Resuscitation (2007) **74**, 332—343

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EXPERIMENTAL PAPER

Resuscitation following severe, controlled hemorrhage associated with a 24 h delay to surgical intervention in swine using a hemoglobin based oxygen carrier as an oxygen bridge to definitive care☆

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Received 13 September 2006; received in revised form 7 December 2006; accepted 7 December 2006

KEYWORDS
Hemorrhage;
Trauma;
Hypovolemic shock;
Resuscitation;
HBOC-201;
Swine

Summary
Objectives: To test our hypothesis that the hemoglobin based oxygen carrier HBOC-201 would have similar or superior efficacy to 6% hetastarch (HEX) as a pre-hospital 'bridging' fluid for hemorrhagic shock when delay to definitive medical care is prolonged to 24 h.
Methods: Twenty-four pigs were anesthetized, instrumented, given a soft tissue injury, and bled 55% estimated blood volume. Pigs were randomized to receive HBOC-201, HEX, or no resuscitation fluids (NON). At 4 h post-injury, surgical sites were repaired and pigs were recovered from anesthesia. Animals were non-invasively monitored, administered blood for anemia or saline for hypotension at 24 and 48 h, and monitored for 72 h.
Results: Survival to 72 h was 87.5% (7/8) in HBOC-201 and HEX pigs compared to 25% (2/8) in NON pigs ($p = 0.01$). Increased mean arterial pressure was observed in the HBOC-201 group ($p < 0.0001$). Cardiac index was highest in HEX pigs (overall $p < 0.001$, HBOC-201 versus HEX $p = 0.002$). Transcutaneous tissue oxygenation was higher with HBOC-201 (overall $p = 0.04$, HBOC-201 versus HEX $p < 0.01$). HBOC-201 and HEX pigs had comparable heart rates, pulmonary pressures, pre-hospital fluid requirements, venous O_2 saturation, base deficit, and lactic acid. Hemoglobin was

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at 10.1016/j.resuscitation.2006.12.018.

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0300-9572/$ — see front matter
doi:10.1016/j.resuscitation.2006.12.018

decreased with HEX (overall $p < 0.0001$, HBOC-201 versus HEX $p < 0.0002$). At 24 h, 14.3% (1/7) HBOC-201 pigs required blood transfusions versus 100% HEX (7/7) and NON (2/2) pigs ($p > 0.001$).
Conclusions: HBOC-201 restored hemodynamics, maintained tissue oxygenation, and decreased blood transfusions in comparison to HEX in severe controlled HS with 24 h delay to simulated hospital care. These results support the potential use of HBOC-201 as a bridging resuscitation fluid for HS.

Introduction

Clinical practice guidelines that emphasize early hemostasis and infusion of red blood cells following profound blood loss are generally accepted as standard care in the management of traumatic hemorrhage.[1,2] These resuscitation strategies lead to an early restoration of adequate oxygen transport, which is paramount in the reversal of shock.[3] Regrettably, blood products such as packed red blood cells or whole blood are not easily deployed and/or used in out-of-hospital environments. Victims of traumatic blood loss must generally await transportation to a hospital before life saving blood transfusions are available, leaving severely injured casualties at a significant survival disadvantage.[4] The use of an oxygen carrying resuscitation fluid to bridge the gap between field resuscitation and definitive in-hospital intervention has been proposed.[5–8]

We have demonstrated previously that the polymerized, bovine, hemoglobin-based oxygen carrier, HBOC-201 (Hemopure®, Biopure Corp., Cambridge, MA) is an effective oxygen carrying resuscitative fluid in several swine models of traumatic hemorrhagic shock (HS) associated with a 4 h delay to definitive intervention.[9–11] Here, we present data confirming the effectiveness of HBOC-201 resuscitation in a model of severe shock with very prolonged (24 h) delay to definitive intervention.

Materials and methods

Study design, setting and population

These experiments were conducted in accordance with 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 (AAALAC).

Study protocol

Instrumentation

Twenty-four male and female Yucatan Mini pigs (~32 kg) (Sinclair Research Center Inc., Columbia, MO) were instrumented as previously reported.[10,11] Animals were allowed free access to food and water until 12 h prior to the experiment. Swine 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. Pigs were intubated and anesthesia was maintained with isoflurane (1–2.5%), FiO_2 0.21 with ventilatory support for apnea (Ohmeda 7800 series ventilator, Datex, Madison, WI) at 15–25 breaths/min and tidal volume 5–10 ml/kg. Body temperature was monitored and maintained using a BAIR hugger device (Model 505, Bair Hugger, MN). Urine output (UO) was measured *via* bladder catheterization. The external jugular vein and carotid artery were catheterized by open cut down 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 (PAC; Edwards Life Sciences, Irvine, CA,) was inserted for continuous hemodynamic and cardiac output (CO) monitoring. Mean arterial pressure (MAP) and heart rate (HR) were measured continuously through an 18 G angiocatheter placed in the carotid artery. A 3–5 cm lower abdominal incision was made, the left rectus abdominus muscle isolated, and a surgical tissue clamp (Kocher) placed over a portion of the muscle in the center of the incision. All surgical procedures were performed using aseptic techniques.

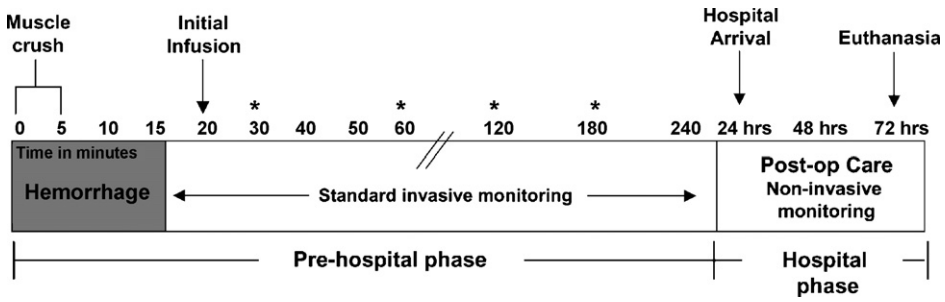

Figure 1 Experimental design. *Infusion times. Fluids were administered at 10 ml/kg over 10 min at room temperature. Blood or saline were provided in the hospital phase. Surviving pigs were sacrificed painlessly at 72 h and tissues collected for histological analysis.

Pre-hospital phase: injury, hemorrhage and resuscitation

Estimated blood volume (EBV) was calculated by weight (kg) $\times$ 65 ml/kg. The Kocher clamp was closed for 5 min to create a soft tissue injury, and pigs were bled 55% EBV *via* the external jugular vein over $\sim$15 min to induce HS (Figure 1). All ''shed'' blood was collected in sterile blood bags containing citrate phosphate dextrose (CPDA-1, Fenwal, Baxter, Deerfield, IL) for possible later re-infusion. Pigs were pre-assigned to one of three treatment groups: Hemoglobin based oxygen carrier (HBOC-201); 6% hetastarch in LR (HEX, Hextend®, Abbott Laboratories, Abbot Park, IL); or no fluids (NON). At 20 min, resuscitated pigs received a bolus infusion of 10 ml/kg of HBOC-201 or HEX over 10 min. Additional bolus infusions of 5 ml/kg were provided at 30, 60, 120, and 180 min post-injury if hypotension (MAP < 60 mmHg) or tachycardia (HR > baseline value [Time 0]) were observed. Fluids were infused at room temperature. At 4 h post-injury, antibiotics (13 mg/kg cephazolin) and analgesics (0.01 mg/kg buprenorphine) were administered. The PAC was removed, the jugular vein introducer secured for post-operative blood sampling and fluid administration, and arterial and bladder catheters removed. Surgical incisions were closed and dressings applied. Animals were allowed to recover from anesthesia and extubated.

Hospital phase: simulated in-hospital care

Hospital arrival was simulated at 24 h post-injury. Non-invasive assessment of vital signs (e.g. MAP and HR) was completed. Pigs received 10 ml/kg autologous shed blood for anemia (hemoglobin [Hb] < 7 g/dl) or 10 ml/kg normal saline for hypotension (MAP < 60 mmHg). Additional infusions were provided at 48 h for anemia or hypotension as needed. Assessment of health status including activity level, feed consumption, and incidence of vomiting was completed post-operatively to 72 h post-injury. Pigs were sacrificed painlessly at 72 h.

Measurements

Standard invasive hemodynamic variables (MAP, HR, CO, mean pulmonary arterial pressure [MPAP]) were monitored until 4 h post-injury. Arterial lactate (LA), base excess (BE), oxygen saturation (Sa_{O_2}), hemoglobin (Hb) and mixed venous oxygen saturation (Sv_{O_2}) were measured every 15 min for the 1st hour and then every 30 min until 4 h with an automatic blood gas analyzer (ABL 705, Radiometer, Copenhagen, Denmark). Calculated data included cardiac index (CI = CO/body surface area), systemic vascular resistance index (SVRI = (MAP $-$ CVP $\times$ 80)/CO), oxygen delivery ($D_{O_2} = CI \times 13.4 \times Hb \times Sa_{O_2}$), oxygen consumption ($V_{O_2} = CI \times 13.4 \times Hb \times Sa_{O_2} - Sv_{O_2}$) and oxygen extraction ratio ($O_{2\,ER} = (V_{O_2}/D_{O_2}) \times 100$). Transcutaneous tissue oxygenation ($tcpO_2$) was measured non-invasively 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. Health status data was scored on a four-point scale from 0 (normal behavior) to 3 (severe inactivity, dyspepsia, or vomiting) and summed across all post-operative days. Blood samples were collected for serum chemistry (Vitros 250 Analyzer, Ortho, France).

Data analysis

The study was designed assuming a statistical power of 80% to detect significant ($\alpha = 0.05$) between-group differences of 50% or greater and to detect

smaller differences (30—40%) with modest statistical power (i.e., 50—70%). Analysis of variance (ANOVA) followed by two-tail Student *t*-tests when appropriate, and χ^2-tests were used to compare continuous and categorical variables, respectively, between groups. For continuous variables, the non-parametric Kruskal—Wallis test was also used when the assumptions of the ANOVA model were unmet. For longitudinal analyses, Cox Proportional Hazards test was used for survival-related outcomes while 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). Overall *p*-values for pre-hospital data sets are for group × time interactions. Overall *p*-values for hospital data sets are from ANOVA analysis with two-tail Student *t*-tests used for group versus group comparisons (Graphpad Software Inc., San Diego, CA). Data are expressed as mean ± standard error of the mean (S.E.M.) for animals alive at time of measurement.

Results

Sex ratio, body weight, percent total blood loss (including blood withdrawn for laboratory assays), and percent hemorrhage of EBV were similar across groups (data not shown). Baseline hemodynamic measurements were similar across groups (Figure 2). Baseline direct and indirect measurements of tissue oxygenation were similar across groups with the exception of $tcpO_2$ at Time 0 (Figure 3). The reason for this finding is unclear but may be due to inconsistencies in the timing of sensor placement and first recorded measurements. However, values were no longer different between groups by Time 5 min (T5) and were similar in all pigs at the end of hemorrhage (T15).

Hemorrhage shock

Hemodynamic responses to hemorrhage were similar in all groups with no significant differences between groups at the end of 55% EBV hemorrhage (T15). HR increased from 140.5 ± 6.0 to 188.9 ± 7.3 bpm and decreases in MAP (76.9 ± 2.1 to 24.5 ± 1.9 mmHg), CI (5.0 ± 0.2 to 3.4 ± 0.3 l/(min m^2)), and MPAP (16.8 ± 0.9 to 8.2 ± 2.6 mmHg) were observed (Figure 2).

Pre-hospital phase

Hemodynamics

Following resuscitation at T20 min, HR decreased in HBOC-201 and HEX but rose in NON pigs throughout the course of the experiment (overall $p < 0.0001$, HBOC-201 versus HEX $p = 0.2$) (Figure 2). Baseline HR was not restored in any group. MAP was highest in the HBOC-201 group, intermediate in HEX, and lowest in the NON group (overall $p < 0.0001$, HBOC-201 versus HEX $p < 0.0001$) (Figure 2). MAP was similar between HBOC-201 and HEX until 60 min when values reached a plateau in the HEX group but continued to rise in the HBOC-201 group. Baseline MAP values were restored in the HBOC-201 group by 45 min (following 2 infusions). CI was higher in HEX — than HBOC-201 — or NON-resuscitated pigs (overall $p < 0.001$, HBOC-201 versus HEX $p = 0.002$) (Figure 2). CI was restored to baseline in the HEX group by 60 min and by 150 min in the HBOC-201 group. CI continued to increase in HEX while remaining at baseline in HBOC-201. CI was markedly depressed in the NON groups throughout the pre-hospital phase. No significant differences were observed between groups over time for MPAP, although values were higher in the HBOC-201 group from 10 min until 3 h post-injury (Figure 2). SVRI values were significantly different between groups over time (overall $p < 0.001$, HBOC-201 versus HEX $p = 0.02$). Following resuscitation, SVRI values gradually increased to return to baseline in the HEX group whereas peak and trough fluctuations were observed in the HBOC-201 and NON groups.

Indirect and direct measurements of tissue oxygenation

LA was significantly lower in the HBOC-201 and HEX groups compared to the NON group (overall $p = 0.005$, HBOC-201 versus HEX $p = 0.3$) (Figure 3). BE was similar across all groups (Figure 3). Sv_{O_2} was similar in the resuscitated groups whereas values were markedly depressed in the NON group (Figure 3). $tcpO_2$ was significantly higher in the HBOC-201 group over time (overall $p = 0.04$, HBOC-201 versus HEX $p = 0.01$) (Figure 3) as was D_{O_2} (overall $p = 0.04$, HBOC-201 versus HEX $p = 0.2$). V_{O_2} and $O_{2\,ER}$ were not different (Figure 3).

Fluid requirements and urine output

Pre-hospital fluid requirements were similar in HBOC-201 (28.0 ± 0.9 ml/kg) and HEX

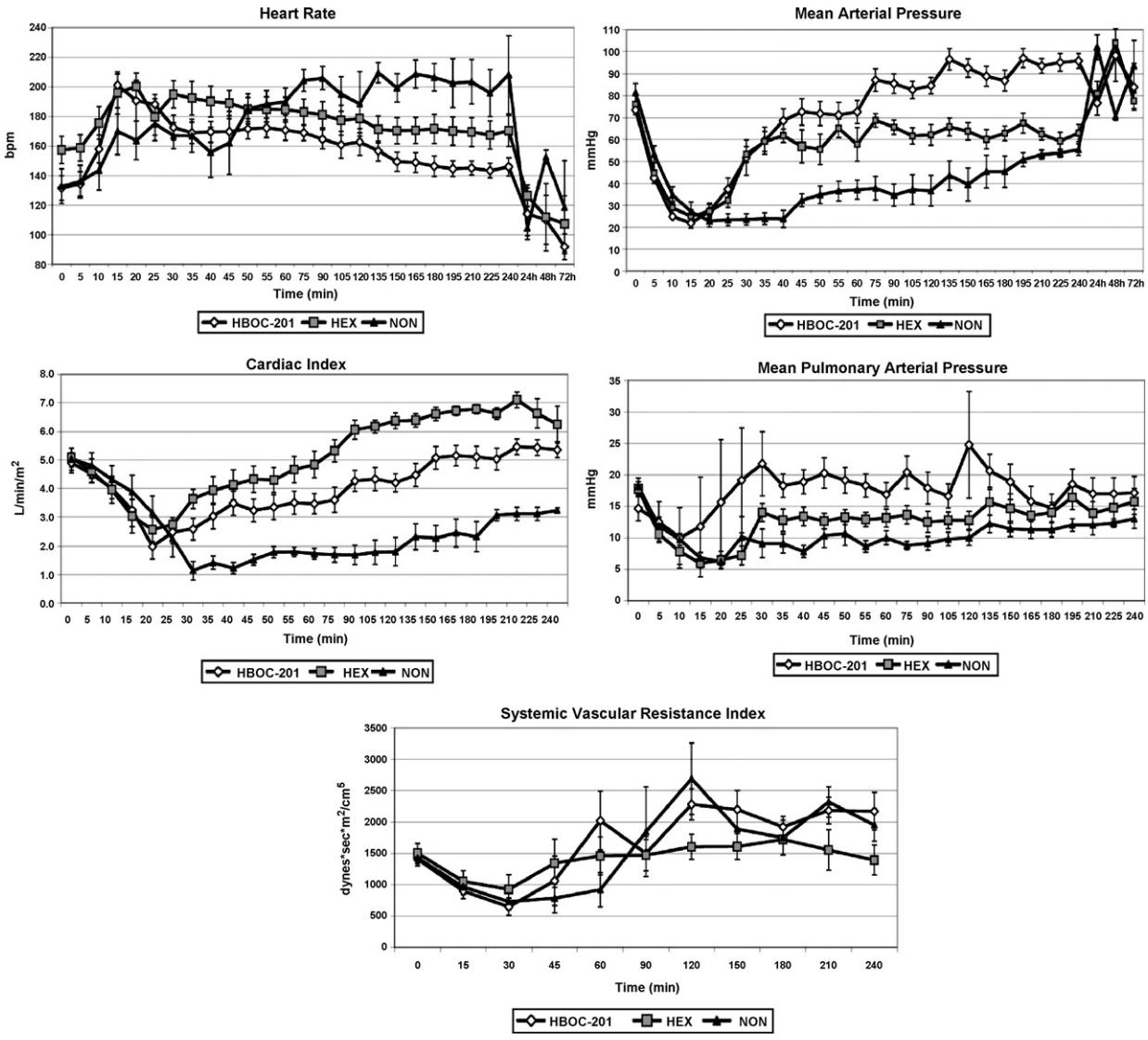

Figure 2 Pre-hospital hemodynamics for HBOC-201, HEX, and NON groups. Values are presented as mean ± S.E.M. Heart rate was significantly higher in the NON group (overall $p < 0.0001$, HBOC-201 vs. HEX, $p = 0.2$). Mean arterial pressure was highest in the HBOC-201 group (overall $p < 0.0001$, HBOC-201 vs. HEX $p < 0.0001$). CI was highest in HEX resuscitated pigs (overall $p < 0.001$, HBOC-201 vs. HEX $p = 0.002$). No significant differences in mean pulmonary pressure was observed between groups over time. Systemic vascular resistance index was significantly different between groups over time (overall $p < 0.001$, HBOC-201 vs. HEX $p = 0.02$).

(30.0 ± 0.0 ml/kg). Total pre-hospital UO was 3.56 ± 0.9, 5.87 ± 0.84, 1.21 ± 0.4 ml/kg in HBOC-201, HEX, and NON, respectively (overall $p = 0.011$, HBOC-201 versus HEX $p = 0.04$).

Hemoglobin

Hb concentration decreased following resuscitation in the HEX group while values remained near baseline in the HBOC-201 and NON groups (overall $p < 0.0001$, HBOC-201 versus HEX $p = 0.002$) (Figure 4).

Pre-hospital survival

At 4 h, survival was 100% (8/8) in HBOC-201 and HEX pigs and 37.5% (3/8) in the NON pigs (Figure 5). Two animals (1 HBOC-201, 1 HEX) suffered respiratory arrest and died on extubation at 5 h. One pig in the NON group failed to regain consciousness post-operatively and was sacrificed painlessly at 6 h. No additional mortality was observed in the hospital phase and survival at 72 h was 87.5% (7/8) in HBOC-201 and HEX groups and 25% (2/8) in NON group ($p = 0.01$).

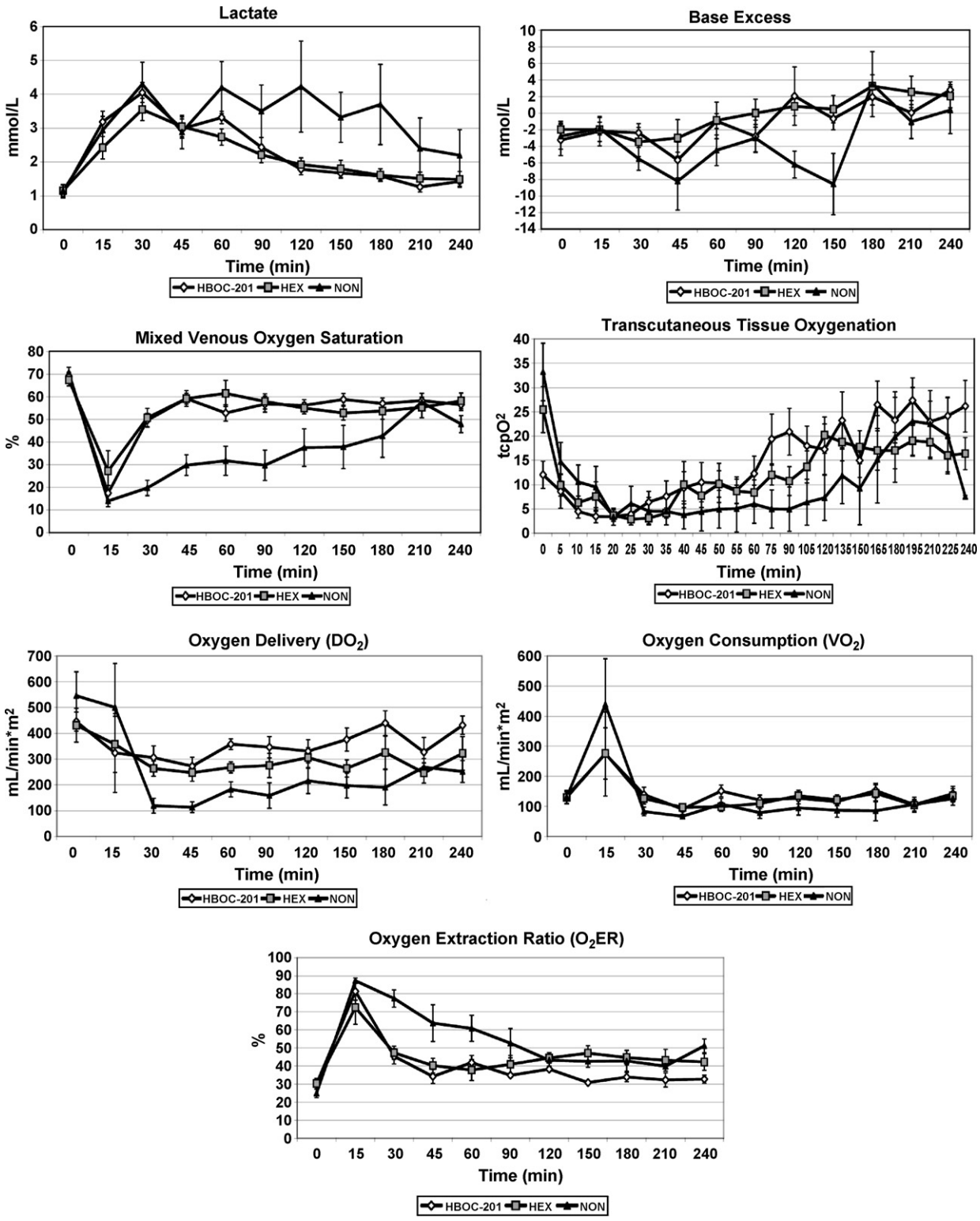

Figure 3 Pre-hospital direct and indirect tissue oxygenation values. Values are presented as mean ± S.E.M. LA was significantly higher in the NON group (overall $p = 0.005$, HBOC-201 vs. HEX $p = 0.3$). No significant differences in BE were observed between groups. Sv_{O_2} was similar in HBOC-201 and HEX but substantially lower in the NON group over time. $tcpO_2$ was significantly higher in the HBOC-201 group over time (overall $p = 0.04$, HBOC-201 vs. HEX $p = 0.01$). D_{O_2} was significantly higher in the HBOC-201 group over time (overall $p = 0.04$, HBOC-201 vs. HEX $p = 0.2$). No differences between groups were observed in V_{O_2} or O_{2ER}.

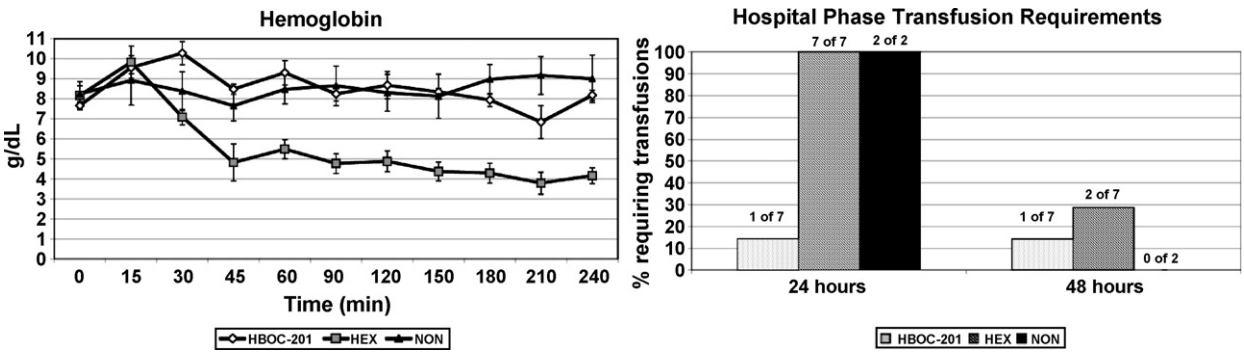

Figure 4 Pre-hospital hemoglobin concentration and hospital phase transfusion requirements for HBOC-201, HEX, and NON groups. Values are presented as mean ± S.E.M. Hb was significantly lower in the HEX group (overall $p < 0.0001$, HBOC-201 vs. HEX $p = 0.002$). At 24 h, HBOC-201 pigs required fewer blood transfusions compared to HEX or NON pigs ($p < 0.001$).

Hospital phase

A trend toward reduced hospital phase total fluid requirements (blood and saline) was observed in the HBOC-201 group compared to HEX and NON (4.2 ± 2.9, 12.9 ± 1.8, 10.0 ± 0.0 ml/kg respectively, overall $p = 0.06$ ANOVA, HBOC-201 versus HEX, $p = 0.03$). At 24 h, 14.3% (1/7) HBOC-201 pigs required blood transfusions compared to 100% of pigs resuscitated with HEX (7/7) or NON (2/2) pigs ($p < 0.001$ ANOVA) (Figure 4). Hospital phase vital signs were not different between groups (data not shown). LA and creatinine were not different between groups in the hospital phase (Table 1). At 24 and 48 h, blood urea nitrogen (BUN) was higher in the HBOC-201 pigs (24 h, overall p = NS ANOVA, HBOC-201 versus HEX, $p = 0.04$, 48 h, overall $p = 0.0495$ ANOVA, HBOC-201 versus HEX $p = 0.044$) (Table 1). Hb was lower in HEX compared to HBOC-201 pigs at 24 h (overall $p = 0.002$ ANOVA, HBOC-201 versus HEX $p = 0.001$) (Table 1). At 48 h, hematocrit was decreased in the HBOC-201 compared to NON group (overall $p = 0.011$, HBOC-201 versus NON $p = 0.009$) (Table 1). Health status data for activity, consumption, and incidence of vomiting were similar between groups (data not shown).

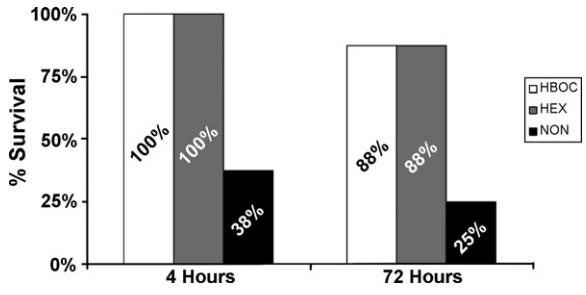

Figure 5 Four hour and 72 h survival ratios. HBOC-201 or HEX resuscitation provided for a significant early as well as late survival advantage in comparison to no intervention ($p = 0.01$). Two animals (1 HBOC-201, 1 HEX) suffered respiratory arrest and died upon extubation at 5 h. One pig in the NON group failed to regain consciousness postoperatively and was sacrificed painlessly at 6 h.

Discussion

Replacement of lost intravascular volume with crystalloid or colloid fluids remains the hallmark of early trauma resuscitation.[12] However, under some clinical conditions this approach is not practical and may even worsen the chances of survival.[13] One example of this situation occurs on the urban battlefield where medics may be required to provide life sustaining care to multiple casualties for hours to days before evacuation to definitive surgical intervention.[4] We have designed our research program to provide novel resuscitation strategies and fluid replacement options for the treatment of severely wounded casualties on the battlefield who can expect prolonged delays to evacuation and definitive surgical intervention. In the present study we have demonstrated that HBOC-201 is an effective, low volume resuscitative agent in a model of severe, controlled, traumatic hemorrhagic shock with a very prolonged delay to definitive surgical intervention.

This study is the fourth experiment in a series of experiments designed to assess the efficacy profile of HBOC-201 resuscitation in militarily relevant models of traumatic HS with escalating severity and increasing transportation delay. Our present results support previous findings in three similar, but less severe swine HS models including; moderate controlled HS (40% EBV with 4 h delay to simulated hospital arrival),[10] severe controlled HS (55% EBV with 4 h delay to simulated hospital arrival[11] and

Table 1 Hospital phase laboratory values for HBOC-201, HEX, and NON groups

Variable	Group	24 h	48 h	72 h
Lactate (mmol/l)	HBOC-201	1.43 ± 0.1	1.53 ± 0.2	1.50 ± 0.2
	HEX	1.67 ± 0.4	1.15 ± 0.1	1.64 ± 0.6
	NON	1.20 ± 0.3	1.15 ± 0.2	1.05 ± 0.1
Creatinine (mg/dl)	HBOC-201	1.36 ± 0.2	0.88 ± 0.2	0.85 ± 0.0
	HEX	1.20 ± 0.1	0.92 ± 0.0	0.79 ± 0.1
	NON	1.05 ± 0.2	0.80 ± 0.1	0.07 ± 0.0
BUN (mg/dl)	HBOC-201	*25.6 ± 2.8	&*21.0 ± 3.0	18.0 ± 4.3
	HEX	*18.9 ± 1.8	&*13.5 ± 1.5	11.9 ± 1.1
	NON	21.5 ± 3.5	&10.5 ± 1.5	12.0 ± 4.0
Hemoglobin (g/dl)	HBOC-201	&*8.91 ± 0.6	8.41 ± 0.4	8.14 ± 0.2
	HEX	&*5.92 ± 0.3	7.54 ± 0.4	7.64 ± 0.5
	NON	&6.44 ± 0.2	9.24 ± 0.0	8.43 ± 0.2
Hematocrit (%)	HBOC-201	16.2 ± 1.1	&^18.9 ± 1.1	21.0 ± 0.9
	HEX	17.3 ± 0.9	&22.2 ± 1.1	22.6 ± 1.6
	NON	18.0 ± 0.8	&#26.5 ± 0.1	23.7 ± 0.0

Values are represented as mean ± S.E.M. &$p < 0.05$, overall; *$p < 0.05$, HBOC-201 vs. HEX; ^$p < 0.05$, HBOC-201 vs. NON; #$p < 0.05$, HEX vs. NON. Lactate and creatinine were not different between groups. At 24 and 48 h, blood urea nitrogen (BUN) was higher in the HBOC-201 pigs (24 h, overall p = NS, HBOC-201 vs. HEX, $p = 0.04$, 48 h, overall $p = 0.0495$, HBOC-201 vs. HEX $p = 0.044$). Hemoglobin was lower in HEX compared to HBOC-201 pigs at 24 h (overall $p = 0.002$, HBOC-201 vs. HEX $p = 0.001$). At 48 h, hematocrit was decreased in the HBOC-201 compared to NON group (overall $p = 0.011$, HBOC-201 vs. NON $p = 0.009$).

severe uncontrolled HS (uncontrolled hemorrhage due to liver injury with 4 h delay to simulated hospital arrival).[9] A review of the results from these experiments demonstrate consistent improvements in multiple key outcome measures across a broad spectrum of injury in our swine models of HS associated with HBOC-201 resuscitation. In all four models, HBOC-201 provided reliable hemodynamic stabilization, restoration of global measures of tissue oxygenation, and improved survival in comparison to control animals (Table 2).

Enhanced survival and reduced morbidity may arguably be considered the most relevant outcome measurements in the assessment of a resuscitation fluid. In the present study, pre-hospital resuscitation with both HBOC-201 and HEX afforded improved survival in comparison to control animals, both early 100% versus 38% (Figure 5) and at 72 h. Equivalent short-term survival has also been reported by other investigators comparing HBOC-201 resuscitation to colloids and crystalloids in less severe models of controlled HS.[14–16] Long-term survival studies of controlled HS have also demonstrated equivalence in survival between pigs administered HBOC-201 and standard I.V. fluids.[17] Fitzpatrick et al. reported equivalent 5-day survival with no evidence of organ dysfunction in swine resuscitated with HBOC-201 or HEX.[18] In a swine model of controlled hemorrhage with concomitant TBI, King and colleagues found that resuscitation with HBOC-301 (less polymerized bovine HBOC, Oxyglobin, Biopure Corp.) allowed animals to be weaned from mechanical ventilation and survive to 72 h with intact neurological function whereas resuscitation using LR was associated with complete failure to wean.[19] A similar TBI and controlled HS study by the same group found resuscitation with a single 6 ml/kg bolus of HBOC-201 restored MAP, and maintained cerebral perfusion pressure and brain oxygenation allowing weaning from the ventilator and extubation at 5.5 h post-injury where as resuscitation using NS, shed blood, and mannitol did not.[20] HBOC-201 resuscitation appears to be particularly beneficial in very severe models of HS. More severe models of uncontrolled hemorrhage have shown a significant survival benefit in swine resuscitated with HBOC-201. In our laboratory, 88% survival to 72 h was observed in HBOC-201 pigs following severe uncontrolled HS compared to 12.5% both HEX and NON pigs.[9] Katz et al. reported survival to 96 h with good functional recovery and normal organ function in swine resuscitated with HBOC-201 following lethal uncontrolled hemorrhage from liver injury. In contrast, 100% mortality by 35 min was observed in swine given no fluid or HEX.[21] In a similar model of liver injury with concomitant TBI and simulated 75 min delay to hospital care, Rice and others found that 62% (8/13) of pigs resuscitated with HBOC-201 survived to 6 h compared to 9% (1/11) resuscitated with LR.[22] These data sug-

Table 2 Comparison of HBOC-201 vs. Hex resuscitation in four HS swine models incorporating delay to definitive care

	Moderate controlled HS[6] (40% EBV with 4 h delay to simulated hospital arrival)	Severe controlled HS[7] (55% EBV with 4 h delay to simulated hospital arrival)	Severe uncontrolled HS[8] (liver injury with 4 h delay to simulated hospital arrival)	Severe controlled HS with prolonged delay (55% EBV with 24 h delay to simulated hospital arrival)
Hemodynamics	Higher MAP, MPAP, and SVRI; decreased CI, less tachycardia with HBOC-201	Higher MAP, MPAP, and SVRI; CI and HR not different with HBOC-201	Higher MAP, MPAP, and SVRI; CI not different; less tachycardia with HBOC-201	Higher MAP and SVRI, decreased CI, HR and MPAP not different with HBOC-201
Tissue oxygenation	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201
Global indirect measures of tissue oxygenation	Similar BD and LA; similar D_{O_2}, and O_{2ER}; elevated V_{O_2} with HBOC-201	Similar BD, LA, D_{O_2}, V_{O_2}, O_{2ER} with HBOC-201	Decreased BD; trend to decreased LA and clearance time; similar V_{O_2}; higher D_{O_2} and MVO_2 with HBOC-201	Similar BD, LA, V_{O_2}, O_{2ER}, elevated D_{O_2} with HBOC-201
Pre-hospital fluid requirements	Lower with HBOC-201	Not different	Same. Lower with HBOC-201 if calculated as ml/kg/survival hour	Not different
Hemoglobin	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201	Higher with HBOC-201
Hospital transfusions	Decreased with HBOC-201	Decreased with HBOC-201	Decreased with HBOC-201	Decreased with HBOC-201
Urine output/hospital renal function	Urine output and BUN not different, creatinine slightly higher with HBOC-201	Lower urine output with HBOC-201, similar creatinine, BUN higher with HBOC-201 at 24 and 48 h	Urine output, BUN, and creatinine not different	Urine output and creatinine not different, BUN higher with HBOC-201 at 24 and 48 h
Survival	Not significantly different (HBOC-201 100%, HEX 88%)	Not significantly different (HBOC-201 100%, HEX 75%)	Higher survival with HBOC-201 (HBOC-201 87.5%, HEX 12.5%)	Not significantly different (HBOC-201 88%, HEX 88%)
Model mortality (NON pigs)	38% (3/8) mortality rate	75% (6/8) mortality rate	87.5% (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 decreased CI, increased tissue oxygenation with similar global indirect measures of oxygenation; lower urine output, similar fluid but decreased transfusion requirements. No difference in survival	HBOC-201 stabilized hemodynamics without compromised CI, improved tissue oxygenation and decreased anaerobic metabolism; 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, decreased CI, increased tissue oxygenation with similar global indirect measures of tissue oxygenation and urine output; similar pre-hospital fluid but decreased transfusion requirements with HBOC-201. No differences in survival

Overview of results from four studies completed by our laboratory investigating the use of HBOC-201 in uncontrolled and controlled hemorrhagic shock models in swine. Modified from Rice et al.[11]

gest that resuscitation of traumatic hemorrhagic shock using HBOC-201 provides for at least equivalent survival in moderately severe controlled HS and superior survival in more severe uncontrolled HS.

Improved survival following HBOC-201 resuscitation may be directly related to the hemodynamic effects of the drug as well as the augmentation of oxygen carrying capacity. HBOC-201 has been shown to rapidly increase MAP to suprabaseline,[9–11,21] baseline,[23] or target blood pressures.[14–18,24] HBOC-201 resuscitation has also been reported to elevate MAP in comparison to crystalloid (LR) and other colloids (hypertonic saline dextran, HSD).[25] However, hemodynamic support resulting from improved vascular tone alone cannot fully explain the improvements in key markers of perfusion. Indeed agents leading strictly to increased BP are likely to be more detrimental than helpful. Successful resuscitation following life-threatening hemorrhage requires not only restoration of blood pressure but also the restoration of adequate tissue oxygenation and resolution of any ongoing O_2 debt. Throughout the development of polymerized hemoglobin solutions for use as a trauma resuscitation fluids, concerns have been raised with regards to the purported vasoactive nature of these products and the resultant effects on pulmonary artery pressures (MPAP effects)[9–11,21] and systematic vascular resistance (SVRI).[9,11,18,23] However, such findings have not been consistently reported in models of controlled hemorrhage. In our moderate controlled HS model, although MPAP was significantly elevated in the HBOC-201 group, higher values for SVRI did not reach statistical significance.[10] In contrast, while SVRI was significantly elevated following HBOC-201 treatment in the current study, MPAP was not significantly increased. This is in concordance with reports by Sampson and others, in which elevated MPAP values did not reach statistical significance following HBOC-201 resuscitation.[16] Moreover, McNeil et al. reported lower MPAP in pigs treated with HBOC-201 in comparison to pigs treated with LR or LR plus shed blood.[15]

In theory, even non-significant increases in MPAP and SVRI could lead to subsequent impairment of tissue oxygenation. In reality, improvements in important biological markers of adequate tissue oxygenation (LA, BE, Sv_{O_2}) have been consistently demonstrated.[9–11,15–17,23] In the current study, consistent with these previous studies, lactate returned to near baseline levels by the end of the pre-hospital phase and lactate clearance was similar between HBOC-201 and HEX groups. These results do contrast with a study by Fitzpatrick, who found slight sustained elevation of lactate levels in a model of controlled hemorrhage with prolonged low-volume resuscitation.[18] It has been reported previously that Sv_{O_2} remains depressed in pigs resuscitated with HBOC-201 in comparison to other fluids.[15,16,23] However, in this study, Sv_{O_2} in the HBOC-201 and HEX groups were similar and remained below baseline in both groups, a finding also reported in our models of moderate controlled HS,[10] severe controlled HS,[11] and severe uncontrolled HS,[9] as well as by Fitzpatrick et al.[18] Furthermore, transcutaneous tissue oxygenation has been consistently higher and values for BE, D_{O_2}, V_{O_2}, and $O_{2\,ER}$ have been equivalent or improved in swine receiving HBOC-201 compared to HEX in experiments completed by our laboratory.[9–11] Based on these data, it appears that, HBOC-201 is at least as effective as HEX resuscitation in restoring tissue oxygenation during HS in swine.

Within the constraints of military combat medicine, the appeal of a resuscitation fluid that could effectively restore hemodynamics and tissue oxygenation with substantial lower volumes than standard resuscitation fluids cannot be overemphasized. Several groups have focused their studies on the evaluation of HBOC-201 as a low volume resuscitation fluid in military relevant HS models,[16,18] because HBOC-201 has often been reported to be fluid sparing in experiments using target pressure-driven, resuscitation.[15–18,23] However, reduction of CO with HBOC-201 resuscitation in comparison to control fluids was observed in those studies and in experiments where a single small volume (6 ml/kg) bolus was administered.[14,25] Furthermore, although Sampson et al. reported no difference in HR between pigs receiving HBOC-201, HEX, LR, hypertonic saline, HDS, or pentastarch following controlled hemorrhage,[16] other investigators have reported sustained tachycardia in pigs resuscitated with low volumes of HBOC-201.[9,11,14,15,18,23,25] Furthermore, low volume, pressure targeted resuscitation using hemoglobin solutions has been associated with worse outcome in comparison to standard fluids.[26] These data suggest that blood pressure may be an inadequate stand-alone criterion for resuscitation using HBOC-201.

In the current study, and others completed by this laboratory, tachycardia in addition to MAP was used as criteria for additional fluid infusions.[9–11] In the present study, recovery to baseline HR values was not achieved during early resuscitation and relative tachycardia was observed throughout the pre-hospital phase in both HBOC-201 and HEX. As a result, HBOC-201 pigs continued

to receive fluid infusions at the later infusion time points (60—180 min) despite having achieved and maintained an adequate MAP. Consequently, pre-hospital fluid requirements were equivalent between the resuscitation groups and CI in the HBOC-201 group, although lower than in the HEX group, was returned to baseline by 150 min. A similar pattern was observed in our more severe models of HS,[9,11] however, in these studies, CI values were not different between HBOC-201 and HEX. Furthermore, in a severe model of exsanguinating liver injury, in which large volume HBOC-201 infusions were administered (180 ml/kg over 45 min), Katz et al. reported CI increased above baseline in HBOC-201 pigs.[21] Based on these data, heart rate as well as blood pressure should be used to guide the administration of additional resuscitation fluid when using HBOC-201 in HS.

An important finding in this study and the other studies completed by this laboratory has been a reduction of hospital phase transfusion requirements in the HBOC-201 pigs.[9—11,22] King et al. reported elimination of the need for RBC transfusions in swine resuscitated with HBOC-301 in their model of controlled hemorrhage and TBI.[19] In a clinical trial of cardiac patients, post-operative infusions with HBOC-201 reduced the need for additional RBC transfusions.[27] Therefore, it appears that in addition to providing an equivalent level of effective resuscitation as HEX, HBOC-201 may provide the added benefit of reducing the immediate need for transfusions in HS casualties. Such a reduction could in effect 'lengthen the bridge' from the pre-hospital setting to definite treatment at higher levels of care. This benefit could be profoundly important to the treatment of combat casualties in the field.

Conclusions based on the data presented here should be tempered by the following limitations. First, experiments were performed under anesthesia, which may have modified the physiological responses to hemorrhage and resuscitation. Second, as the model is a controlled hemorrhage model incorporating minor tissue crush injury, the results may not be generalizable to HS with more severe concomitant traumatic injuries (e.g. brain, vascular, solid organ injury). Third, the pre-hospital scenario simulated in this model in which no fluid therapy is available for over 20 h, is extremely severe and is, at most, a rare occurrence in combat or urban settings. However, since as our model was designed specifically to address the question of efficacy in a scenario of profound delay to definitive care, it was necessary that the model be appropriately rigorous. Lastly, although U.S. Special Operations Forces often use 6% hydroxyetheyl starch for HS resuscitation, crystalloid rather than colloid resuscitation is more commonly used in the civilian pre-hospital setting.

Conclusions

In this swine model of severe controlled HS with 24 h delay to definitive surgical intervention, in comparison with HEX, HBOC-201 stabilized hemodynamics and increased cutaneous tissue oxygenation (despite a mild decrease in CI), and had similar effects on global indirect measures of tissue oxygenation, urine output, pre-hospital fluid requirements, post-operative health status, and survival, while decreasing in hospital transfusion requirements. These results are consistent with findings from previously reported studies of controlled hemorrhage in swine from our laboratory, other investigators and in models of uncontrolled hemorrhage and indicate that pre-hospital resuscitation with HBOC-201 is at lease as efficacious as HEX or similar fluids. Furthermore, these results support the potential use of HBOC-201 as a bridging fluid for HS in the austere environment of military combat. Human clinical trials should be conducted to confirm these findings in traumatic HS patients.

Disclosures

The studies described herein were entirely funded by the U.S. Government (Office of Naval Research Work Unit Number 602236N.4426.W26.A0241). Test materials were provided free of charge under a Materials Transfer Agreement by Biopure Corp. (HBOC-201) and Abbott Laboratories, Chicago, IL (HEX). The Naval Medical Research Center authors have no financial or other competing interests related to this article. Dr. G. McGwin has no financial or competing interests. The opinions expressed in this paper are those of the authors and do not reflect the official policy of the Departments of the Navy, Army, and Defense, or the U.S. Government.

Conflict of interest

None of the authors have any financial or personal relationships with other people or organizations or other conflict of interest that could inappropriately influence (bias) this work.

Acknowledgments

The authors thank HM1 Benjamin Esperat, Ms. Noemy Carballo, and Mr. Mike Hammett, for their excellent technical support.

References

1. Balogh Z, Caldwell E, Heetveld M, et al. Institutional practice guidelines on management of pelvic fracture-related hemodynamic instability: do they make a difference? J Trauma 2005;58:778—82.
2. Martel MJ, MacKinnon KJ, Arsenault MY, et al. Hemorrhagic shock. J Obstet Gynaecol Cancer 2002;24:504—20.
3. Kaplan LJ, Philbin N, Arnaud F, Rice J, Dong F, Freilich D. Resuscitation from hemorrhagic shock: fluid selection and infusion strategy drives unmeasured ion genesis. J Trauma 2006;61:90—7.
4. Mabry RL, Holcomb JB, Baker AM, et al. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. J Trauma 2000;49:515—28.
5. Levy JH. The use of haemoglobin glutamer-250 (HBOC-201) as an oxygen bridge in patients with acute anaemia associated with surgical blood loss. Expert Opin Biol Ther 2003;3:509—17.
6. Arnoldo BD, Minei JP. Potential of hemoglobin-based oxygen carriers in trauma patients. Curr Opin Crit Care 2001;7:431—6.
7. Gould SA, Moore EE, Hoyt DB, et al. The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable. J Am Coll Surg 2002;195:445—52.
8. Hall RI. The utility of hemoglobin based oxygen carriers (HBOC)—can animal studies help? Can J Anaesth 2005;52:895—8.
9. Gurney J, Philbin N, Rice J, et al. 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. J Trauma 2004;57:726—38.
10. Philbin N, Rice J, Gurney J, 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 2005;66:367—78.
11. Rice J, Philbin N, McGwin G, et al. Bovine polymerized hemoglobin versus Hextend resuscitation in a swine model of severe controlled hemorrhagic shock with delay to definitive care. Shock 2006;26:302—10.
12. Hiippala S. Replacement of massive blood loss. Vox Sang 1998;74(Suppl. 2):399—407.
13. Rhee P, Koustova E, Alam HB. Searching for the optimal resuscitation method: recommendations for the initial fluid resuscitation of combat casualties. J Trauma 2003;54:S52—62.
14. 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 2003;54:242—52.
15. 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 2001;50:1063—75.
16. 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 2003;55:747—54.
17. York GB, Eggers JS, Smith DL, et al. 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 2003;55:873—85.
18. Fitzpatrick CM, Biggs KL, Atkins BZ, et al. Prolonged low-volume resuscitation with HBOC-201 in a large-animal survival model of controlled hemorrhage. J Trauma 2005;59:273—81.
19. King DR, Cohn SM, Proctor KG. Resuscitation with a hemoglobin-based oxygen carrier after traumatic brain injury. J Trauma 2005;59:553—60.
20. Patel MB, Feinstein AJ, Saenz AD, Majetschak M, Proctor KG. Prehospital HBOC-201 after traumatic brain injury and hemorrhagic shock in swine. J Trauma 2006;61:46—56.
21. Katz LM, Manning JE, McCurdy S, et al. HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. Resuscitation 2002;54:77—87.
22. Rice J, Stern S, Philbin N, et al. Resuscitation with HBOC-201 vs. LR following severe uncontrolled hemorrhage and traumatic brain injury (TBI) in swine. In: Proceedings of the 35th Critical Care Congress of the Society of Critical Care Medicine (SCCM). 2006 [Ref Type: Abstract].
23. 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 2004;199:693—701.
24. Manning JE, Katz LM, Brownstein MR, Pearce LB, Gawryl MS, Baker CC. Bovine hemoglobin-based oxygen carrier (HBOC-201) for resuscitation of uncontrolled, exsanguinating liver injury in swine. Carolina Resuscitation Research Group. Shock 2000;13:152—9.
25. Lee SK, Morabito D, Hemphill JC, et al. Small-volume resuscitation with HBOC-201: effects on cardiovascular parameters and brain tissue oxygen tension in an out-of-hospital model of hemorrhage in swine. Acad Emerg Med 2002;9:969—76.
26. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL. Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. Shock 2005;23:337—43.
27. Levy JH, Goodnough LT, Greilich PE, 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 2002;124:35—42.