 1,692 ± 203 mL; pentastarch, 1,695 ± 200 mL). Also of note, animals receiving HBOC-201 achieved an MAP of 60 mm Hg with a small initial bolus and little if any further product administration. In all other groups, there were continued fluid requirements throughout the resuscitation period to maintain MAP. Urinary output was significantly reduced in the HBOC-201 group (HBOC-201, 105 ± 13 mL; LR, 1,096 ± 466 mL; HSD, 1,491 ± 355 mL; hetastarch, 399 ± 61 mL; and pentastarch, 757 ± 218 mL).

Hemodynamics

All groups were equivalent at baseline and showed a similar hemodynamic response to hemorrhage. There were no differences between the LR, HSD, hetastarch, pentastarch, and HBOC-201 groups with regard to heart rate or MAP during the resuscitation phase. Each of these groups showed an increase in cardiac output (CO) in response to resuscitation; however, animals in the HBOC-201 group continued to have markedly depressed CO, whereas in all other groups CO returned to or exceeded baseline levels (Fig. 3). There was a trend toward decreased pulmonary artery wedge pressure and increased mean pulmonary artery pressures in the HBOC-201 group, but these differences did not reach statistical significance (Figs. 4 and 5).

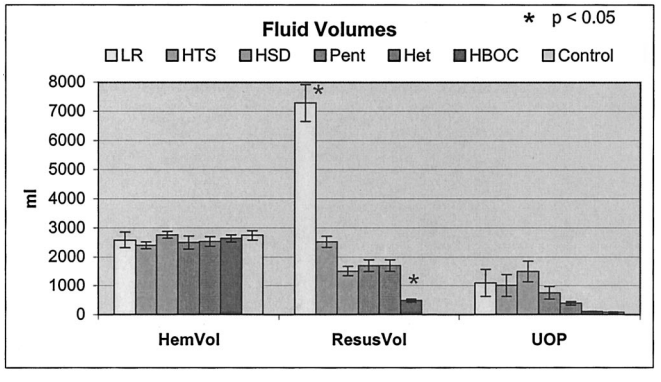

Fig. 2. *Mean fluid volumes ± SE (mL). *p < 0.05, ANOVA. Volume of blood hemorrhaged, volume of resuscitation fluid administered, and urine output volumes are shown. Hemorrhage volumes are equal. Resuscitation volume is significantly greater in the LR group and significantly lower in the HBOC group. LR, lactated Ringer's; HTS, hypertonic saline 7.5%; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

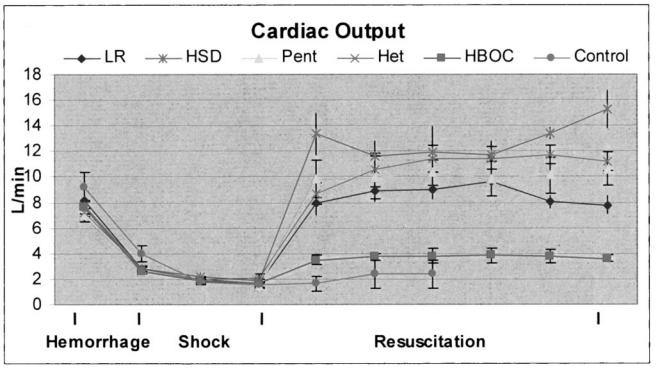

Fig. 3. *Mean cardiac output (CO) ± SE (L/min). *p < 0.05, ANOVA. Hemorrhage, shock, and resuscitation phases are shown. CO levels return to near baseline levels in all resuscitation groups except the HBOC group, where CO levels are significantly lower. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

Metabolic Markers

All groups showed a marked metabolic response to shock. Lactic acidosis developed equally in all groups during

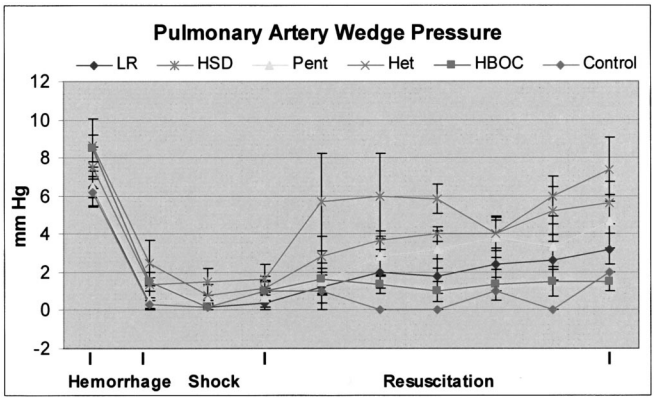

Fig. 4. *Mean pulmonary artery wedge pressure (PAWP) ± SE (mm Hg). Hemorrhage, shock, and resuscitation phases are shown. PAWP increases during resuscitation but does not return to baseline in any group. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

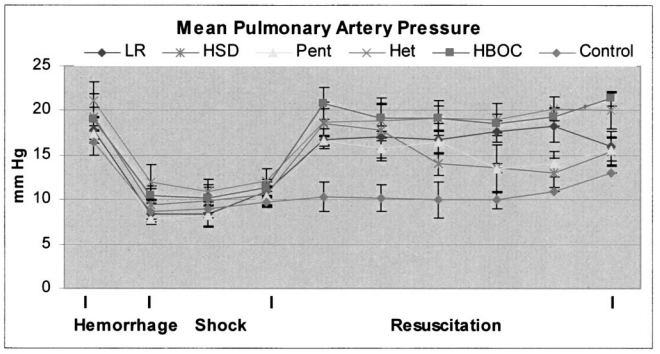

Fig. 5. *Mean pulmonary artery mean pressure (PAM) ± SE (mm Hg). Hemorrhage, shock, and resuscitation phases are shown. PAM is restored to baseline during resuscitation. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

the shock phase. In the LR, HSD, hetastarch, pentastarch, and HBOC-201 groups, lactate levels peaked 30 minutes into resuscitation and gradually decreased during resuscitation (Fig. 6). Lactate levels were equivalent among these groups during the resuscitation period. Base excess (BE) levels mirrored lactate levels during both shock and resuscitation (Fig. 7). All groups were equivalent at baseline and during shock. During resuscitation, BE levels in the HSD group were significantly lower than those in the hetastarch, pentastarch, and HBOC-201 groups. Otherwise, there were no differences in BE levels between the LR, hetastarch, pentastarch, and HBOC-201 groups.

Oxygen Consumption

All groups showed marked reduction in mixed venous oxygen saturation (Svo_2) and oxygen consumption (VO_2) in response to hemorrhage. Svo_2 levels were equivalent at baseline and throughout hemorrhage in all groups. LR, HSD,

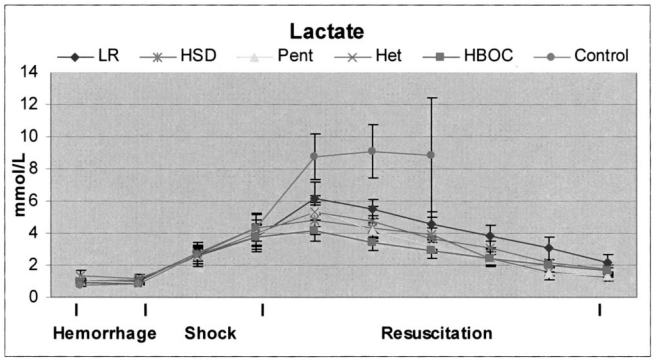

Fig. 6. *Mean serum lactate ± SE (mmol/L). Hemorrhage, shock, and resuscitation phases are shown. Lactate levels peak early in the resuscitation phase and return to baseline at the completion of the resuscitation phase in all resuscitation groups. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

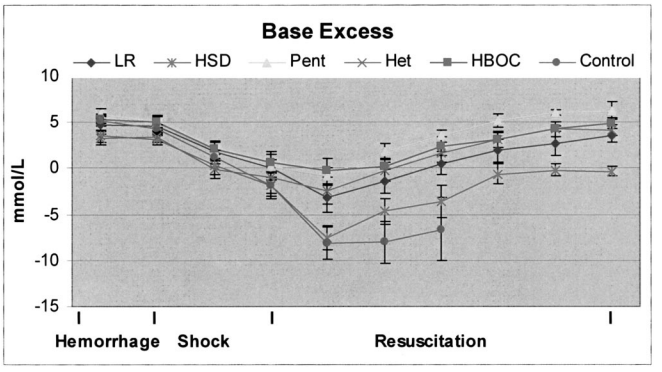

Fig. 7. *Mean base deficit ± SE (mmol/L). Hemorrhage, shock, and resuscitation phases are shown. Base deficit levels peak early in the resuscitation phase and return to baseline at the completion of the resuscitation phase in all resuscitation groups. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

hetastarch, pentastarch, and HBOC-201 groups all showed significant increases in Svo_2 after initial resuscitation, but Svo_2 levels remained significantly reduced in all these groups (Fig. 8). This effect was most pronounced in the HBOC-201 group, whose Svo_2 levels were significantly lower than in any other group throughout the resuscitation period. VO_2 levels were equivalent in all groups at baseline and throughout hemorrhage. VO_2 levels were equivalent in the LR, HSD, hetastarch, pentastarch, and HBOC-201 groups during resuscitation (Fig. 9). In these groups, VO_2 levels returned to baseline in response to resuscitation.

DISCUSSION

In this experiment, we expand on previous work studying the use of HBOC-201 in resuscitation for hemorrhagic shock.

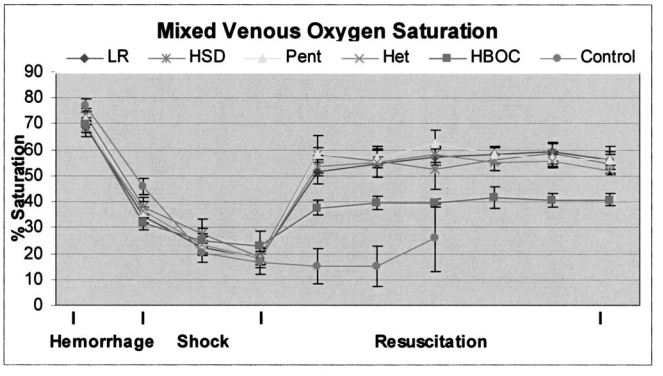

Fig. 8. *Mean mixed venous oxygen saturation (Svo_2) ± SE (%). *p < 0.05, ANOVA. Hemorrhage, shock, and resuscitation phases are shown. Svo_2 levels increase with resuscitation but do not return to baseline in any group. HBOC resuscitation results in a significantly smaller increase in Svo_2. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

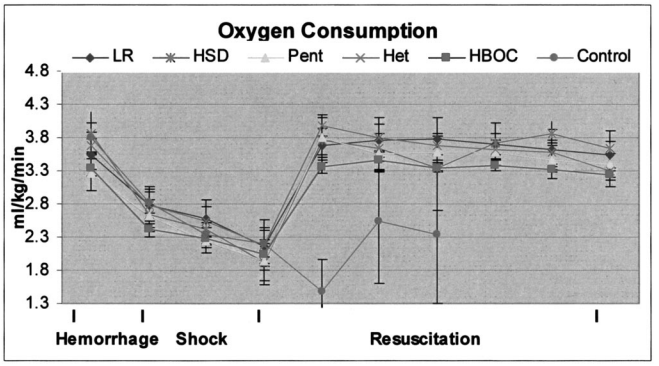

Fig. 9. *Mean oxygen consumption ± SE (mL/kg/min). Hemorrhage, shock, and resuscitation phases are shown. Baseline oxygen consumption is restored in all resuscitation groups. LR, lactated Ringer's; HSD, hypertonic saline 7.5%/Dextran-70 6%; Pent, pentastarch 6%; Het, hetastarch 6%; HBOC, HBOC-201; Control, no resuscitation.*

McNeil et al.[18] showed that hypotensive resuscitation with HBOC-201 was effective at reversing anaerobic metabolism. Resuscitation with HBOC-201 to an MAP of 60 mm Hg restored baseline levels of lactate and base deficit at significantly lower volumes than standard resuscitation fluid. Subsequent experiments in a survival model have shown no clinically significant end-organ effects of resuscitation with HBOC-201 despite the relative hypovolemia and low urine output during the resuscitative period in these animals (G. B. York et al., unpublished data).

HBOC-201 would be an ideal primary resuscitative fluid for military use. It is free from many of the logistical constraints associated with blood product usage and could be readily available in the far-forward tactical environment. Current military protocols for field resuscitation of combat casualties calls for an initial bolus of a low-volume resuscitative agent and rapid evacuation to a higher echelon of care. This study was designed to compare HBOC-201 to other low-volume resuscitative fluids in a well-established large-animal model of controlled hemorrhagic shock. The idea behind the study was to determine the one best fluid to use in forward-deployed military operations where logistic constraints limit the amount and variety of fluids available. To our knowledge, this is the first experiment to directly compare HBOC-201 to a variety of low-volume resuscitative agents in hemorrhagic shock.

This model produced a severe injury that was almost uniformly fatal if left untreated. Resuscitation with HTS 7.5% alone proved to be ineffective and subsequently lethal in this model. Although administration of HTS 7.5% resulted in an initial increase in MAP, continued administration was not effective in restoring MAP to the 60-mm Hg threshold. Animals in the HTS 7.5% group received an average volume of 2,517 mL during resuscitation. This ultimately led to a severe hypernatremia and a hyperchloremic acidosis with resulting severe base deficit and death of the animal. It is important to realize that this is not the usual approach to hypertonic solutions in resuscitation protocols. Usually, an initial small bolus (250 mL, or 4 mL/kg) of HTS 7.5% followed by isotonic fluid administration as needed is all that is required to initially stabilize blood pressure and improve acid base equilibrium.[19–21] This regimen has been tested in clinical studies that have shown a trend toward increased survival using these agents during initial resuscitation; however, no statistically significant effect was proven.[21] In this model, we were studying the effects of the various fluids using a single therapeutic agent paradigm most applicable to the tactical combat casualty care setting. The fact that HTS 7.5% was ineffective in restoring MAP to the modest level required in this model was unexpected. However, previous studies have shown the volume expansion effects of HTS 7.5% to be transient over the 30-minute period postinfusion.[22,23] This may help partially explain the poor response to HTS 7.5% seen in this model. Future experiments will use traditional regimens for HTS 7.5% resuscitation. This will more accurately allow us to make comparisons between HTS 7.5% and HBOC-201 with regard to resuscitation volumes required.

In the remaining groups, LR, HBOC-201, HSD, pentastarch, and hetastarch, hypotensive resuscitation was effective. Mean arterial pressure of 60 mm Hg was rapidly achieved; oxygen consumption was restored to baseline levels, and acidosis resolved. It is interesting to note that although all the animals in the HTS group did not survive resuscitation, five of six animals in the HSD group survived with reversal of anaerobic metabolism. In this group, the animals responded with an increase in MAP to 60 mm Hg with initial small volumes, with total resuscitation volumes for the 4-hour period of 1,508 ± 159 mL. Although hypernatremia did develop in these animals, severe hyperchloremic acidosis did not develop and the animals maintained base deficits within the normal range. The reason for the difference in survival between the HTS 7.5% and HSD groups is not entirely clear, although this finding has been described in other experiments. The addition of dextran to HTS adds a colloid component to the fluid that helps maintain plasma volume and may help prolong the hemodynamic effects seen.[23–25] In a swine model of hemorrhagic shock different from the one used here, animals resuscitated with HSD showed 96-hour survival rates of 100% compared with only 53% for HTS 7.5%.[26] The results of this study seem to support the superiority of HSD over HTS 7.5%; however, caution should be exercised when drawing conclusions from this study because these agents were not used in a conventional fashion in this protocol.

There were significant differences in the resuscitation volume required. HBOC-201 was effective at the smallest volume (< 2 units of HBOC-201), and of particular interest was the fact that HBOC-201 produced an increase in MAP that was maintained throughout the study period, with little or no further fluid requirement. In all other groups, after the initial restoration of MAP, there were continued fluid requirements throughout the study period to maintain MAP. The

ability of HBOC-201 to sustain increases in MAP over prolonged periods could be important in situations with prolonged prehospital times involving multiple casualties and limited numbers of personnel to care for them. If, after initial hemorrhage control, persistent (several hours) hemodynamic stability could be obtained with a single infusion of HBOC-201 without continued titration requirement, overall efficiency of field resuscitation could be dramatically improved.

Although HBOC-201 did restore MAP at the lowest volume, this agent did not restore cardiac output to baseline levels. This is consistent with results previously published with this model.[18] By comparison, the LR, hetastarch, pentastarch, and HSD groups showed return to baseline levels of cardiac output during resuscitation. The lack of improvement of CO after resuscitation with HBOC-201 most likely reflects relative hypovolemia in these animals given the low volumes of resuscitation fluid required and subsequent decreased ventricular filling pressures. Urine output was significantly lower in the HBOC-201 group and, although these differences did not reach statistical significance, pulmonary artery wedge pressures were lowest in the animals resuscitated with HBOC-201. The effect of HBOC-201 on CO has been evaluated in other studies. Models of hemorrhagic shock using a set volume of HBOC-201 resuscitation have shown restoration of CO to baseline values after HBOC-201 infusion.[27] Experiments using isovolemic hemodilution with HBOC-201 have shown mixed results,[12,28] with one study showing no effect on CO and the other showing decreases in CO. In our experiments, the increase in MAP at low volumes can be explained by the fact that HBOC-201 administration acts to directly augment vascular tone via scavenging of nitric oxide.[29,30] There is concern that this property may make these agents harmful in the setting of resuscitation from hemorrhagic shock. The effect of HBOC-201 on pulmonary artery pressures is a further concern. In this experiment, we saw a restoration of baseline mean pulmonary artery pressures in all resuscitation groups, with no statistically significant differences between groups. This finding is consistent with another animal model where pulmonary artery pressure was restored to baseline[31] and a human trial where pulmonary vascular resistance was not significantly affected by HBOC-201 administration.[32] However, in this model, the vasoactive properties combined with the oxygen-carrying capacity of the product appear to contribute to the superiority of HBOC-201 in reducing the resuscitation volumes necessary to reverse anaerobic metabolism. Any potential deleterious effects of the vasoconstrictive property of HBOC-201 were not realized in this model.

The reversal of anaerobic metabolism and restoration of baseline oxygen consumption despite inadequate volume replacement can be attributed to the oxygen-carrying and off-loading properties of HBOC-201. HBOC-201 has a P_{50} of 38 mm Hg compared with 26.5 mm Hg for human red blood cells. Therefore, HBOC-201 has a lower affinity for oxygen that favors release at the tissue level. Previous work in this same model has shown that oxygen delivery is consistently decreased during resuscitation with HBOC-201 compared with standard resuscitation protocols using LR plus shed blood (unpublished observations). The presence of HBOC-201 in the plasma increases the arterial oxygen content of the blood, whereas the lower CO seen in our animals offsets this increase, resulting in lower delivery. However, despite lower oxygen delivery, our findings show normalization of oxygen consumption after resuscitation with HBOC-201 with decreases in serum lactate measurements. The most likely explanation for these findings is enhanced off-loading of oxygen at the tissue level resulting in higher oxygen extraction ratios for the animals resuscitated with HBOC-201. This observation is supported by data showing HBOC-201 increases oxygen extraction in low-flow states[33] and tissue oxygen tension in resuscitation from hemorrhage.[31] In addition, these results mimic previous studies showing maintenance of oxygen consumption despite decreased oxygen delivery in animals hemodiluted with HBOC-201.[28] Increases in oxygen extraction also help explain the persistent significant decrease in Sv_{O_2} levels in animals resuscitated with HBOC-201.

The effectiveness of HBOC-201 was not evident by some traditional clinical markers. Cardiac output, Sv_{O_2}, and urine output levels all suggested inadequacy of resuscitation; however, laboratory markers of cellular ischemia and global oxygen consumption suggest satisfactory cellular oxygenation. Therefore, future use of HBOC-201 as a primary resuscitative agent will require a reanalysis of what constitutes clinically sufficient resuscitation from hemorrhagic shock. Traditional clinical markers will not satisfactorily reflect the adequacy of resuscitation with HBOC-201 and will lead to higher than necessary volumes of replacement. Therefore, although more emphasis will need to be placed on laboratory values and newer technologies allowing direct measurements of tissue oxygenation in the hospital, the field medic will need to continue to judge adequacy of perfusion by mentation and vital signs.

In this experiment, HBOC-201 as a primary resuscitative agent reversed markers of anaerobic metabolism at significantly smaller volumes when compared with other low-volume resuscitative fluids. In addition, one bolus infusion of HBOC-201 was all that was necessary to adequately resuscitate these animals after hemorrhage control. This finding, in addition to the known logistical advantages of universal compatibility, stability at extremes of temperature, and long-shelf life, show HBOC-201 to have several advantages over traditional resuscitation fluids. The ideal paradigm for treatment of combat casualties in the tactical field military setting would involve the use of one fluid for resuscitation purposes. Protocols using combinations of agents, such as a low-volume hypertonic and/or hyperoncotic fluid followed by isotonic fluids, are more complex and more difficult to administer in situations where there may be several casualties needing care and many distractions (e.g., enemy fire). The

ability to administer one agent, potentially as a bolus injection after hemorrhage control, which reliably restores adequate perfusion at low volume would greatly simplify this task and decrease demands on the combat medic.

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