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BOVINE POLYMERIZED HEMOGLOBIN VERSUS HEXTEND RESUSCITATION IN A SWINE MODEL OF SEVERE CONTROLLED HEMORRHAGIC SHOCK WITH DELAY TO DEFINITIVE CARE

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ABSTRACT—To compare the efficacy of low-volume resuscitation with bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in an intermediate severity combat-relevant hemorrhagic shock swine model with a simulated delay to hospital care. Twenty-four anesthetized pigs were hemorrhaged 55% estimated blood volume in conjunction with a 5-min rectus abdominus crush. At 20 min, pigs were resuscitated with 10 mL/kg of HBOC-201 or HEX or nothing (NON); resuscitated pigs received additional infusions (5 mL/kg) at 30, 60, 120, or 180 min if hypotension or tachycardia persisted. Pigs were monitored for a 4-h "prehospital" period. At 4-h, hospital arrival was simulated: surgical sites were repaired, blood, or saline provided, and pigs were recovered from anesthesia. Pigs were monitored for 72 h and then killed for histological evaluation. One hundred percent (8/8) of HBOC-201–, 75% (6/8) of HEX-, and 25% (2/8) of NON-resuscitated pigs survived to 72 h ($P = 0.007$ overall, HBOC vs. HEX $P > 0.05$). Mean arterial pressure and mean pulmonary arterial pressure were highest in the HBOC-201 group ($P < 0.001$), and HR was lowest ($P < 0.001$). HBOC-201– and HEX-resuscitated pigs had comparable cardiac index and prehospital fluid requirements. HBOC-201 pigs had higher transcutaneous tissue oxygen tension, $P < 0.001$) and lower urine output ($P < 0.001$). At simulated hospital arrival, no HBOC-201 pigs required additional fluids or blood transfusion. In contrast, 100% of HEX pigs required blood transfusions ($P < 0.01$). In this swine model of controlled hemorrhage with low-volume resuscitation and delayed definitive care, HBOC-201 pigs had improved hemodynamics, transcutaneous tissue oxygen tension, and transfusion avoidance compared with HEX.

KEYWORDS—Hemorrhage, HBOC, resuscitation, shock, trauma, pig

INTRODUCTION

In military conflict as well as urban settings, transport times can be prolonged and arrival at definitive care delayed. This can be particularly pronounced on the battlefield where prehospital transport times are often longer than in urban trauma. The current standard of care for prehospital resuscitation is infusion with asanguinous fluids (i.e., crystalloids or colloids) in attempts to stabilize blood pressure and to restore circulating volume. However, this approach is suboptimal because although they replenish intravascular volume, asanguinous fluids lack oxygen carrying capability and can leave patients with significant lactic acidosis and base deficit abnormalities (1, 2).

Hemoglobin-based oxygen carriers (HBOC) are chemically modified hemoglobin solutions that can restore blood volume and carry oxygen, properties vital to salvage casualties of hemorrhagic shock (HS). Bovine polymerized hemoglobin HBOC-201 (Hemopure, Biopure Corporation, Cambridge, Mass) is an ultra-purified, bovine polymerized hemoglobin that is stable at room temperature, has a long shelf-life, does not require typing, is easily administered, and ultra-purified, leading to a low risk for transmission of communicable pathogens. HBOC-201 has been shown to be an efficacious low-volume resuscitative fluid that is superior, in some cases, to standard asanguinous fluids (3–6). In addition, HBOC-201 may decrease transfusion requirements at hospital arrival (5–8).

We recently presented data from a 40% controlled hemorrhage model and a severe uncontrolled hemorrhage model *via* liver crush/laceration (5, 6). The current model is intermediate in severity compared with the 2 aforementioned studies. In both previous models, hemodynamics and tissue oxygenation were improved, and blood transfusion requirements decreased with HBOC-201. We also demonstrated a significant survival advantage after HBOC-201 resuscitation in the severe uncontrolled hemorrhage in comparison with colloid resuscitation (5). The present study was designed to determine if the benefits of HBOC-201 observed in the less severe model of controlled HS would translate in a more severe model of controlled HS (55% EBV), incorporating tissue injury and a 4-h simulated delay to definitive care.

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MATERIALS AND METHODS

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals,'' Institute of Laboratory Animals Resources, National Research Council, National Academy Press (1996). The study was approved by the Naval Medical Research Center/Walter Reed Army Institute of Research Institutional Animal Care and Use Committee, and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International.

Animal preparation

Animals were prepared as previously reported (6). Briefly, 24 male and female Yucatan mini pigs (~40 kg) (Sinclair Research Center, Inc, Columbia, Mo) were fed and allowed access to water *ad libidum* until 12 h before experimentation. Animals were sedated and induced to a surgical plane of anesthesia with intramuscular ketamine hydrochloride (33 mg/kg) and atropine sulfate (0.05 mg/kg), followed by mask ventilation with isoflurane (3.0%) with FiO_2 1.0 to facilitate endotracheal intubation. Anesthesia was maintained *via* isoflurane (1%–2.5%), FiO_2 0.21, and ventilatory support for apnea (Ohmeda 7800 series ventilator; Datex, Madison, Wis) at 15 to 25 breaths/min and tidal volume 5 to 10 mL/kg. Rectal temperature was monitored and body heat maintained using a Bair hugger device (Model 505, Bair Hugger, Minn). Urine output was measured *via* bladder catheterization. The external jugular vein and carotid artery were catheterized by open technique for vascular access. A 9-F introducer sheath was placed in the external jugular vein using Seldinger technique and a 7.5-F pulmonary artery catheter (Edwards Life Sciences, Irvine, Calif) was inserted for continuous hemodynamic and cardiac output (CO) monitoring. An 18-Ga angiocatheter was placed in the carotid artery, and mean arterial pressure (MAP) and heart rate (HR) continuously transduced. A 3- to 5-cm lower abdominal incision was made, and the left rectus abdominus muscle located. The rectus sheath was bluntly mobilized, and a surgical tissue clamp Kocher clamp placed on a standardized portion of the muscle in the center of the incision. All surgical procedures were performed using aseptic techniques.

Simulated prehospital phase: tissue injury, hemorrhage, and resuscitation

Estimated blood volume (EBV) was calculated by weight (kg) × 65 mL/kg. The Kocher clamp was closed for 5 min to create a soft tissue injury, and pigs were hemorrhaged 55% EBV *via* the external jugular vein and/or carotid artery at a constant rate over approximately ~15 min to induce HS (Fig. 1). All "shed'' blood was collected in sterile blood bags containing citrate phosphate dextrose (CPDA-1, Fenwal, Baxter, Deerfield, Ill) for possible later reinfusion. Pigs were preassigned to 1 of 3 treatment groups: hemoglobin-based oxygen carrier (HBOC-201, Hemopure, Biopure Corp, Cambridge, MA); 6% hetastarch in lactated Ringer's solution (HEX, Hextend, Abbott Laboratories, Abbot Park, IL); or no resuscitation fluid (NON). At 20 min, resuscitated pigs were administered 10 mL/kg of HBOC-201 or HEX over 10 min. Additional infusions of 5 mL/kg were provided at 30, 60, 120, and 180 min postinjury if hypotension (MAP < 60 mmHg) or tachycardia (HR > baseline value [time 0]) were observed. Fluids were infused at room temperature.

Simulated hospital phase: recovery and long term survival

Hospital arrival was simulated at 4 h. Animals were administered 13 mg/kg cephazolin (antibiotic) and 0.01 mg/kg buprenorphine (analgesic), as well as 10 mL/kg autologous shed blood for anemia (hemoglobin (Hb) < 7 g/dL) or 10 mL/kg normal saline for hypotension (MAP < 60 mmHg). The pulmonary artery catheter was removed, jugular vein introducer secured for postoperative blood sampling and fluid administration, and arterial and bladder catheters removed. Surgical incisions were closed, and dressings were applied. Animals were extubated and recovered from anesthesia. Noninvasive monitoring and health status were assessed up to 72 h postinjury. Pigs received additional antibiotic, analgesic, and 10 mL/kg shed blood or saline as needed for anemia or hypotension. Pigs were euthanized for necropsy and histological analysis at the end of the experiment. Methods and results of the histopathologic data have been previously published (9).

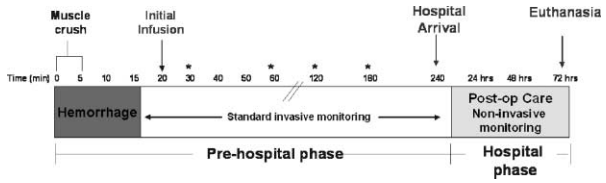

FIG. 1. **Experimental design.** The muscle crushed, and start of the hemorrhage denoted the beginning of the experiment (time 0). Fluid resuscitation was initiated at 20 min (10 mL/kg over 10 min) and additional infusions (*) (5 mL/kg over 10 min) were provided for MAP less than 60 mmHg or heart rate (HR) more than baseline. Prehospital care was simulated between 15 and 240 min; then hospital arrival was simulated; surgical sites were repaired whole blood or normal saline infused if required for anemia (Hb <7 g/dL) or hypotension (MAP <60 mmHg); and animals recovered from anesthesia. Animals were euthanized at 72 h.

Data collection

Standard invasive and noninvasive hemodynamic parameters were monitored for 240 min during the simulated prehospital phase (Fig. 1). Transcutaneous tissue oxygenation ($tcpO_2$) was noninvasively measured with a TCM4 Tina monitor (Radiometer, Copenhagen, Denmark) using (4) Clark type polarographic electrodes (data represent mean values) positioned bilaterally on the upper torso and on the inner thighs. Arterial and mixed venous blood gases were measured with an automatic analyzer (ABL 705, Radiometer, Copenhagen, Denmark). Blood samples were collected for complete blood counts (Pentra 60 C±, ABX, Aisniers, France) and serum chemistries (Vitros 250 Analyzer, Ortho). Blood tests results are reported only if plasma Hb (B-Hb method, Hemocue, Angelholm, Sweden) was below interference levels. Oxygen consumption (VO_2), delivery (DO_2), and extraction ratio (O_2ER) were calculated using the following formulas:

$$VO_2 = (\ CO \times 13.4 \times \text{hemoglobin} \times [SaO_2\ S\bar{v}O_2])/100$$

$$DO_2 = (\ CO \times 13.4 \times \text{hemoglobin} \times SaO_2)/100$$

$$O_2ER = VO_2/DO_2(\times 100)$$

Cardiac index (CI) was calculated as CO divided by body surface area. Systemic vascular resistance index (SVRI) was calculated as MAP − CVP × 80/CO.

Statistical analysis

Analysis of variance and chi-square tests were used to compare continuous and categorical variables, respectively, between groups. For continuous variables, the nonparametric Kruskal-Wallis test was also used when the assumptions of the analysis of variance model were unmet. For longitudinal analyses, Cox Proportional Hazards was used for survival-related outcomes, whereas mixed statistical models were used for continuous outcome measurements. For the mixed statistical model, estimates were obtained for treatment group and time as well as their interaction. The dependence of measures taken within the same subjects over time was accounted for using the standard syntax of the PROC MIXED procedure in SAS (SAS Institute, Inc, Cary, NC). Data are expressed as mean ± SEM for animals alive at time of measurement. For analysis of data over time, unless otherwise noted, P values reported are the results of a time-by-group interaction.

RESULTS

General characteristics including sex distribution, body weight, hemorrhage volume, percent hemorrhage of EBV, and percent total blood loss (including blood drawn for laboratory analysis) were similar across groups (Table 1).

Hemorrhagic shock

The 55% EBV hemorrhage resulted in similar hemodynamic insult in all groups. Before intervention, there were no significant hemodynamic group differences. In response to hemorrhage, MAP decreased 75% (78.3 ± 3.2 to 19.5 ± 1.5 mmHg, $P < 0.001$), HR increased 32% (121.7 ± 4.7 to 161.2 ± 6.8 bpm, $P < 0.001$), CI decreased 43% (5.2 ± 0.3 to 2.9 ± 0.4 L/min per m^2, $P < 0.001$), mean pulmonary arterial pressure (MPAP) decreased 41% (15.3 ± 1.2 to 9.0 ± 2.2 mmHg, $P = 0.01$), and $tcpO_2$ decreased 81% (27.0 ± 2.5 to 5.2 ± 1.5 mmHg, $P < 0.001$) (Figs. 2 and 3, chi-squared analysis).

Simulated prehospital phase

Hemodynamic response to intervention—After resuscitation, MAP was higher in HBOC-201 compared with the HEX or NON (Fig. 2, $P < 0.001$). As briefly noted by Johnson and

TABLE 1. **Demographics and hemorrhage volume**

Group	Sex ratio (M/F)	Weight (kg)	Hemorrhage volume (mL)	% EBV	% Total blood loss (Including Laboratory Samples)
HBOC-201	4:4	43.7 ± 6.8	1560.4 ± 687.4	54.8 ± 0.2	60.3 ± 1.4
HEX	4:4	36.6 ± 13.0	1309.7 ± 616.5	55.0 ± 0.0	61.0 ± 1.2
NON	4:4	39.5 ± 6.9	1406.6 ± 694.9	54.9 ± 0.1	60.4 ± 1.9

Baseline data and demographics for HBOC-201 (HBOC), 6% HEX and NON experimental groups. Values (except sex ratio) are presented as mean ± SEM. There were no significant between groups.

associates (9), a substantial rise in MAP was observed in HBOC-201 pigs after the first infusion, was restored to baseline by 75 min, and remained elevated throughout the remainder of the prehospital phase. Although MAP was higher in HEX as compared with NON pigs, MAP did not return to baseline in either of these groups. All pigs had elevated HR throughout the prehospital phase with the NON pigs having significantly higher HR (Fig. 2, $P < 0.001$); a trend toward decreased tachycardia over time was observed with HBOC-201 versus HEX ($P = 0.05$). CI was similar in both treatment groups, returning to baseline by 150 min, although there was a trend toward increased CI with HEX versus HBOC-201 resuscitation ($P = 0.07$, group); CI remained depressed in the NON group throughout the prehospital phase (Fig. 2, $P < 0.001$). SVRI, as calculated by $[(MAP - CVP) \times 80]/CO$, increased after resuscitation with HBOC-201, was restored to baseline by 90 min and continued to rise throughout the prehospital phase, remaining higher than HEX pigs ($P = 0.06$). The overall SVRI curve was significantly different between groups ($P < 0.05$, group). MPAP was higher in HBOC-201 compared with HEX and NON pigs ($P < 0.001$) (Fig. 2). CVP was higher in HBOC-201 compared with HEX or NON pigs ($P < 0.05$).

Indirect and direct measures of tissue oxygenation—Transcutaneous tissue oxygenation remained below baseline in all groups during the first 60 min of the prehospital phase (Fig. 3). However, HBOC-201 pigs' $tcpO_2$ values surpassed baseline after 60 min and remained significantly higher than HEX and NON pigs ($P < 0.001$). Lactate was not different between groups (Fig. 3). Transcutaneous tissue oxygenation correlated with MAP ($r = 0.59$), but observed group differences remained significant despite adjustment for MAP ($P = 0.04$). Transcutaneous tissue oxygenation also correlated (inversely) with lactate ($r = -0.54$). Arterial oxygen saturation (O_2 sat) was lower in HBOC-201 compared with HEX pigs ($P = 0.04$). Mixed venous O_2 sat was similar in HBOC-201 and HEX pigs, initially decreasing but returning to baseline with resuscitation (Fig. 3). However, mixed venous O_2 sat did not achieve baseline in either the HBOC-201 or the HEX group. Oxygen consumption, delivery, and O_2ER were not different between groups (Fig. 4). Base excess was similar in HBOC-201 and HEX pigs and appeared higher than in NON ($P = 0.07$) (Fig. 4).

Renal function—As reported by Johnson and colleagues (9), urine output was higher in the HEX group over time ($P < 0.001$). Total urine output was highest in the HEX, intermediate in HBOC-201, and lowest in the NON group (Fig. 5). Blood urea nitrogen was higher in the NON group toward the end of the prehospital phase ($P = 0.05$); there were no

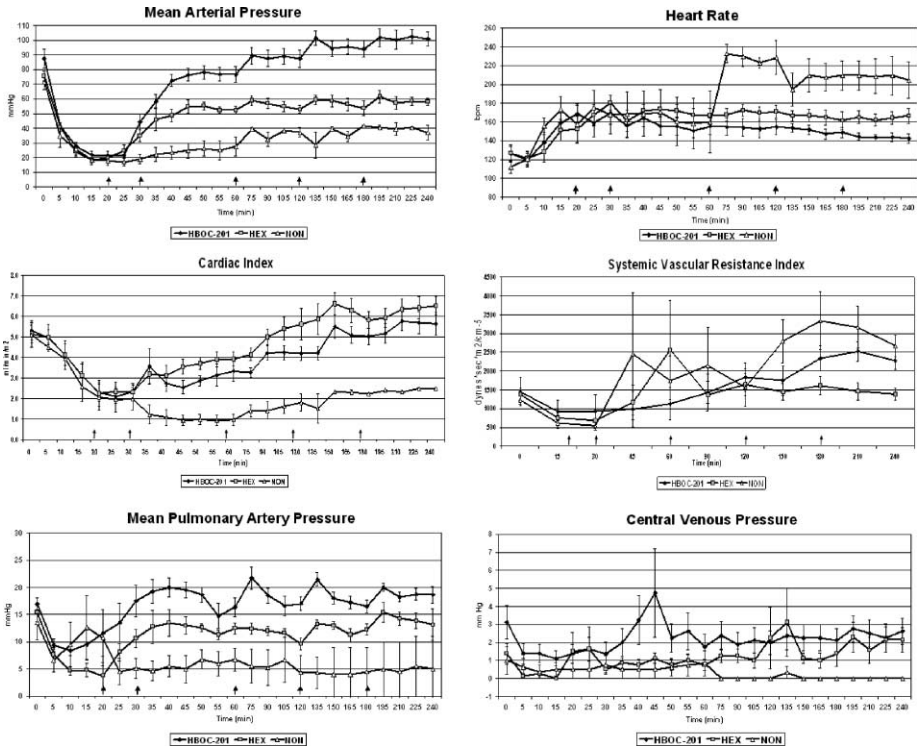

FIG. 2. **Hemodynamics changes in pigs resuscitated with HBOC-201, HEX or NON.** Values are presented as mean ± SEM. There were significant overall differences in MAP ($P < 0.001$, time-by-group), heart rate ($P < 0.001$, time-by-group), systematic vascular resistance index ($P < 0.01$, group), MPAP ($P < 0.001$, time-by-group), and central venous pressure ($P < 0.05$, time-by-group). Cardiac index was similar between HBCO-201 and HEX but higher than NON pigs ($P < 0.001$, time-by-group) and SVRI was different beetween groups ($P < 0.05$, group). Arrows indicate time of infusions. Groups are represented as HBOC-201 (—◆—), (—□—), NON (—△—).

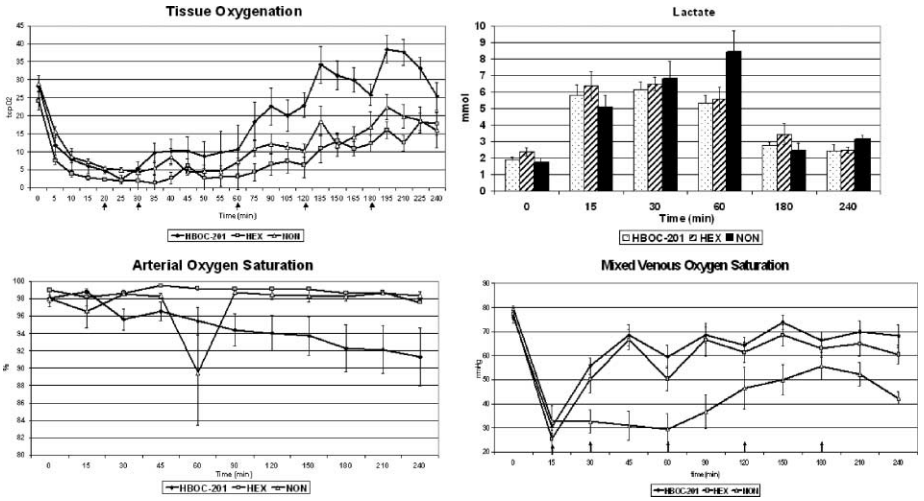

FIG. 3. **Direct and indirect measures of tissue oxygenation.** Values are presented as mean ± SEM. Transcutaneous tissue oxygenation ($tcpO_2$) was higher in HBOC-201 pigs ($P < 0.01$). Lactate was not different between groups. Arterial oxygen saturation was lower in HBOC-201 pigs ($P = 0.04$). Mixed venous oxygen saturation was not different between groups. Arrows indicate times of infusion. Groups are represented as: HBOC-201 (–◆–), HEX (–□–), NON (—△—); HBOC-201 (–□–), HEX (▨), NON (■).

differences between HBOC-201 and HEX (Fig. 5). There were no group differences in creatinine during the prehospital phase (data not shown).

Fluid requirements—As reported by Johnson and colleagues (9), total prehospital fluid requirements were similar in the HBOC-201 and HEX groups (27.5 ± 1.9 vs. 28.8 ± 0.8 mL/kg, respectively) (Fig. 6).

Hemoglobin and hematocrit—Hemoglobin was higher in HBOC-201 compared with HEX pigs during the prehospital phase ($P < 0.001$) (Fig. 7). Hematocrit was similar in HBOC-201 and HEX pigs and was lower than NON pigs ($P < 0.03$).

Hospital phase—At simulated hospital arrival, blood transfusion requirements were lower in HBOC-201 versus HEX pigs (12.5% vs. 100%, respectively, Fig. 8, $P < 0.001$) and saline requirements were lower compared with NON pigs (0% vs. 100%, respectively, $P = 0.02$). There were no differences in blood or saline requirements at 24 or 48 h. MAP was similar at 24 h but higher in HBOC-201 versus HEX pigs at 48 h ($P = 0.03$, Table 2). However, these differences were not observed at 72 h. HR was not different between groups at 24 or 72 h. At 48 h, HBOC-201 pigs were less tachycardic than HEX ($P = 0.04$) or NON ($P < 0.01$) pigs. Body temperature was not different among groups at time point. There were no differences in vomiting incidence, activity level, or feed consumption between groups (data not shown). Lactate was similar at all posthospital arrival time points across groups (Table 3). With the exception of HBOC-201 pigs having slightly higher creatinine levels compared with NON pigs at 24 h, there were no other differences in creatinine levels (Table 3). Blood urea nitrogen was higher in HBOC-201 versus HEX pigs at 24 ($P = 0.02$) and 48 ($P = 0.06$) h but not at 72 h. Hemoglobin was higher in HBOC-201 compared with HEX ($P = 0.06$) or NON ($P < 0.01$) pigs at 24 h, but not at 48 or 72 h (Table 3). Hematocrit was higher in HEX compared with

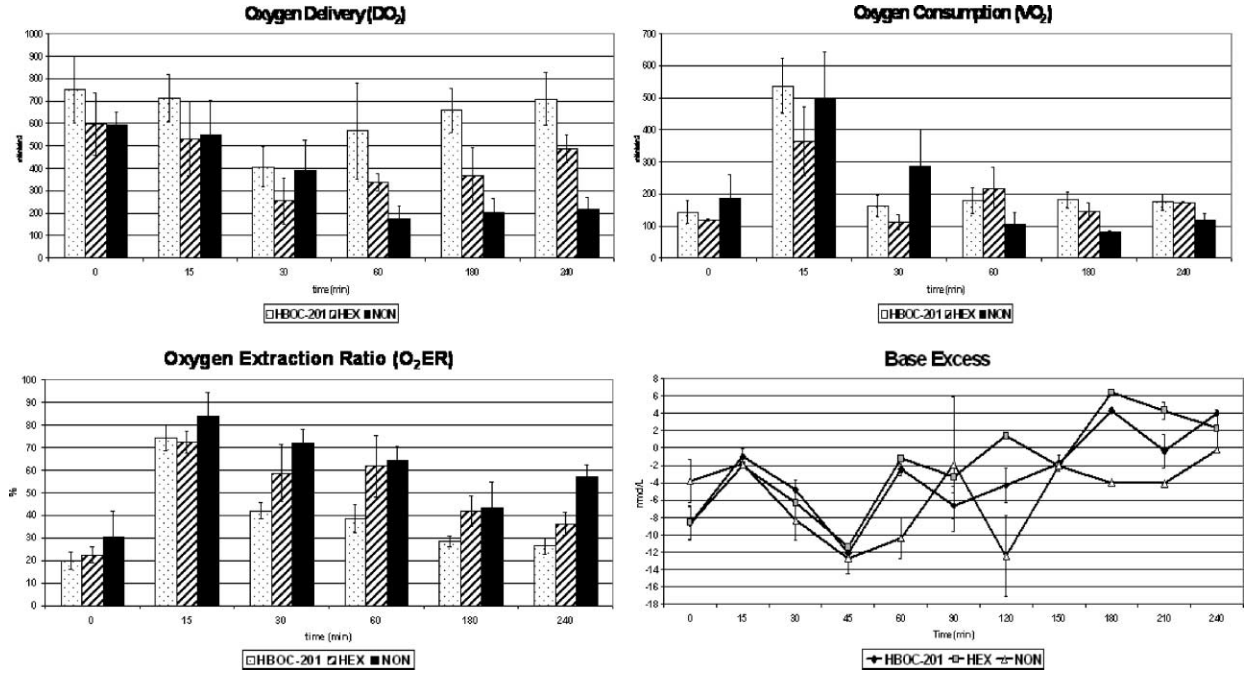

FIG. 4. **Measures of tissue oxygenation.** Values are presented as mean ± SEM. No significant differences were observed on DO_2, VO_2, O_2ER, or base excess. Arrows indicate times of infusion. Groups are represented as: HBOC-201 (–◆–) HEX (–□–), NON (—△—); HBOC-201 (□), HEX (▨), NON (■).

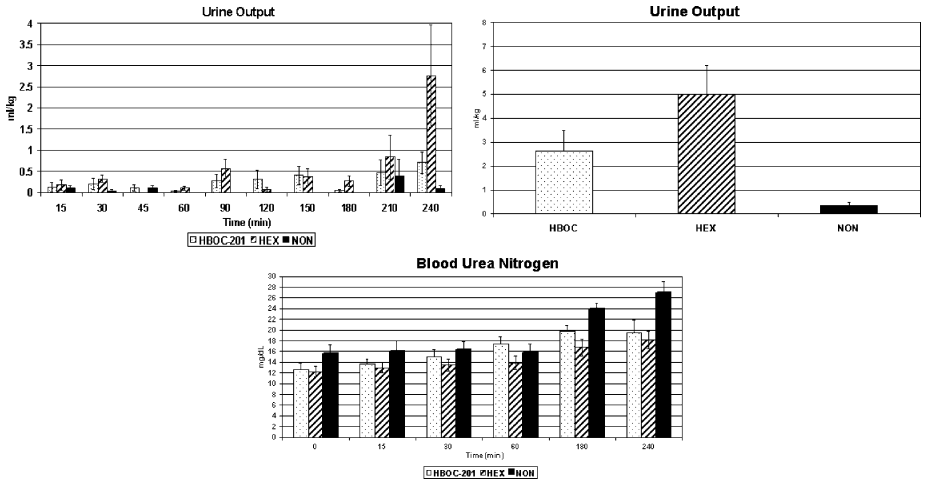

FIG. 5. **Renal function.** Values are presented as mean ± SEM. Urine Output (mL/kg) over time and total output were higher in HEX pigs ($P < 0.001$). Blood urea nitrogen was higher in NON pigs ($P = 0.05$). Groups are represented as HBOC-201 (□), HEX (▩), NON (■).

HBOC-201 ($P < 0.01$) or NON ($P = 0.05$) pigs at 24 h. At 48 h, hematocrit was significantly higher in HEX versus HBOC-201 pigs ($P < 0.01$). No differences in hematocrit were observed at 72 h.

Survival—One hundred percent (8/8) of HBOC-201–, 75% (6/8) of HEX-, and 25% (2/8) of NON-resuscitated pigs survived to 72 h (Fig. 9, overall $P = 0.007$, HBOC-201 vs. HEX $P > 0.05$). Mean survival times were 72.0 ± 0.0, 54.9 ± 11.3, and 19.0 ± 12.6 h for HBOC-201, HEX, and NON pigs, respectively (overall $P = 0.002$, HBOC-201 versus HEX $P > 0.05$).

DISCUSSION

In the present model of severe controlled hemorrhage with 55% EBV loss, low-volume resuscitation with HBOC-201 resulted in rapid restoration of MAP, HR, and CI, and improved $tcpO_2$ compared with HEX resuscitation. Furthermore, animals receiving HBOC-201 required fewer blood transfusions. These findings are consistent with studies previously reported by our laboratory and other investigators in models of less or more severe hemorrhage (3–6).

The present study represents the third model of HS reported by our group designed to evaluate HBOC-201 versus 6% hetastarch (HEX) resuscitation. The overriding goal of this series of experiments was to establish the efficacy of HBOC-201 resuscitation for the prehospital treatment of HS, incorporating a simulated delayed evacuation to definitive care with a range of hemorrhage severity. HEX was chosen as the comparator fluid because it has been identified by the US Special Operations Forces as their standard of care for resuscitation of battlefield casualties with hemorrhagic shock. Our aim was to investigate HBOC-201 in a wide range of experiments to establish the breadth and depth of HBOC-201 efficacy for a broad range of hemorrhage severities in combat relevant scenarios. We previously reported a similar controlled hemorrhage model with moderate severity (40% EBV hemorrhage) and a severe uncontrolled hemorrhage with solid organ injury (liver crush/laceration) (5, 6). The current model represents an intermediate severity hemorrhagic shock insult, as evidenced by group survival rate of 25% (2/8) in NON-resuscitated animals as compared with 62.5% (5/8) in our 40% EBV and 12.5% (1/8) in our liver injury models.

As was observed in the 40% EBV model, blood transfusion requirements were significantly reduced at hospital arrival (4 h). This is consistent with human trials in which blood transfusion requirements were less when patients were provided HBOC-201 instead of LR or blood (7, 8). In addtion, over the 3-day simulated hospital phase, only 1 HBOC-201 pig required blood transfusions as compared with all HEX animals. These data, at least in this animal model, suggest that HBOC-201 is a safe and effective resuscitative fluid for the early treatment of hemorrhagic shock, and support the contention that such a fluid may offer a significant survival benefit in the setting of civilian trauma or military field care when blood is not available.

In some previous studies, investigators reported decreased CI with HBOC-201 resuscitation (10–15). This is consistent with our 40% EBV model; however, in the present more severe model, CI was similar in HBOC-201 and HEX pigs.

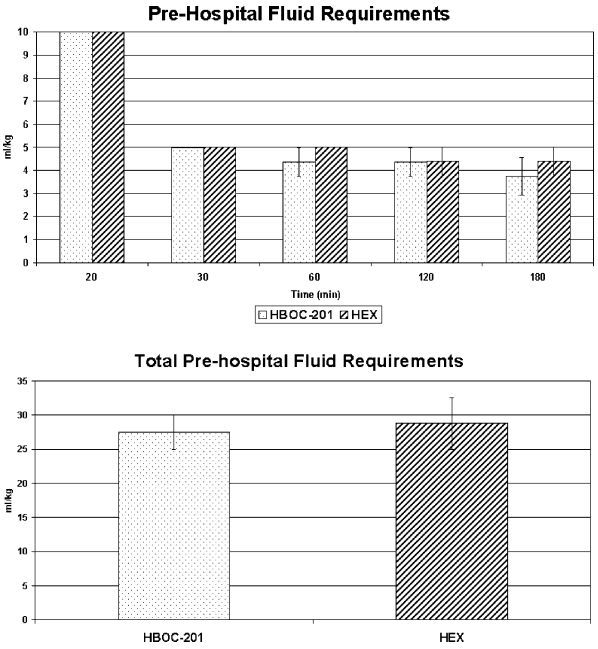

FIG. 6. **Prehospital fluid requirements.** Values are represented as mean ± SEM. There were no differences in prehospital fluid requirements. Groups are represented as HBOC-201 (□), HEX (▩), NON (■).

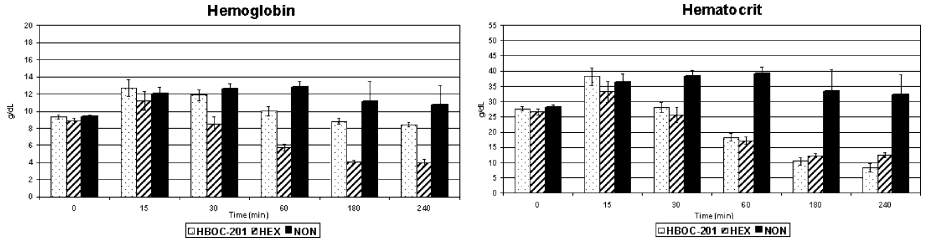

FIG. 7. **Prehospital hemoglobin and hematocrit.** Values are presented as mean ± SEM. Hemoglobin was higher over time in HBOC-201 than HEX pigs ($P < 0.001$), whereas hematocrit was similar. Groups are represented as HBOC-201 (□), HEX (▩), NON (■).

These apparently conflicting results may be due to differences in overall infusion volume. In previous studies and in our 40% EBV model, the volume of HBOC-201 provided was markedly decreased versus the comparative fluid. In the present more severe model, infusion volumes were similar in both HEX and HBOC-201 groups (Table 4). HBOC-201 and HEX pigs all met criteria for and received infusions at all infusion opportunities. In the less severe 40% EBV model, pigs received approximately ~18 mL/kg HBOC-201, whereas in the current model, pigs received almost double the volume (~30 mL/kg). Other controlled hemorrhage models with varying severity (40%–52% EBV hemorrhage) reported that pigs resuscitated with HBOC-201 required less fluids to return to and maintain baseline MAP (6, 12). Similar results have also been reported in more severe models of uncontrolled hemorrhage, including the liver injury model performed at our institution, when fluid requirements were calculated on a mL/kg per survival hour basis (3–5). In the current model, however, criteria for additional fluid infusion were based on both MAP and HR because we appreciated potential risk of underresuscitation when BP is used alone, especially with a somewhat vasoactive resuscitation fluid. Pigs in all groups were hypotensive and tachycardic following hemorrhage and received the first and second fluid infusions. While HEX pigs were reinfused primarily for hypotension and tachycardia, HBOC-201 pigs met fluid reinfusion criteria due mainly to tachycardia. The addition of HR as an infusion trigger in the present study led to similar infusion volumes of HBOC-201 and HEX, likely accounting for the similarities in CI for HBOC-201 and HEX pigs.

A trend toward improved survival was seen among HBOC-201 animals compared with HEX (100% vs. 75%, respectively); however, this survival advantage did not reach statistical significance. Although, it is estimated based on these data that if more animals were added to each group (n = 22), the survival benefit of HBOC-201 observed by other investigators in more severe models, may be observed in the current model (3–5). Metabolically, HBOC-201 and HEX pigs performed similarly in response to treatment. Indicators of global tissue oxygenation and perfusion were not significantly different, although tissue oxygenation, as measured by TCOM, was more rapidly restored and was significantly higher in HBOC-201 versus HEX pigs. This is consistent with our previous experiments of moderate severity hemorrhage model (40% EBV), in which we observed similarly that $tcpO_2$ tissue oxygenation was increased with HBOC-201 in the absence of significant differences in DO_2, O_2ER, lactic acid, or base deficit (BD) compared with HEX-resuscitated pigs. In the current model, pigs receiving HBOC-201, maintained DO_2 and had lower O_2ER throughout the experiment whereas HEX pigs had decreased DO_2 and increased O_2ER. Although these differences did not reach statistical significance, the trends do suggest a better state of systemic perfusion with HBOC-201 resuscitation. Our findings are consistent with reports by Standl and colleagues (16), who demonstrated that HBOC-201 is more potent than blood at restoring tissue oxygenation in hind limb muscle tissue in hemorrhaged dogs. This is in agreement with a rabbit hemorrhage model that reported more rapid restoration of tissue oxygenation compared with a starch colloid (17, 18). Masuno and colleagues (19) also reported higher tissue oxygenation with HBOC-201 compared with saline resuscitation. In contrast to the present findings, these studies also demonstrated improved correction of BD correlating with improved tissue oxygenation. We believe that the similar metabolic performance seen presently between HBOC-201 and HEX animals relates to the severity of injury. As with many disease processes of variable intensity, large differences in outcome parameters become more evident with increasing severity. In hemorrhaged animals, it is likely that metabolic benefits of HBOC-201 resuscitation may be directly proportional to the severity of hemorrhage and to the consequent metabolic deficit

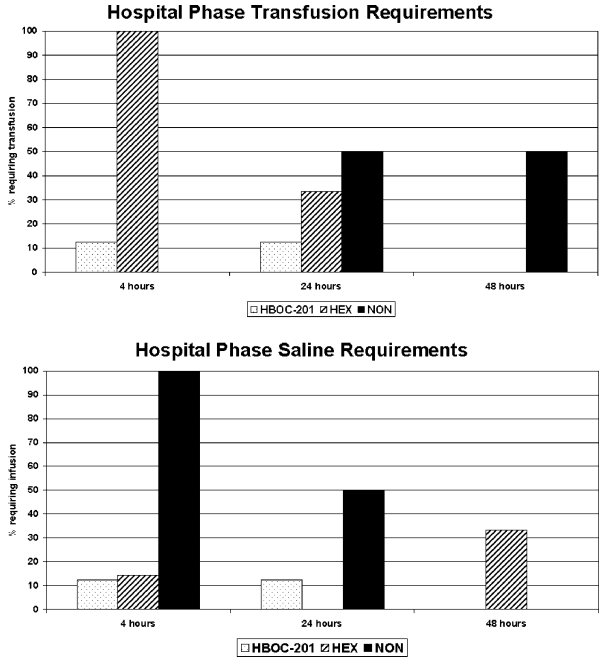

FIG. 8. **Posthospital fluid requirements.** Values are presented as mean ± SEM. Blood transfusion requirements we decreased in HBOC-201 pigs at 4 h compared with HEX pigs ($P < 0.001$). Saline infusion requirements were decreased in HBOC-201 pigs at 4 h compared with NON pigs ($P = 0.01$). There were no differences in blood or saline requirements at 24 or 48 h. Groups are represented as HBOC-201 (□), HEX (▩), NON (■).

TABLE 2. **Posthospital arrival vital signs**

Variable	Group	24 h	48 h	72 h
MAP (mmHg)	HBOC-201	88.3 + 6.8	*96.4 + 8.9	86.3 + 11.2
	HEX	76.3 + 7.9	*66.7 + 6.7	67.3 + 10.3
	NON	69.5 + 6.5	106.0 + 18.0	67.0 + 2.0
HR (bpm)	HBOC-201	91.0 + 9.3	*†109.0 + 10.8	106.8 + 3.0
	HEX	95.3 + 11.8	*133.8 + 11.7	119.5 + 16.0
	NON	103.0 + 5.0	†171.5 + 23.5	115.5 + 32.5
Temperature (°F)	HBOC-201	100.6 + 0.4	101.9 + 0.5	102.0 + 0.3
	HEX	102.2 + 0.3	102.1 + 0.5	102.7 + 0.6
	NON	101.0 + 1.8	102.1 + 0.9	102.5 + 0.5

Vital signs for HBOC-201, HEX, and NON groups during the hospital phase (24–72 h). Values are presented as mean ± SEM. *$P < 0.05$, HBOC-201 versus HEX. †$P < 0.05$, HBOC-201 versus NON.

at the time of intervention. This is supported by our study that incorporated liver injury and uncontrolled hemorrhage in which we demonstrated that HBOC-201 improved $tcpO_2$, decreased lactic acid, improved base deficit, and increased mixed venous oxygen saturation and pO_2 versus HEX resuscitation (5). The $tcpO_2$ group differences remained significant even when adjusted for MAP; thus, the higher $tcpO_2$ in the HBOC-201 animals can be considered independent of MAP. Moreover, our results showed moderate to strong correlation between $tcpO_2$ and lactate. Thus, in agreement with results reported by Tatevossian and colleagues (20), we believe that $tcpO_2$ is a useful and sensitive clinical tool, especially in less severe hemorrhage in which milder physiological perturbations related to tissue oxygenation may not be apparent clinically, and that

TABLE 3. **Posthospital arrival hematology values**

Variable	Group	24 h	48 h	72 h
Lactate (mmol/L)	HBOC-201	1.5 + 0.1	1.5 + 0.1	1.4 + 0.1
	HEX	1.7 + 0.4	1.3 + 0.1	1.5 + 0.2
	NON	1.3 + 0.6	2.7 + 1.8	0.2 + 0.2
Creatinine (mg/dL)	HBOC-201	†1.4 + 0.1	1.1 + 0.1	0.9 + 0.1
	HEX	1.2 + 0.1	1.0 + 0.1	0.9 + 0.1
	NON	†0.9 + 0.1	0.8 + 0.1	0.8 + 0.1
BUN (mg/dL)	HBOC-201	*23.4 + 2.3	*18.7 + 2.7	17.7 + 3.7
	HEX	*15.7 + 1.5	*12.7 + 0.9	10.8 + 0.7
	NON	17.0 + 2.0	14.5 + 0.5	13.5 + 1.5
Hemoglobin (g/dL)	HBOC-201	*†8.5 + 0.3	8.6 + 0.3	7.6 + 0.4
	HEX	*7.2 + 0.6	8.9 + 0.3	8.4 + 0.7
	NON	†5.3 + 0.1	8.1 + 2.6	6.5 + 1.2
Hematocrit (%)	HBOC-201	*15.3 + 0.6	*19.4 + 0.9	19.3 + 1.7
	HEX	*‡21.7 + 1.8	*26.7 + 1.0	25.1 + 2.3
	NON	‡16.1 + 0.6	24.2 + 8.2	18.5 + 5.3

Laboratory values for HBOC-201, HEX, and NON groups during the hospital phase (24–72 h). Values are presented as mean ± SEM.
*$P < 0.05$, HBOC-201 versus HEX. †$P < 0.05$, HBOC-201 versus NON.
‡$P < 0.05$, HEX versus NON.
BUN indicates blood urea nitrogen.

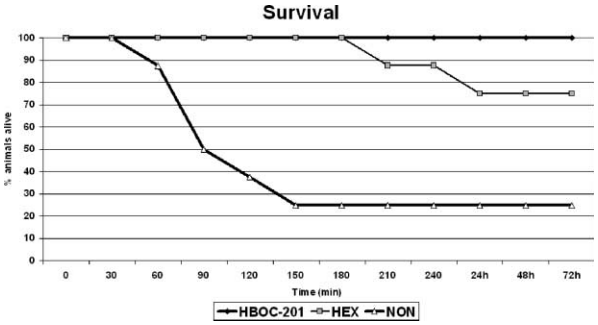

FIG. 9. **Stepwise Percent Survival to 72 h.** Overall group survival was significantly different ($P > 0.001$). There were no differences in survival rate between HBOC-201 and HEX. Groups are represented as HBOC-201 (□), HEX (–□–), NON (■).

$tcpO_2$ should supplement traditional indirect measures of tissue oxygenation such as lactic acid (LA) and BD (20).

Concerns of possible vasoactivity after resuscitation with an HBOC exist because increased systemic and pulmonary pressure have been reported in animal and human studies (10, 12, 13, 21–24). In our previously reported models of moderate controlled and severe uncontrolled hemorrhage, we observed slight, but likely clinically insignificant, rises in MPAP and SVRI. In a recent article by Sampei and colleagues (25), it was reported that significant nitric oxide scavenging, attributed to HBOC (a sebacyl cross-linked Hb product), increased vasoactivity in vascular beds with porous (fenestrated) endothelium (i.e., splanchnic, renal, and neurohypophysis), but not in vasculature with tight endothelial junctions such as cardiac tissue. Therefore, it could be that HBOC vasoactivity may vary in different types of vascular beds. However, it is unclear whether these findings are relevant to the observed rises in pressures (MAP, MPAP, SVRI) in the current study, and additional investigation of this mechanism is warranted. In the model reported herein, an increase in MPAP from 17 (baseline value) to a maximum of 21.75 mmHg (~22% increase) above baseline was observed in HBOC-201 animals, suggesting a small degree of pulmonary vasoconstriction. However, this relatively small degree of increases were likely not physiologically deleterious in these severely hemorrhaged animals, given the fact that evidence of global and local hypoperfusion (i.e., increased lactic acid, failure of lactate clearance, and diminished $tcpO_2$) were not evident in this model. Moreover, $tcpO_2$ was substantially increased through the course of resuscitation, suggesting that microcirculatory flow was increased, as opposed to being decreased, as would be expected with detrimental vasoconstriction.

Attempts at new formulations of HBOCs have been sought to alleviate or eliminate the possible vasoactivity. Recently, Young and colleagues (26) reported results from a swine hemorrhage model comparing resuscitation with a polyethylene glycol conjugated Hb (MalPEG) solution versus Ringer's acetate or 10% pentastarch. They reported improved survival and no incidence of systemic vasoconstriction with MalPEG resuscitation. Malhorta and colleagues (27) evaluated a novel HBOC (rHb2.0) in a swine model of uncontrolled hemorrhage and reported no hypertensive response after resuscitation while maintaining CO and DO_2.

TABLE 4. **Comparison of HBOC-201 versus hextend resuscitation in 3 HS models**

	Moderate controlled HS (40% EBV)	Severe uncontrolled HS (liver injury)	Severe controlled HS (55% EBV)
Hemodynamics	Higher MAP, MPAP, and SVRI; decreased CI; less tachycardia with HBOC-201	Higher MAP, MPAP, and SVRI; CI not different; less tachycardia with HBOC-201	Higher map, MPAP, and SVRI; CI and HR not different with HBOC-201
Tissue oxygenation	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201
Global indirect measures of tissue oxygenation	Similar BD and LA; similar DO_2, and O_2ER; elevated VO_2 with HBOC-201	Decreased BD; trend to decreased LA and clearance time; similar VO_2; higher DO_2 and MVO_2 with HBOC-201	Similar BD, LA, DO_2, VO_2, O_2ER with HBOC-201
Hemorrhage volume	N/A (40% EBV controlled hemorrhage)	Decreased prehospital and total blood loss	N/A (55% EBV controlled hemorrhage)
Fluid requirements	Lower with HBOC-201	Same. Lower with HBOC-201 if calculated on a mL/kg per survival hour basis.	Not different
Hemoglobin	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201
Transfusion requirements	Decreased with HBOC-201	Decreased with HBOC-201	Decreased with HBOC-201
Renal Function	Urine output and BUN not different, creatinine slightly higher with HBOC-201	Urine output, BUN, and creatinine not different.	Lower urine output with HBOC-201, similar BUN and creatinine
Survival	Not significantly different (HBOC-201 100%, HEX 88%)	Higher survival with HBOC-201 (HBOC-201 87.5%, HEX 12.5%)	Not significantly different (HBOC-201 100%, HEX 75%)
Mortality (in NON-resuscitated, control pigs)	38% (3/8) mortality rate	88% (7/8) mortality rate	75% (6/8) mortality rate
Conclusions	HBOC-201 stabilized hemodynamics, decreased CI, increased higher tissue oxygenation, similar global indirect measures of tissue oxygenation and urine output; decreased fluid and blood transfusion requirements with HBOC-201. Slight vasoactivity. No differences in survival.	HBOC-201 stabilized hemodynamics without compromised CI, improved tissue oxygenation and decreased anaerobic metabolism (global measures of tissue oxygenation); similar urine output; decreased fluid and blood transfusion requirements; lower blood loss despite vasoactive changes with HBOC-201. Survival dramatically improved.	HBOC-201 stabilized hemodynamics without decreased CI, higher tissue oxygenation, and similar global indirect measures of oxygenation; lower urine output, similar fluid but decreased transfusion requirements. No difference in survival.

LA, lactic acid; MVO_2, mixed venous oxygen saturation.

In summary, the benefits of HBOC-201 resuscitation that were observed in our less severe model of 40% controlled hemorrhage and in our more severe model of uncontrolled hemorrhage were also observed in the current intermediate severity model with controlled 55% EBV hemorrhage. HBOC-201 restored hemodynamics more rapidly, increased $tcpO_2$, and decreased blood transfusion requirements, while maintaining cardiac index and oxygen transport, and metabolic parameters. Although urine output was lower in HBOC-201 pigs, no impairment of renal function or oliguria was observed. In light of our 40% and 55% EBV controlled and uncontrolled HS studies, HBOC-201 resuscitation appears to be increasingly beneficial as the severity of hemorrhage progresses. Further studies, including human trials of HS resuscitation, are warranted to validate the safety and efficacy of HBOC-201 resuscitation and to define the optimal parameters of its use in prehospital care.

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REFERENCES

1. Davis JW, Parks SN, Kaups KL, Gladen HE, O'Donnell-Nicol S: Admission base deficit predicts transfusion requirements and risk of complications. *J Trauma* 41:769–774, 1996.
2. Rutherford EJ, Morris JA Jr, Reed GW, Hall KS: Base deficit stratifies mortality and determines therapy. *J Trauma* 33:417–423, 1992.
3. Manning JE, Katz LM, Pearce LB, Batson DN, McCurdy SL, Gawryl MS, Baker CC: Selective aortic arch perfusion with hemoglobin-based oxygen carrier-201 for resuscitation from exsanguinating cardiac arrest in swine. *Crit Care Med* 29:2067–2074, 2001.
4. Katz LM, Manning JE, McCurdy S, Pearce LB, Gawryl MS, Wang Y, Brown C: HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. *Resuscitation* 54:77–87, 2002.
5. Gurney J, Philbin N, Rice J, Arnaud F, Dong F, Wulster-Radcliffe M, Pearce LB, Kaplan L, McCarron R, Freilich D: A hemoglobin based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in an uncontrolled liver injury hemorrhagic shock swine model with delayed evacuation. 57:726–738, 2004.
6. Philbin N, Rice J, Gurney J, McGwin G, Arnaud F, Dong F, Johnson T, Flournoy WS, Ahlers S, Pearce LB, et al.: A hemoglobin-based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in a moderate severity hemorrhagic shock swine model with delayed evacuation. *Resuscitation* 66:367–378, 2005.
7. Sprung J, Kindscher JD, Wahr JA, Levy JH, Monk TG, Moritz MW, O'Hara PJ: The use of bovine hemoglobin glutamer-250 (Hemopure) in surgical patients: results of a multicenter, randomized, single-blinded trial. *Anesth Analg* 94: 799–808, 2002. table of contents.
8. Levy JH, Goodnough LT, Greilich PE, Parr GV, Stewart RW, Gratz I, Wahr J,

Williams J, Comunale ME, Doblar D, et al.: Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: results of a randomized, double-blind trial. *J Thorac Cardiovasc Surg* 124:35–42, 2002.
9. Johnson T, Arnaud F, Dong F, Philbin N, Rice J, Asher L, Arrisueno M, Warndorf M, Gurney J, McGwin G, et al.: Bovine polymerized hemoglobin (HBOC-201) resuscitation in three models of hemorrhagic shock with militarily relevant evacuation—effects on histopathology and organ function. *Crit Care Med* 34:1464–1474, 2006.
10. Sampson JB, Davis MR, Mueller DL, Kashyap VS, Jenkins DH, Kerby JD: A comparison of the hemoglobin-based oxygen carrier HBOC-201 to other low-volume resuscitation fluids in a model of controlled hemorrhagic shock. *J Trauma* 55:747–754, 2003.
11. York GB, Eggers JS, Smith DL, Jenkins DH, McNeil JD, Mueller D, Josephs JD, Kerby JD: Low-volume resuscitation with a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) provides adequate tissue oxygenation for survival in a porcine model of controlled hemorrhage. *J Trauma* 55:873–885, 2003.
12. McNeil CJ, Smith LD, Jenkins LD, York MG, Josephs MJ: Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) leads to reversal of anaerobic metabolism. *J Trauma* 50:1063–1075, 2001.
13. Fitzpatrick CM, Savage SA, Kerby JD, Clouse WD, Kashyap VS: Resuscitation with a blood substitute causes vasoconstriction without nitric oxide scavenging in a model of arterial hemorrhage. *J Am Coll Surg* 199: 693–701, 2004.
14. Khan AK, Jahr JS, Nesargi S, Rothenberg SJ, Tang Z, Cheung A, Gunther RA, Kost GJ, Driessen B: Does lead interfere with hemoglobin-based oxygen carrier (HBOC) function? A pilot study of lead concentrations in three approved or tested HBOCs and oxyhemoglobin dissociation with HBOCs and/or bovine blood with varying lead concentrations. *Anesth Analg* 96: 1813–1820, 2003. table of contents.
15. Knudson MM, Lee S, Erickson V, Morabito D, Derugin N, Manley GT: Tissue oxygen monitoring during hemorrhagic shock and resuscitation: a comparison of lactated Ringer's solution, hypertonic saline dextran, and HBOC-201. *J Trauma* 54:242–252, 2003.
16. Standl T, Freitag M, Burmeister MA, Horn EP, Wilhelm S, Am Esch JS: Hemoglobin-based oxygen carrier HBOC-201 provides higher and faster increase in oxygen tension in skeletal muscle of anemic dogs than do stored red blood cells. *J Vasc Surg* 37:859–865, 2003.
17. Boura C, Caron A, Longrois D, Mertes PM, Labrude P, Menu P: Volume expansion with modified hemoglobin solution, colloids, or crystalloid after hemorrhagic shock in rabbits: effects in skeletal muscle oxygen pressure and use versus arterial blood velocity and resistance. *Shock* 19:176–182, 2003.
18. Standl T, Lipfert B, Reeker W, Schulte am Esch J, Lorke DE: Acute effects of complete blood exchange with ultra-purified hemoglobin solution or hydroxyethyl starch on liver and kidney in the animal model. *Anasthesiol Intensivmed Notfallmed Schmerzther* 31:354–361, 1996.
19. Masuno T, Moore EE, Cheng AM, Moore PK, Grant AR, Johnson JL: Prehospital hemoglobin-based oxygen carrier resuscitation attenuates post-injury acute lung injury. *Surgery* 138:335–341, 2005.
20. Tatevossian RG, Wo CC, Velmahos GC, Demetriades D, Shoemaker WC: Transcutaneous oxygen and CO2 as early warning of tissue hypoxia and hemodynamic shock in critically ill emergency patients. *Crit Care Med* 28:2248–2253, 2000.
21. Gulati A, Barve A, Sen AP: Pharmacology of hemoglobin therapeutics. *J Lab Clin Med* 133:112–119, 1999.
22. Schultz SC, Hamilton IN Jr, Malcolm DS: Use of base deficit to compare resuscitation with lactated Ringer's solution, Haemaccel, whole blood, and diaspirin cross-linked hemoglobin following hemorrhage in rats. *J Trauma* 35:619–625, 1993. discussion 625–626.
23. Shannon M: Oral Presentation. Army Sponsored Blood Substitute Meeting, WRAMC, Washington, DC, 2003.
24. Viele MK, Weiskopf RB, Fisher D: Recombinant human hemoglobin does not affect renal function in humans: analysis of safety and pharmacokinetics. *Anesthesiology* 86:848–858, 1997.
25. Sampei K, Ulatowski JA, Asano Y, Kwansa H, Bucci E, Koehler RC: Role of nitric oxide scavenging in vascular response to cell-free hemoglobin transfusion. *Am J Physiol Heart Circ Physiol* 289:H1191–H1201, 2005.
26. Young MA, Riddez L, Kjellstrom BT, Bursell J, Winslow F, Lohman J, Winslow RM: MalPEG-hemoglobin (MP4) improves hemodynamics, acid-base status, and survival after uncontrolled hemorrhage in anesthetized swine. *Crit Care Med* 33:1794–1804, 2005.
27. Malhotra AK, Kelly ME, Miller PR, Hartman JC, Fabian TC, Proctor KG: Resuscitation with a novel hemoglobin-based oxygen carrier in a swine model of uncontrolled perioperative hemorrhage. *J Trauma* 54: 915–924, 2003.