A hemoglobin-based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in a moderate severity hemorrhagic shock swine model with delayed evacuation ☆

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Abstract

Objective: To evaluate the efficacy of HBOC-201 for resuscitation of hemorrhagic shock in a swine model incorporating soft tissue injury and delayed evacuation.

Methods: A muscle crush injury and 40% estimated blood volume controlled hemorrhage was completed in 24 Yucatan mini-pigs. Pigs were untreated or resuscitated with HBOC-201 or 6% hetastarch (HEX) at 20 min. Invasive hemodynamics and clinical variables were monitored for 4 h (pre-hospital phase) and subsequent fluid infusions were administered for severe hypotension or tachycardia. Animals were recovered from anesthesia and monitored non-invasively to 72 h (hospital phase).

Results: 100% (8/8) of HBOC-201-, 88% (7/8) of HEX-, and 63% (5/8) of non-resuscitated pigs, survived to 72 h ($p = 0.27$). Mean arterial pressure, mean pulmonary arterial pressure and systemic vascular resistance index were higher in HBOC-201 pigs. By 90 min, cardiac index was restored to baseline in the HBOC-201 group and was 1.4-fold greater than baseline in the HEX group. HBOC-201 pigs had lower fluid requirements than HEX pigs (18.8 ± 1.8 and 29.9 ± 1.1 ml/kg, $p < 0.001$) in the pre-hospital phase and required fewer blood transfusions (1.3 ± 1.3 and 9.4 ± 0.6 ml/kg, respectively, $p < 0.001$) in the hospital phase. Urine output and blood creatinine were comparable in HBOC-201 and HEX pigs. Tissue oxygenation levels were highest in the HBOC-201 group.

Conclusions: As HBOC-201 restored hemodynamics and tissue oxygenation and decreased fluid requirements, in comparison with HEX, HBOC-201 was at least as efficacious and possibly a superior resuscitative fluid in a military-relevant delayed evacuation hemorrhagic shock swine model.

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Keywords: Fluid therapy; Hemorrhage; Hypovolemia; Resuscitation; Shock; Trauma

1. Introduction

Investigation of hemoglobin-based oxygen carriers (HBOC) for use as primary resuscitation fluids for hemorrhagic shock (HS) causalities in the pre-hospital setting, where blood is not available (rural trauma or military operations), has recently gained prominence in trauma research [1–4]. HBOC are chemically modified hemoglobin solutions

(e.g., polymerized or conjugated) that are universally compatible. HBOC can be heat-treated, and therefore the risk of transmission of communicable pathogens is low. A bovine polymerized HBOC (HBOC-201) is room temperature stable with a long shelf-life (~3 years). HBOC offer benefits over standard intravenous (IV) fluids (i.e., crystalloids or colloids) because they have oxygen carrying as well as volume expansion capacity. In animal models of HS, HBOC are efficacious at low volumes, thereby decreasing logistical constraints, resuscitation-related hemodilution, fluid overload, and consequent complications [5,6]. They can be administered easily by simple IV administration without special training. Furthermore, some HBOC have been evaluated in phase I, II, and III clinical trials for trauma and non-trauma uses [7–10].

In the austere environment of military combat, prolonged evacuation times, logistical constraints, as well as the devastating nature of combat injuries frequently present extreme challenges to pre-hospital medical providers. Research evaluating HBOC as resuscitation fluids for military combat HS casualties has generated significant interest recently. The use of HBOC as low volume, universally compatible, and oxygen carrying resuscitative fluids could significantly improve pre-hospital care of HS casualties.

The aims of the present study were to: 1. develop a military relevant pre-hospital swine HS model incorporating controlled hemorrhage, soft tissue injury, limited resuscitation volume, and delayed arrival to definitive care and 2. use this model to test our hypotheses that resuscitation with HBOC-201 would stabilize hemodynamics, decrease fluid requirements, improve key surrogates of tissue perfusion, and decrease morbidity and mortality, in comparison with the standard resuscitative fluid carried by *US Special Operations Forces*, 6% hetastarch in LR (Hextend®).

2. 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 (NMRC)/Walter Reed Army Institute of Research (WRAIR) Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC).

2.1. Animal preparation

Twenty-four male and female Yucatan Mini pigs (~26 kg) (Sinclair Research Center, Inc., Columbia, MO) were used. Feed and water were withheld 12–14 h prior to initiation of the experiment. Animals were sedated and anesthesia induced 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 tracheal intubation. Anesthesia was maintained via isoflurane (1–2.5%) with FiO_2 0.21. Animals were allowed to breathe spontaneously throughout the experiment. Anesthesia-induced apnea was addressed with ventilatory support (Ohmeda 7800 series ventilator, Datex, Madison, WI) at 12–15 breaths/min and tidal volume 5–10 ml/kg. No significant differences were observed in pCO_2 or pH prior to hemorrhage. Body temperature was monitored and maintained at $99 \pm 1\,^{\circ}F$ with a thermal blanket. Bladder catheterization for urine collection was accomplished in females via insertion of a Foley catheter, directly into the urethra and in males via laproatomy and direct bladder catheterization. The right external jugular vein and carotid artery were dissected and isolated. 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. A 20 G angiocatheter was placed in the carotid artery and mean arterial pressure (MAP) and heart rate (HR) were continuously monitored. A 3–5 cm lower abdominal incision was made and the left *rectus abdominus* muscle located. The rectus sheath was mobilized bluntly and a surgical tissue clamp (Kocher) placed on a standardized portion of the muscle in the center of the incision. All surgical procedures were performed using aseptic techniques. Blood volume (ml) was estimated as: EBV = animal weight (kg) × 65 ml/kg.

2.2. Pre-hospital phase: tissue injury, hemorrhage and resuscitation

The Kocher clamp was closed for 5 min to crush the portion of the rectus abdominus muscle to create a soft tissue injury, and pigs were bled by 40% of their EBV via the external jugular vein and/or the carotid artery over 15 min to induce HS (Fig. 1). The blood volume was withdrawn at a rate that decreased over time in a stepwise fashion with half of the target hemorrhage volume occurring in the first 5 min and the remaining volume over the next 10 min. 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 allocated randomly to one of three treatment groups: hemoglobin-based oxygen carrier (HBOC, HBOC-201, Hemopure®, Biopure Corp., Cambridge, MA); 6% hetastarch in LR (HEX, Hextend®, Abbott Laboratories, Abbot Park, IL); or no fluids (NON). At 20 min, resuscitated pigs were administered 10 ml/kg of HBOC (equivalent to ~1/2 the Hb load of one unit of PRBCs) or HEX over 10 min. Additional 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.
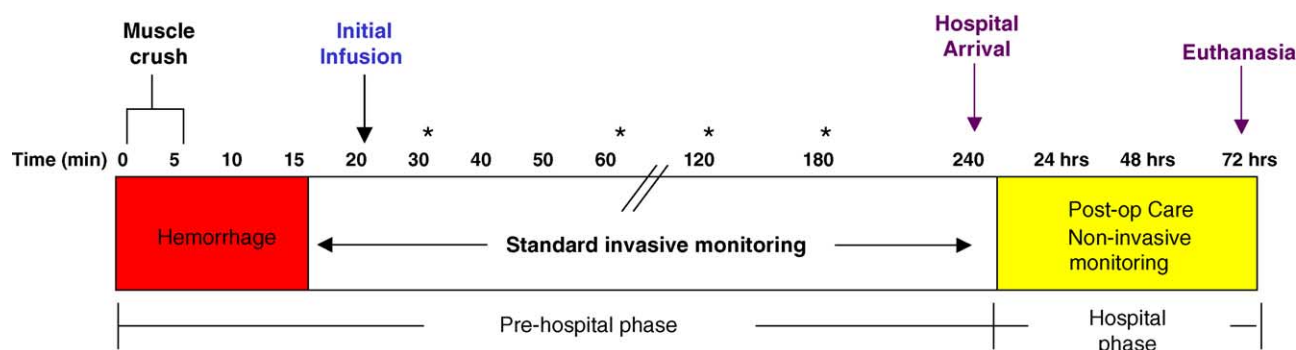
Fig. 1. Experimental design. The muscle crush and start of the hemorrhage denoted the beginning of the experiment (time 0). Fluid resuscitation was initiated at 20 min (10 ml/kg over 10 min) and additional infusions (*) (5 ml/kg over 10 min) were provided for mean arterial pressure (MAP) < 60 mmHg or heart rate (HR) > baseline. Pre-hospital 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.

2.3. Hospital phase: recovery and long term survival

To assess HBOC-201 in the context of militarily relevant prolonged transportation delay, 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 without anemia. The PAC was removed, jugular vein introducer secured for postoperative blood sampling and fluid administration, and arterial and bladder catheters removed. Surgical incisions were closed and surgical dressings applied. Animals were extubated and recovered from anesthesia. Vital signs and general health status (including feed consumption, incidence of vomiting, and overall physical activity) were assessed 24, 48, and 72 h post-injury. At these later time points, pigs received additional antibiotic, analgesic, and 10 ml/kg shed blood or saline as needed for anemia or hypotension. Pigs were euthanized 72 h post-injury for necropsy and histological analysis.

2.4. Data collection

Standard invasive and noninvasive hemodynamic parameters were monitored for 240 min during the simulated pre-hospital phase (Fig. 1). Transcutaneous tissue oxygenation (tcpO$_2$) was noninvasively measured with a TCM4 Tina monitor (Radiometer, Copenhagen, Denmark) using four Clark type polarographic electrodes (data represent mean values) positioned bilaterally on the upper torso and on the inner thighs. Arterial (ABG) (carotid) and mixed venous (MVBG) blood gases (from PA catheter) were measured with an automatic analyzer (ABL 705, Radiometer). Oxygen consumption, delivery, and extraction ratio were calculated using the following formulas:

$$VO_2 = \frac{cardiac\ output \times 13.4 \times hemoglobin \times [SaO_2 - S\bar{v}O_2]}{100}$$

$$DO_2 = \frac{cardiac\ output \times 13.4 \times hemoglobin \times SaO_2}{100}$$

$$O_2ER = \frac{VO_2}{DO_2} \times 100$$

Blood samples were collected for complete blood counts (CBC, Pentra 60 C+, ABX, France) and serum chemistries including lactate (Vitros 250 Analyzer, Ortho).

2.5. Statistical analysis

The statistical techniques were either cross-sectional or longitudinal in nature. The cross-sectional analyses compared outcomes of interest at a single point in time or aggregated over time. For these comparisons, analysis of variance (ANOVA) 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 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, N.C.). Data are expressed as mean ± standard error of the mean (S.E.M.) for animals alive at time of measurement.

3. Results

Animals in HBOC, HEX, and non-treatment groups were similar in terms of sex distribution, body weight, hemorrhage volume, hemorrhage percent of EBV, and percent total blood loss including blood drawn for laboratory analysis (Table 1).

Table 1
Demographics and hemorrhage volume

Group	Gender ratio (M/F)	Weight (kg)	Hemorrhage volume (ml)	Hemorrhage (%) EBV	Total blood loss (including lab samples) (%)
HBOC	3/5	26.7 ± 1.3	695.8 ± 35.1	40.0 ± 0.08	50.7 ± 0.7
HEX	4/4	27.1 ± 1.3	705.2 ± 34.5	40.0 ± 0.00	51.0 ± 0.7
NON	5/3	26.1 ± 1.8	678.6 ± 47.7	40.0 ± 0.00	51.3 ± 0.8

Gender ratio, weight, hemorrhage volume, hemorrhage % estimated blood volume (EBV), and percent total blood loss for HBOC, HEX, and NON groups. Values (except gender ratio) are presented as group means ± S.E.M. There were no significant differences between groups.

3.1. Hemorrhagic shock

All animals responded similarly to hemorrhage and no significant differences were observed between groups at time 0 and time 15 (Fig. 2). Following 40% EBV hemorrhage, there was a 61% decrease in MAP (66.7 ± 2.6–26.0 ± 2.1 mmHg, $p < 0.001$), 15% increase in HR (144.6 ± 5.0–$170.3 \pm$ 6.7 bpm), 39% decrease in cardiac index (CI) (4.9 ± 0.6–3.0 ± 0.3 L/min/m^2, $p < 0.03$), 61% decrease in mean pulmonary arterial pressure (MPAP) (18.0 ± 0.2–11.2 ± 0.5 mmHg), and 32% decrease in pulmonary arterial wedge pressure (PAWP) (8.1 ± 6.7–5.5 ± 5.3 mmHg). Additionally, an 80% decrease in tcpO$_2$ (10.8 ± 1.5–2.1 ± 0.8 mmHg) was observed following hemorrhage in all animals ($p < 0.006$).
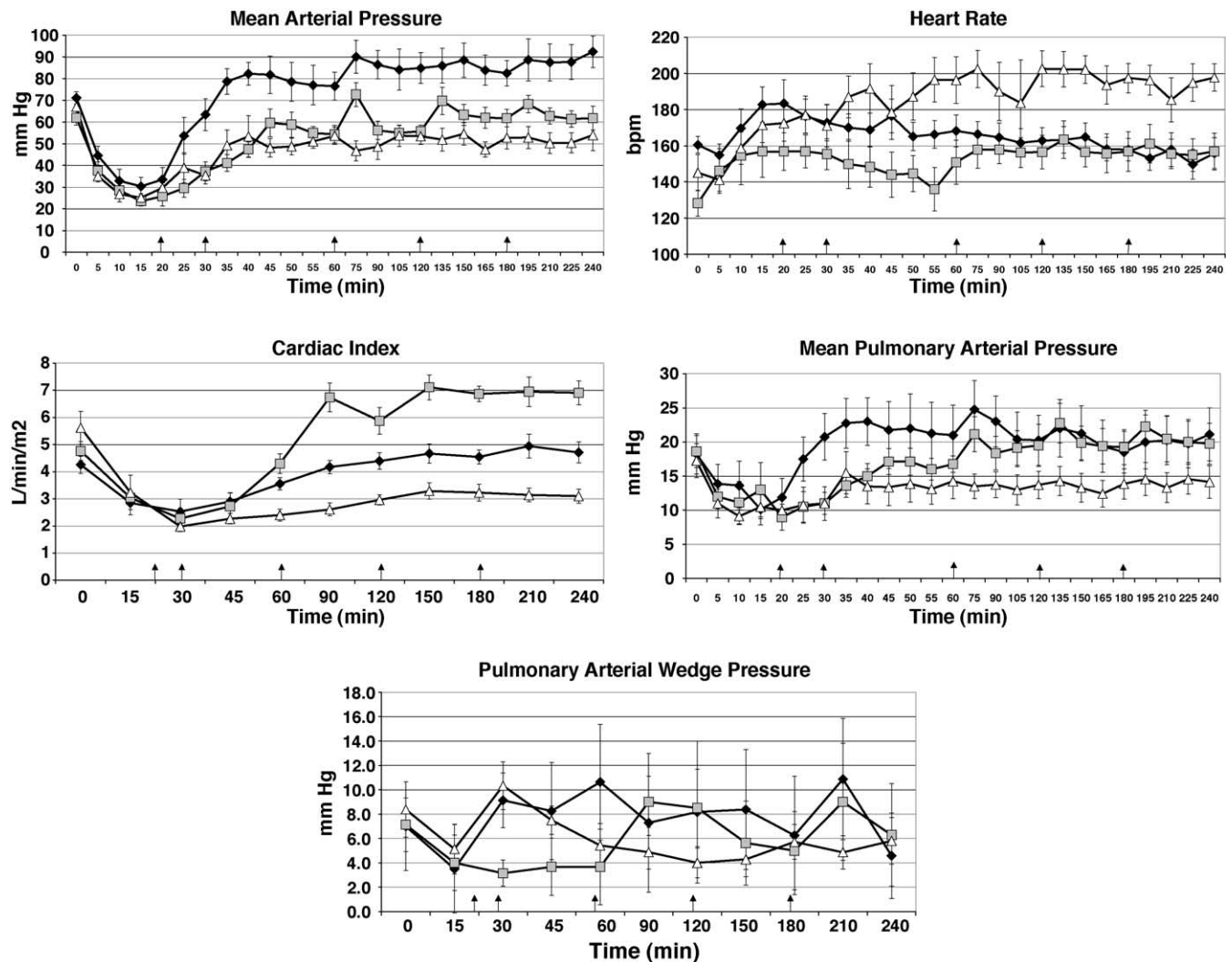

Fig. 2. Hemodynamic changes in swine with controlled hemorrhage resuscitated with HBOC, HEX, or NON (no resuscitation). Values are presented as group mean ± S.E.M. The start of the experiment is denoted as time 0 and arrows indicate times of infusion throughout the 4-h pre-hospital phase. There were significant time by group interactions for mean arterial pressure ($p = 0.002$), heart rate ($p < 0.0001$), cardiac index ($p < 0.0001$), and mean pulmonary arterial pressure ($p = 0.02$). Wedge pressure approached significance over time ($p = 0.05$). Groups are represented as HBOC (◆), HEX (▢), and NON (△).

3.2. Pre-hospital phase

3.2.1. Hemodynamics

Mean arterial pressure (MAP) was significantly different between groups over time during the pre-hospital phase ($p = 0.002$) (Fig. 2). At 35 min, MAP was restored to baseline in the HBOC group (78.6 ± 5.9 mmHg) and remained elevated throughout the pre-hospital phase. In the HEX group, MAP did not reach baseline until 135 min although a transient post-infusion spike in MAP was observed following the 60-min infusion. In NON pigs, MAP was auto-regulated such that MAP increased following hemorrhage to ~ 52 mmHg without resuscitation; however, MAP never returned to baseline in this group.

HR was significantly different between groups over time ($p < 0.001$). In HBOC pigs, HR returned to baseline by 165 min while HEX and NON pigs remained tachycardic throughout the pre-hospital phase. CI in the groups differed over time ($p < 0.001$). In HBOC pigs, CI was restored by 120 min and remained slightly above baseline until 4 h. CI was highest in HEX pigs, and by 90 min was significantly higher than in HBOC pigs (6.7 ± 0.5 versus 4.2 ± 0.3 L/min/m^2 respectively, $p < 0.001$); CI in NON pigs remained markedly depressed throughout the pre-hospital phase. A marked increase in systemic vascular resistance index (SVRI) was observed in the HBOC group immediately following the initial infusion although values were not significantly different between groups over time (data not shown). MPAP was significantly different between groups over time ($p = 0.02$). In the HBOC group, a rapid and sustained increase in MPAP was observed following the first infusion; reaching baseline by 30 min. In the HEX group, MPAP returned to baseline by 120 min and subsequently was similar to the HBOC group. In contrast, MPAP remained below baseline in the NON group for the entire pre-hospital phase. PAWP approached significance between groups over time ($p = 0.05$), and was lower in the HEX pigs in comparison to HBOC and NON pigs at the 30 and 45 min time points. Although differences were insignificant after these time points, substantial variability was observed in the PAWP values in all groups.

3.2.2. Fluid requirements

In comparison to HEX pigs, HBOC pigs had decreased fluid requirements at 60, 120, and 180 min ($p < 0.05$) (Fig. 3). Total pre-hospital fluid requirements (18.8 ± 1.8 and 29.9 ± 1.1 ml/kg in the HBOC and HEX pigs, respectively) were also significantly decreased ($p = 0.001$).

3.2.3. Indirect and direct measures of tissue oxygenation

O$_2$ consumption (VO$_2$) was significantly different between groups over time ($p = 0.02$). At 60 min, VO$_2$ was higher in HBOC (105.2 ± 11.9 ml/min/m^2) compared to HEX pigs (91.8 ± 9.7 ml/min/m^2). O$_2$ delivery (DO$_2$) and O$_2$ extraction ratio (O$_2$ER) were not significantly different between groups ($p = 0.67$ and 0.09, respectively) (Fig. 4), although, DO$_2$ was consistently higher in HBOC than in HEX
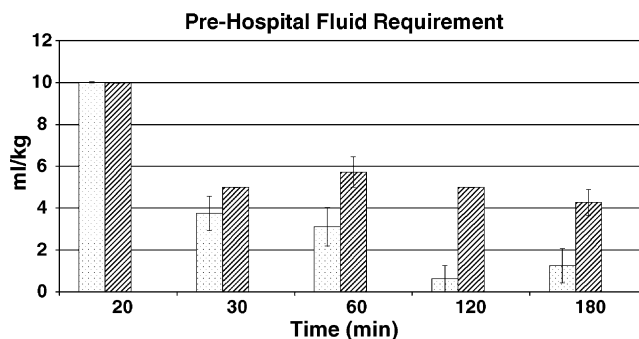

Fig. 3. Total fluid requirements during the pre-hospital phase. All pigs received an initial infusion of 10 ml/kg at time 20. Additional fluids were provided at 5 ml/kg for hypotension or tachycardia at time 30, 60, 120, and 180. Values are presented as group means ± S.E.M. HBOC pigs had significantly decreased fluid requirements at Time 60, 120, and 180 ($p < 0.05$). NON pigs were not administered fluids in the pre-hospital phase. Groups are represented as HBOC (□), HEX (▨).

or NON pigs at all time points from 30 min. Although not significantly different between groups, blood lactate (LA) levels in all groups were elevated during the first hour of the pre-hospital phase before returning to baseline (Fig. 4). The tcpO$_2$ was significantly different between groups over time ($p < 0.001$). The tcpO$_2$ levels were 26.2 ± 6.6 mmHg in HBOC-treated pigs and 9.8 ± 3.6 mmHg in HEX-treated pigs at 60 min, and remained significantly higher in HBOC pigs compared to HEX or NON pigs throughout the pre-hospital phase (Fig. 4). Base excess (BE) was not significantly different between groups (Fig. 4). Mixed venous O$_2$ saturation (SⅴO$_2$) was consistently higher in the HBOC group although no statistically significant difference was observed between groups (Fig. 4).

3.2.4. Renal function

Urine output (UO) during the pre-hospital phase was similar in HBOC and HEX pigs (3.2 ± 0.4 versus 3.5 ± 0.7 ml/kg, respectively) (Fig. 5). Although the difference was not significant, UO was higher in these groups compared to NON pigs (1.6 ± 0.8 ml/kg). Blood creatinine measurements in HEX pigs were unchanged throughout the pre-hospital phase and slight but non-significant increases were observed in the NON pigs at 3 and 4 h (Table 2). Laboratory assay interference from HBOC-201 precluded evaluation of the creatinine values in the HBOC group during the pre-hospital phase. Blood urea nitrogen (BUN) levels were elevated in all groups during the pre-hospital phase with no significant differences between groups (Table 2).

3.2.5. Hemoglobin and hematocrit

Baseline Hb and hematocrit (Hct) were similar across groups (Table 3). HBOC pigs received hemoglobin loads of ~ 1 g/kg after the 1st infusion and a total of 2.35 g/kg after all infusions. From 30 min, Hb was significantly reduced only in HEX pigs (HBOC versus HEX, $p < 0.001$; HEX versus NON, $p = 0.02$) and reached a nadir of 4.9 ± 0.2 at 240 min. In con-
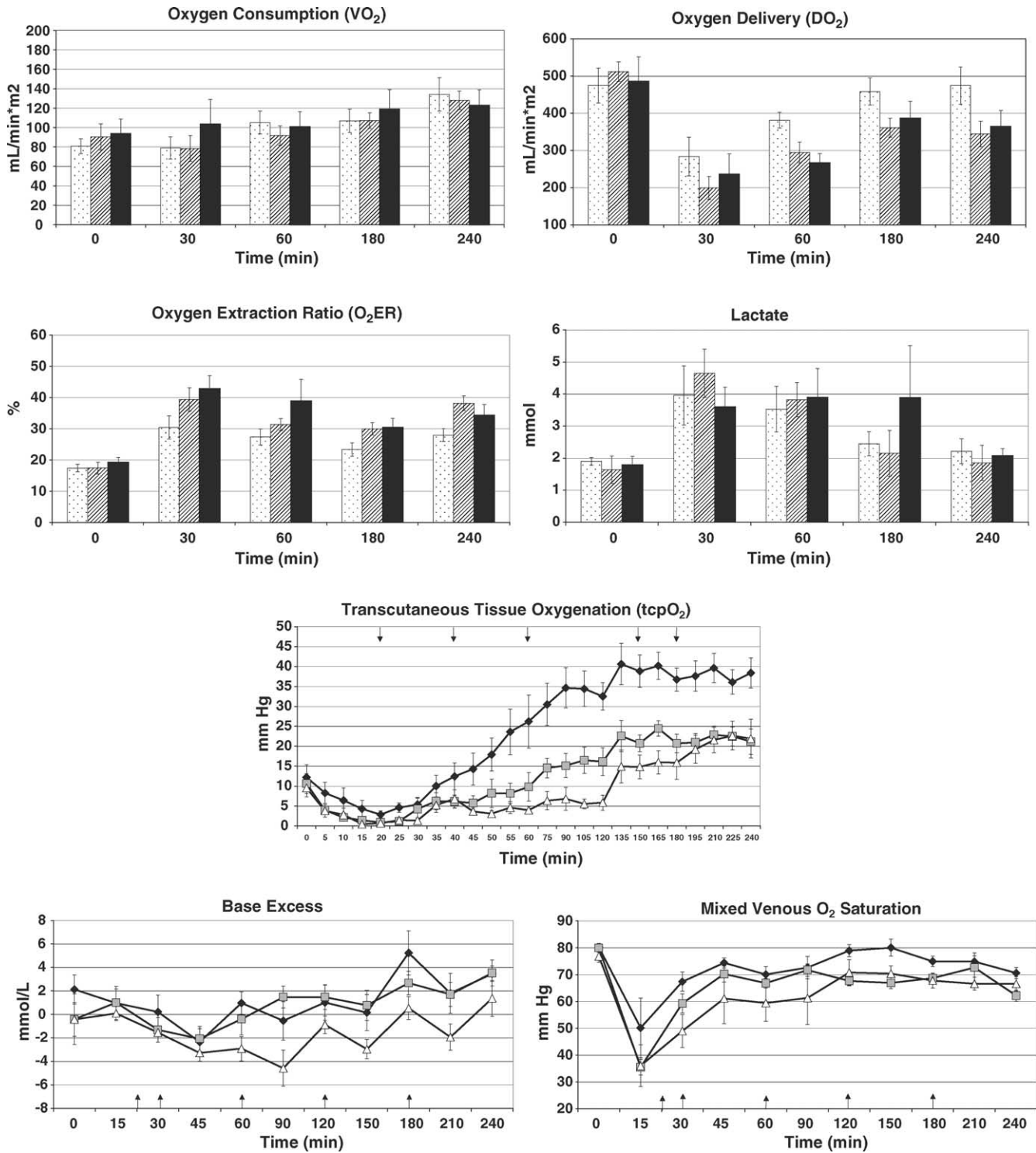
Fig. 4. Direct and indirect measures of tissue oxygenation. Values are presented as group means ± S.E.M. Oxygen consumption was significantly different between groups over time ($p = 0.02$). Tissue oxygenation as measured by transcutaneous oxygen pressure was significantly higher in the HBOC group over time ($p < 0.001$). Arrows indicated times of infusion. Groups are represented as HBOC (▥) HEX (▨) and NON (■), HBOC (◆), HEX (▢), and NON (△).

trast, Hb remained fairly constant throughout the pre-hospital phase in HBOC and NON pigs, and was not significantly different from baseline at 240 min ($p = 0.15$). Hct dropped significantly from baseline in HBOC and HEX pigs ($p < 0.001$) but was elevated in NON pigs at 60 and 180 min.

3.3. Hospital Phase

At simulated hospital arrival (4 h), total fluid requirements (blood and saline) were reduced in HBOC compared to HEX or NON pigs ($p < 0.001$) (Fig. 6, top). At this time point,
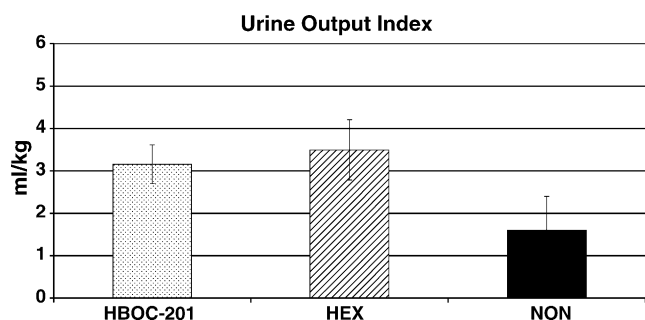
Urine Output Index

Fig. 5. Total urine output during the pre-hospital phase. Urine was collected via bladder catheterization. Values are presented as group means ± S.E.M. No significant differences were observed between groups.

HBOC pigs did not meet criteria for fluid infusions (anemia or hypotension), whereas all HEX pigs required saline (2/8) or blood (6/8), and all NON pigs required saline (7/7). Blood transfusion requirements (determined by Hb levels) were significantly less in HBOC (1.3 ± 1.3 ml/kg) compared to HEX pigs (9.4 ± 0.6 ml/kg) ($p < 0.001$) during the hospital phase (Fig. 6, bottom). Blood Hb was highest and the Hct lowest in the HBOC group at 24 h compared to the HEX and NON groups (Table 4). Hb and Hct remained below baseline (see Table 3) in all groups at 72 h.

During the hospital phase (Table 4), MAP, HR, and temperature were similar among surviving pigs in all groups. Temperatures were elevated in all pigs at 24, 48, and 72 h compared to the pre-hospital phase. LA and creatinine were not different between groups in the hospital phase. Postoperative observations indicated that pigs in the HBOC group were more active compared to pigs in the HEX or NON

Fluid Requirement (4, 24, 48 hours)

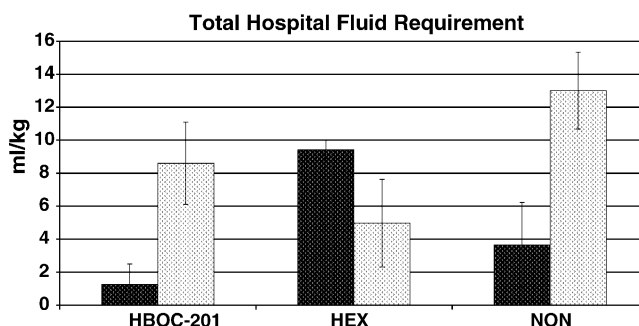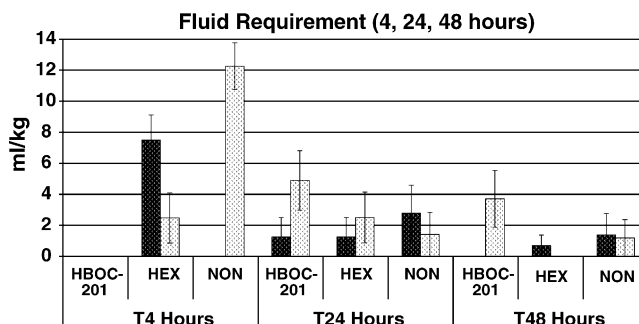**Total Hospital Fluid Requirement**

Fig. 6. Fluid requirements at 4, 24, and 48 h (top) and total hospital fluid requirements (bottom). Pigs were administered 10 ml/kg blood for anemia (hemoglobin levels < 7 g/dl) or saline for hypotension (mean arterial pressure < 60 mmHg) at 4, 24, and 48 h. Blood and saline requirements were significantly reduced in HBOC pigs at 4 h ($p < 0.001$, top) compared to HEX and NON pigs. Blood transfusion requirements were significantly less in HBOC pigs compared to HEX pigs in the hospital phase ($p < 0.001$, bottom). Values are presented as group means ± S.E.M. Fluids are represented as (blood (▦) and saline (▥)).

Table 2
Renal function variables

Variable	Group	Baseline	30 min	60 min	180 min	240 min
Creatinine (mg/dl)	HBOC	0.8 ± 0.0	ND	ND	ND	ND
	HEX	0.8 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.0
	NON	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.3 ± 0.1	1.4 ± 0.1
Blood Urea Nitrogen (mg/dl)	HBOC	14.4 ± 1.7	11.6 ± 2.2	12.3 ± 2.3	19.4 ± 1.9	20.1 ± 3.3
	HEX	11.6 ± 1.2	12.8 ± 1.1	13.0 ± 1.2	15.5 ± 1.8	16.9 ± 1.9
	NON	11.0 ± 1.4	13.8 ± 2.0	13.4 ± 1.5	16.0 ± 2.7	18.9 ± 3.0

Creatine and blood urea nitrogen levels for HBOC, HEX, and NON groups. Values are presented as group means ± S.E.M. Laboratory assay interference from HBOC-201 precluded evaluation of the creatinine values in the HBOC group during the pre-hospital phase (indicated as ND in the table). No significant differences were observed between groups.

Table 3
Hemoglobin and hematocrit

Variable	Group	Baseline	30 min	60 min	180 min	240 min
Hemoglobin (g/dl)	HBOC	10.7 ± 0.3	11.0 ± 0.4	10.5 ± 0.4	9.6 ± 0.2	9.6 ± 0.2
	HEX	10.4 ± 0.3	8.4 ± 0.4	6.7 ± 0.5	5.0 ± 0.3	4.9 ± 0.2
	NON	9.9 ± 0.4	10.4 ± 0.4	11.5 ± 0.5	11.1 ± 0.5	10.9 ± 0.6
Hematocrit (%)	HBOC	30.4 ± 0.9	25.0 ± 1.1	21.3 ± 1.1	17.0 ± 0.8	16.5 ± 0.8
	HEX	29.3 ± 0.7	23.5 ± 1.2	18.6 ± 1.5	14.0 ± 1.0	13.5 ± 0.7
	NON	28.3 ± 1.0	29.7 ± 1.0	33.2 ± 1.4	32.0 ± 1.4	27.9 ± 4.5

Hemoglobin and hematocrit values for HBOC, HEX, and NON groups during the pre-hospital phase. Values are presented as group means ± S.E.M. Hemoglobin was significantly reduced in HEX pigs compared to HBOC ($p < 0.001$) and NON pigs ($p = 0.02$) from time 30. Hematocrit was significantly reduced from baseline in HBOC and HEX pigs ($p < 0.001$) compared to NON pigs from time 30.

Table 4
Post-hospital physiologic variables

Variable	Group	24 h	48 h	72 h
MAP (mmHg)	HBOC	78.2 ± 7.2	67.4 ± 7.8	82.1 ± 10.6
	HEX	73.6 ± 7.8	93.8 ± 8.8	74.4 ± 12.6
	NON	70.8 ± 15.7	105.6 ± 10.3	73.6 ± 6.7
HR (bpm)	HBOC	98.7 ± 15.2	105.5 ± 8.8	125.3 ± 13.6
	HEX	108.7 ± 10.1	128.0 ± 9.0	145.7 ± 17.4
	NON	121.6 ± 5.1	134.2 ± 10.7	121.2 ± 11.6
Temp (^{o}F)	HBOC	101.2 ± 0.3	101.9 ± 0.5	102.5 ± 0.5
	HEX	101.6 ± 0.3	102.7 ± 0.1	102.4 ± 0.2
	NON	101.6 ± 0.2	102.1 ± 0.5	102.1 ± 0.9
Hemoglobin (g/dl)	HBOC	8.7 ± 0.5	8.7 ± 0.3	7.6 ± 0.5
	HEX	8.4 ± 0.3	8.9 ± 0.5	9.7 ± 0.4
	NON	8.0 ± 0.6	7.8 ± 0.4	7.8 ± 0.4
Hematocrit (%)	HBOC	18.9 ± 0.7	22.0 ± 1.5	20.5 ± 1.1
	HEX	24.1 ± 0.8	25.1 ± 1.6	27.5 ± 0.8
	NON	23.0 ± 1.8	22.0 ± 1.5	22.2 ± 1.0
Lactate (mmol/l)	HBOC	2.0 ± 0.3	2.1 ± 0.4	1.4 ± 0.1
	HEX	1.5 ± 0.4	3.6 ± 1.7	1.5 ± 0.5
	NON	1.5 ± 0.3	1.1 ± 0.2	1.1 ± 0.2
Creatinine (mg/dl)	HBOC	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
	HEX	0.9 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
	NON	1.0 ± 0.1	0.8 ± 0.0	0.8 ± 0.1

Hemodynamic and laboratory values for HBOC, HEX, and NON groups during the hospital phase (4–72 h). Values are presented as group means ± S.E.M. No significant differences were observed between groups.

groups. Feed consumption was decreased and the incidence of vomiting was higher in HBOC compared to HEX and NON pigs (data not shown).

3.4. Survival

Survival to simulated hospital arrival was 100% (8/8) in both the HBOC and HEX groups compared to 88% (7/8) in the NON group ($p = 0.27$). There were no significant survival differences between groups upon stratification based on gender (data not shown). Lower BE was associated with survival to simulated hospital arrival ($p = 0.03$). In the HBOC, HEX, and NON groups, respective survival rates to 72 h were 8/8 (100%), 7/8 (88%), and 5/8 (63%) (Fig. 7) ($p = 0.3$). The
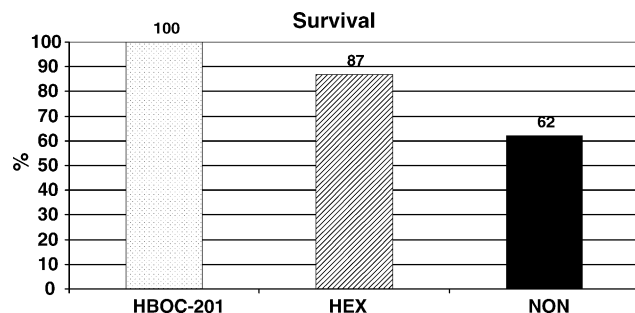

Fig. 7. Percent survival to 72 h for pigs resuscitated with HBOC (☐) HEX (▨) and NON (■). 8/8 HBOC pigs, 7/8 HEX pigs, and 5/8 NON pigs survived to the study end point (72 h). No significant differences were observed between groups.

three pigs that died after hospital arrival (one HEX and two NON pigs) never recovered from anesthesia or had immediate post-operative serious complications, met criteria for early euthanasia, and thus did not survive to the 24-h time point.

4. Discussion

Extensive evaluation of HBOC-201 and Oxyglobin® (FDA-approved veterinary HBOC) has been completed in large animal models of controlled hemorrhage (Table 5). In the present model of controlled hemorrhage with 40% EBV loss we demonstrated that low volume resuscitation with HBOC-201 restored hemodynamics, including CI, improved tissue oxygenation, maintained urine output, stabilized mixed venous oxygen saturation, and decreased fluid and blood transfusion requirements despite evidence of vasoactivity.

The data demonstrate that pigs resuscitated with HBOC-201 maintained hemodynamics with fewer infusions than pigs resuscitated with HEX. For example, 15 ml/kg of HBOC-201 versus 25 ml/kg of HEX was required to restore baseline MAP. With a similar hemorrhage volume (40% EBV), Driessen et al. reported comparable results, observing that 1/3 less Hb-200 (Oxyglobin) than HEX was required to restore blood pressure and central venous pressure in dogs [11]. Although both groups ultimately received 30 ml/kg of resuscitation fluid, dogs resuscitated with Hb-200 had improved blood pressures compared to HEX resuscitated dogs. McNeil et al. observed that less HBOC (7.4 ml/kg) was needed to resuscitate hemorrhaged pigs to a target MAP of 60 mmHg, when compared to blood (24.1 ml/kg) [3]. They observed that, compared to blood, lower volumes of HBOC-201 allowed equivalent improvement in metabolic parameters (i.e., pH, BE, and LA) and led to a reversal of anaerobic metabolism. Although we are uncertain as to whether decreased fluid requirements will translate into a direct clinical benefit (e.g., less hemodilution coagulopathy/thrombopathy or ARDS), any diminution in the volume of fluid that military corpsmen/medics need to carry in the field is significant.

On the battlefield, where blood is often unavailable and logistical constraints limit the amount of supplies that can be carried, a fluid that offers physical (e.g., no refrigeration requirement) and physiological (i.e. restores hemodynamics, reverses anaerobic metabolism) benefits over other resuscitation therapies at lower volumes would be extremely valuable. Recent interest in the use of HBOC by the military for the treatment of combat casualties has emphasized the potential of HBOC to provide adequate low-volume resuscitation and alleviate immediate need for blood transfusion. Further, civilian clinical trials to evaluate transfusion requirements following HBOC infusions in a hospital setting have been completed. In a randomized clinical trial in surgical patients comparing intraoperative infusions of HBOC-201 and LR, no differences were observed in post-operative trans-

Table 5
Recent publications of large animal controlled hemorrhage models investigating HBOC-201 or oxyglobin for resuscitation of hemorrhagic shock

Study	Year	Model	HBOC	n	Interventions	Resuscitation	Monitoring (h)	Outcome in HBOC groups
Cheung [21]	2004	Dogs	Oxyglobin	9	Hemorrhaged 40% EBV to MAP 50 mmHg over 30–45 min	Oxyglobin (OXY) 10 ml/kg vs. shed blood 30 ml/kg vs. hetastarch (HEX) 30 ml/kg 6%	3	Restoration of microvascular and systemic variables without restoration of total oxygen content
Fitzpatrick [18]	2004	Swine	HBOC-201	27	Hemorrhaged to MAP 30 mmHg and held for 45 min	HBOC-201 vs. blood vs. Lactated Ringers (LR) + blood	4	MAP restored to baseline, significantly reduced fluid requirement, lactate and BE normalized, cardiac output (CO) depressed, heart rate (HR), pulmonary vascular resistance (PVR), systemic vascular resistance (SVR), and methemoglobin elevated, mixed venous oxygen saturation ($S\bar{v}O_2$) lower, persistent vasoconstriction
Driessen [11]	2003	Dogs	Oxyglobin	24	Hemorrhaged 40% EBV over 30 min (average MAP 50 mmHg) held for 60 min	OXY 30 ml/kg vs. OXY 20 ml/kg/HEX 10 mg/kg vs. OXY 10 ml/kg/HEX 20 ml/kg vs. HEX 30 ml/kg	3	MAP, mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), pulmonary wedge pressure (PAWP), SVRI elevated, more rapid reversal of anaerobic cell metabolism, CI lower, no augmentation in total hemoglobin
Knudson [17]	2003	Swine	HBOC-201	30	Hemorrhaged to MAP 40 mmHg and held for 20 min	HBOC-201 6 ml/kg vs. LR 12 ml/kg vs. hypertonic saline dextran (HSD) 4 ml/kg	2	MAP restored and maintained above baseline, CO remained below baseline, liver pO_2 significantly lower, persistent tachycardia (in all groups)
Sampson [4]	2003	Swine	HBOC-201	42	Hemorrhaged to MAP of 30 mmHg and held for 45 min	HBOC-201 vs. hypertonic saline (HTS) vs. HSD vs. pentastarch vs. HEX vs. LR vs. no fluid, to MAP of 60 mmHg	4	Reversed anaerobic metabolism, lower fluid requirement, increased tissue oxygenation, 100% survival, lower CO, $S\bar{v}O_2$, and urine output (UO)
York [2]	2003	Swine	HBOC-201	24	Hemorrhaged to MAP of 30 mmHg and held for 45 min	HBOC-201 to 60 mmHg vs. shed blood to baseline MAP vs. shed blood to 60 mmHg vs. LR + blood to baseline MAP	72	Tissue oxygenation restored, equivalent survival, significantly less fluid required, normalization of arterial pH and serum lactate, no long-term organ dysfunction, CO and UO lower, mild hepatocellular damage
Lee [19]	2002	Swine	HBOC-201	7	Hemorrhaged to MAP of 40 mmHg and held for 20 min	HBOC-201 6 ml/kg bolus	2	MAP and CO restored, brain oxygen pressure increased over baseline
McNeil [3]	2001	Swine	HBOC-201	30	Hemorrhaged to MAP of 30 mmHg and held for 45 min	HBOC-201 vs. LR vs. LR + blood vs. blood to MAP of 50 or 60 mmHg or baseline	4	Reversal of anaerobic metabolism in HBOC 60 mmHg group as measured by lactate, base excess, and arterial pH, no differences in mortality, lower CO, pulmonary arterial wedge pressure, and MAP, significantly lower $S\bar{v}O_2$, worse outcome in HBOC 50 mmHg group
Moon [22]	2001	Pregnant sheep	Oxyglobin	15	Hemorrhaged 20 ml/kg sequentially over 1 h	OXY 20 ml/kg vs. HEX 20 ml/kg vs. shed blood 20 ml/kg over 30 min	2	Maternal and fetal oxygen content restored to baseline, maternal blood pressure increased above baseline, maternal oxygen saturation decreased, no change in fetal plasma-free hemoglobin

fusion requirements between study groups [12]. Thirty-six of 42 (85.7%) HBOC patients versus 21 of 26 (80.8%) LR patients required blood transfusion during their hospitalization period. However, in a randomized, double blind trial comparing allogenic RBCs with HBOC-201 post-operatively in cardiac patients, transfusion avoidance was demonstrated in the HBOC-201 group: following initial infusion with HBOC-201 or RBCs, 33 of 50 (66%) HBOC-201- versus 48 of 48 (100%) RBCs-subjects required additional blood transfusions [13]. Also, HBOC-201 patients required fewer transfusions than patients initially infused with RBCs (1.72 versus 2.19 units, respectively, $p = 0.05$). We observed a similar and also significant decrease in transfusion requirements postoperatively in HBOC-201- compared with HEX-resuscitated pigs up to 48 h post-injury. Based on our results, the use of HBOC-201 could reduce the need for blood transfusions and further conserve the limited supply of blood in a combat field setting, thus allowing available blood to go to highest priority casualties. Additionally, decreasing the need and demand for blood would reduce logistic difficulties and risk of continually supplying fresh blood to forward field hospitals, thus, avoiding scenarios where available blood supplies are rapidly exhausted precluding adequate resuscitation of all HS casualties.

Reduction of CO and/or UO has been reported by other investigators using HBOC-201 for resuscitation from HS [3,11,13,17,18]. York et al. showed that low-volume resuscitation with HBOC-201 (targeted MAP 60 mmHg) in swine restored tissue oxygenation and blood pH; however, CO and UO were lower compared with shed blood [2]. In previous research by the same group, CO in the HBOC-201 group did not return to baseline throughout the experiment, and decreased UO and $S\bar{v}O_2$ were also observed in comparison to the HEX group [4]. In our model, CI in pigs resuscitated with HBOC-201 did return to baseline and UO was comparable to the HEX group; however, these pigs received larger volumes of HBOC-201 (~16.9 ml/kg) than pigs in the Sampson experiments (~7.9 ml/kg). In both experiments, HEX resuscitated pigs had higher CO compared to HBOC pigs; however, HEX pigs received significantly larger volumes of fluid compared to HBOC pigs in these experiments (~27.5 ml/kg) and in our study (30.0 ml/kg). Despite these differences in fluid volumes and CO, measured metabolic variables were similar between HBOC and HEX-treated pigs in both experiments.

Due to shunting of blood from the integument, cutaneous oxygenation should be a sensitive indicator of global perfusion and an early manifestation of physiological compensation to HS. Although not the indicator for resuscitation in this study, direct and indirect measures of tissue oxygenation were similar or better ($tcpO_2$) in the HBOC-201 group. Noninvasive monitoring has been evaluated as a substitute for invasive PA catheters when their use is impractical (i.e., bioimpedance for CO, SaO_2 for pulmonary function, BP as a marker of overall circulatory status, and $tcpO_2$ for tissue perfusion) [14]. In general, $tcpO_2$ has been reported to correlate with $S\bar{v}O_2$, however, peripheral shunting in late shock can lead to mis-

leading relatively elevated $S\bar{v}O_2$ [15]. In clinical trials, $tcpO_2$ has correlated well with survival in critical emergency room patients (including blunt trauma) [14,16]. Shoemaker found that $tcpO_2$ distinguished survivors (mean $tcpO_2$ index 206) from non-survivors (mean $tcpO_2$ index 92) better than other noninvasive monitoring techniques ($p < 0.001$).

Overall, in our experiment, HBOC-201 resuscitation resulted in similar global and indirect (e.g., VO_2, DO_2, lactate, and base deficit) but improved direct (i.e., $tcpO_2$) measures of tissue oxygenation, in comparison with HEX. These results may reflect the moderate severity of this model, in which tissue oxygenation per se was improved, but compensatory mechanisms precluded detecting global systemic differences. It may be surprising that $tcpO_2$ stabilized even in NON pigs, but presumably, autotransfusion and other compensatory mechanisms led to increases in MAP, CO, DO_2, and O_2ER, and consequently improved $tcpO_2$.

Other investigators using more invasive techniques to monitor tissue oxygenation have reported contradictory findings. Using deltoid muscle tissue oxygen probes, Knudson et al. observed that PO_2 returned to baseline similarly in pigs resuscitated with 6 ml/kg of HBOC-201 or 12 ml/kg of LR [17]. However, pigs were ventilated with 100% O_2 and the beneficial effects of HBOC-201 on tissue oxygenation may have been compromised. Lee et al. evaluated small volume HBOC-201 (6 ml/kg) resuscitation on brain tissue oxygenation following hemorrhage, and observed increased brain pO_2 over baseline values in pigs receiving 100% O_2 [19]. In our model, where pigs were maintained with 21% O_2, $tcpO_2$ returned to baseline after only two HBOC-201 (15 ml/kg) versus three HEX (20 ml/kg) infusions. Hare et al. observed increased brain tissue oxygenation following hemodilution with a hemoglobin raffimer (Hemolink™) and observed increased oxygenation without increased regional cerebral blood flow [20].

In contrast to other investigators, we did not observe differences between HBOC-201 and HEX groups regarding indirect indicators of tissue oxygenation (i.e., LA, BE, $S\bar{v}O_2$). However, in previous experiments with more severe HS, we observed a trend toward more rapid lactate clearance and significantly improved base excess in HBOC-201- in comparison to HEX-resuscitated pigs [1]. Manning et al. reported a decrease in blood lactate levels with HBOC-201 in a similar swine uncontrolled HS liver injury model [5]. Diminished base deficit following HBOC-201 resuscitation was also reported by the same research group [6]. Although other investigators observed decreased $S\bar{v}O_2$ following HBOC-201 resuscitation [3,4], we did not observe significant differences between groups.

Hemoglobin solutions have vasoactive properties (systemic and pulmonary hypertension) reportedly due to hemoglobin's high affinity for nitric oxide (especially due to tetrameric hemoglobin) and subsequent smooth muscle contraction. While increases in MPAP and SVRI following infusion of HBOC-201 have been observed [4,13], some investigations did not report systemic or pulmonary effects

with HBOC-201 [3,17,19]. It is possible that volume of infusion may play a role in the pressure effect of HBOC-201 as lower volumes do not appear to result in observed effects on pulmonary pressure. For example, Lee et al. resuscitated pigs with 6 ml/kg HBOC-201 and did not see an increase above baseline of pulmonary artery diastolic pressure [19]. Although systemic and pulmonary vasoconstriction could result in decreased cardiac output and tissue perfusion and oxygenation, tissue oxygenation was actually improved in this model. Cardiac output was lower in HBOC-201—than HEX-treated pigs, but it was restored to baseline. As well, increased bleeding was not seen in a previously reported uncontrolled hemorrhage solid organ injury model [1]. Although, mildly increased pulmonary arterial pressure was present it is unclear to what extent it is clinically significant.

The results of this study should be tempered by a number of limitations. First, as the study was not powered for survival, there may have been significant differences that were undetected in this small study (i.e., type II error). Second, the strict criteria for fluid and blood infusions ensured control for the purposes of group comparisons, but may be overly simplistic for extrapolation to real clinical scenarios. For example, use of anemia alone as the blood transfusion criterion in this study often precluded need for blood transfusions in NON pigs despite loss of 40% of blood volume. Third, although hetastarch is a good control fluid in terms of colloidal properties, crystalloid fluids are more widely used in the pre-hospital setting in the U.S. However, McNeil et al.'s results were similar in a swine study comparing HBOC-201 and LR [3].

5. Conclusions

In summary, in a moderately severe controlled hemorrhage swine model incorporating soft tissue injury and simulated 4-h delay to definitive medical care, low volume HBOC-201 resuscitation stabilized hemodynamics, decreased fluid and blood transfusion requirements, and increased transcutaneous tissue oxygenation, in comparison to HEX or no resuscitation. However, indirect global measures of tissue oxygenation (e.g., lactic acidosis) and survival were similar with HBOC-201 and HEX. Thus, overall, HBOC-201 was at least as efficacious as HEX and possibly superior, as a pre-hospital resuscitative fluid for hemorrhagic shock. Further clinical evaluation is warranted to confirm these findings in humans with traumatic HS.

Conflict of interest statements

L. Bruce Pearce is an employee of Biopure Corp. and has financial interest in the subject material, HBOC-201. Dr. Pearce's contribution to this manuscript was limited to study design, protocol authorship, and editorial review. The Naval Medical Research Center (NMRC) and Biopure Corp. have a

Cooperative Research and Development Agreement for evaluation of HBOC-201 in trauma clinical trials, and a *Material Transfer Agreement* for supply of HBOC-201 material for pre-clinical studies. There are no transfers of funds in either of these agreements.

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