Prehospital hemoglobin-based oxygen carrier resuscitation attenuates postinjury acute lung injury

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Background. *Crystalloid infusion has been the standard prehospital fluid resuscitation in the United States for the past 35 years, but the emergence of a safe and effective hemoglobin-based oxygen carrier (HBOC) may change that practice. The purpose of this in vivo study is to simulate an existing multicenter prehospital trial of HBOC versus crystalloid to determine the effects in a controlled 2-event construct of postinjury multiple organ failure.*

Methods. *Rats underwent hemorrhagic shock (30 mm Hg × 45 min) and were resuscitated over 2 hours in a clinically relevant design: 2 × volume of shed blood (SB) using normal saline (NS) in the first 30 minutes; 1/2 volume of SB in the next 30 minutes; another 2 × SB volume with NS over the remaining 60 minutes. Study groups represented alternative fluid strategies during the first hour of resuscitation: (1) Inhospital SB (standard resuscitation), (2) Inhospital HBOC, (3) Prehospital SB, and (4) Prehospital HBOC. Global physiologic response was assessed via tissue oxygenation (near infrared spectroscopy) and arterial base deficit, and pulmonary response, via lung polymorphonuclear neutrophil accumulation and vascular permeability.*

Results. *Prehospital HBOC resuscitation provided the most efficient recovery of tissue oxygenation and correction of base deficit, had the greatest reduction in pulmonary polymorphonuclear neutrophil accumulation, and abrogated acute lung injury. Prehospital SB and Inhospital HBOC regimens afforded intermediate lung protection, compared with standard resuscitation.*

Conclusions. *The findings in this controlled in vivo study suggest prehospital HBOC resuscitation improves the recovery from postshock oxygen debt and reduces postinjury organ dysfunction. (Surgery 2005;138:335-41.)*

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CRYSTALLOID INFUSION has remained the standard initial fluid resuscitation for hemorrhagic shock in the United States for the past 35 years, predicated on the landmark studies of Shires et al.[1] Moreover, crystalloid is currently the mainstay for prehospital resuscitation because of the prohibitive logistics in providing a blood transfusion. However, as originally suggested by Cannon's observations[2] in World War I, recent studies have questioned crystalloid loading to improve systemic blood pressure in the field because it may promote further blood loss.[3] In contrast, as demonstrated by the extensive investigations of Wiggers[4] in the 1940s, delayed resuscitation can result in irreversible shock, and, in contemporary studies, protracted oxygen debt has been associated with a systemic inflammatory response that can precipitate multiple organ failure (MOF).[5-7] The availability of a safe hemoglobin-based oxygen carrier (HBOC) that can both restore circulating blood volume and transport oxygen offers a new approach to this dilemma. Recent in-hospital FDA-approved Phase II clinical studies have established potential clinical utility of a polymerized human hemoglobin solution (PolyHeme, Northfield Laboratories, Evanston, Ill) as an oxygen carrier for the resuscitation of patients with acute blood loss.[8] During these investigations it became apparent that PolyHeme averts the effects of stored red blood cell (RBC) transfusion on the innate immune response in severely injured

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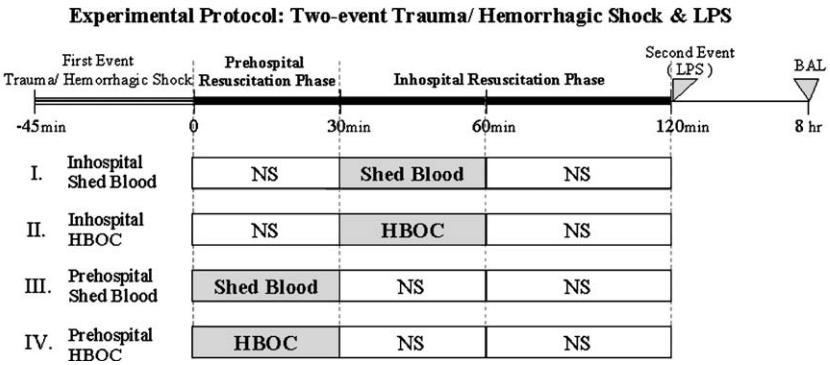

Fig 1. Group I (Inhospital SB) reflects standard resuscitation: NS at 2 × SB volume, followed by one-half SB volume, and then NS at 2 × SB volume; Group II (Inhospital HBOC) represents use of a HBOC in the hospital: NS at 2 × SB volume, HBOC at one-half SB volume, and then NS at 2 × SB volume; Group III (Prehospital SB) simulates the availability of stored blood in the field: one-half SB volume, followed by NS at 2 × SB volume, and NS at 2 × SB volume; and Group IV (Prehospital HBOC) illustrates our current clinical trial: HBOC at one-half SB volume, followed by NS at 2 × SB volume, and NS at 2 × SB volume. *LPS,* Lipopolysaccharide; *BAL,* bronchoalveolar lavage; *NS,* normal saline; *HBOC,* hemoglobin-based oxygen carrier.

patients;[9] this finding was confirmed in vitro.[10] On the basis of these collective data, a prehospital Phase III multicenter trial was initiated in January 2003 and has now surpassed the third interim analysis. The purpose of the present in vivo study is to simulate this prehospital trial of initial HBOC versus crystalloid resuscitation to determine the effects in a controlled 2-event construct of post-injury MOF.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (Harlan Laboratories, Madison, Wis) weighing 300 to 350 g were housed under barrier-sustained conditions and allowed free access to food and water before use. All animals were maintained in accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals,* and this study was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee.

Materials. Polymerized human hemoglobin solution (PolyHeme) was kindly provided by Northfield Laboratories; 0.9% injection grade normal saline (NS) was purchased from Baxter Healthcare (Deerfield, Ill). All other materials were purchased from Sigma-Aldrich Corp. (St. Louis, Mo) unless otherwise specified.

Two-event trauma/shock experiment (Fig 1). Animals were anesthetized with 50 mg/kg pentobarbital sodium (Abbott Labs, North Chicago, Ill) administered intraperitoneally. The right femoral artery and vein were cannulated aseptically with polyethylene (PE-50) tubing. The arterial catheter was used for continuous blood pressure monitoring. The animal's body temperature was kept at 37°C with the use of a heat lamp. To induce tissue injury and shock, we opened the abdomen through a midline incision and withdrew blood from the arterial catheter into a syringe containing 100 units of heparin sodium (American Pharmaceutical Partner Inc, Schaumburg, Ill). The blood pressure was reduced to 30 mm Hg in 10 minutes and maintained at that pressure for 45 minutes. At the end of the shock period, the animals were resuscitated over 2 hours by using NS, shed blood (SB) or HBOC. The first resuscitation phase, designated as "Prehospital," was the 30 minutes immediately after shock; the groups were randomized to receive NS, SB, or HBOC. The second resuscitation phase, designated as "Inhospital," consisted of 2 segments: a 30-minute segment in which groups were randomized to receive NS, SB, or HBOC, followed by a 60-minute segment during which NS at 2 × the SB volume was administered in all groups. In animals resuscitated with SB, the volume of blood infused was one half that of SB withdrawn during shock. The volume administered to animals resuscitated with the HBOC gave an equivalent amount of hemoglobin (Hb) as that in one-half SB volume. The HBOC contained 10 g/dL Hb in a balanced electrolyte solution. Consequently, achieving an equivalent Hb content required the SB infusion to range from 5 to 6 mL, whereas the required range in HBOC was 6 to 7 mL. The difference was adjusted in NS volume during the ensuing study period. Thus, all study animals were administered equivalent resuscitation

Table I. Hemodynamic response: trauma/hemorrhagic shock

		Mean arterial pressure (mm Hg)				
		Baseline	*Shock*	*Prehospital Phase*	*Inhospital Phase*	
	N			*30 min*	*60 min*	*120 min*
Inhospital SB	5	101.7 ± 3.2	30.8 ± 0.7	80.0 ± 5.1	107.4 ± 3.5	108.2 ± 5.3
Inhospital HBOC	5	99.5 ± 3.0	29.8 ± 0.7	77.2 ± 6.5	112.2 ± 3.7†	106.2 ± 5.2
Prehospital SB	5	99.5 ± 1.0	30.5 ± 0.5	90.8 ± 7.3	97.3 ± 3.9	107.2 ± 3.3
Prehospital HBOC	5	101.5 ± 2.8	31.3 ± 0.6	113.8 ± 4.8*	108.5 ± 4.0	119.5 ± 3.9‡

SB, Shed blood; *HBOC,* hemoglobin-based oxygen carrier.
*P <.05 vs other groups.
†P <.05 vs Prehospital SB.
‡P <.05 vs Inhospital HBOC.

volume and equivalent Hb concentration. For the second event, animals were injected with 100 μg/kg of lipopolysaccharide (*Salmonella enteritidies*) through the femoral vein after the 2-hour resuscitation period.

Tissue oxygenation and blood biochemistry. Tissue oxygenation was monitored noninvasively on the animals' left hind limbs by near infrared spectroscopy (NIRS) with the use of the InSpectra Tissue Spectrometer (Hutchinson Technology Inc, Hutchinson, Minn. pH and base deficit (BD) were measured with the use of the I-STAT portable clinical analyzer (I-STAT Corp, East Windsor, NJ).

Lung neutrophil accumulation. At the end of resuscitation or 6 hours after LPS administration, lung tissue was harvested, snap-frozen, and stored at −70°C until use. The tissue sample was thawed, weighed, and homogenated in 10 mL of 20 mmol/L potassium phosphate buffer (PPB) and centrifuged at 40,000*g* for 30 minutes. The pellet was sonicated for 90 seconds in 4 mL of 50 mmol/L PPB containing 0.5 g/dL hexadecyltrimethyl ammonium bromide, incubated at 60°C for 2 hours, and centrifuged. The supernatant (5 μL) was added to 145 μL of 50 mmol/L PPB containing 0.167 mg/mL O-dianisidine with 0.0005% hydrogen peroxide; the absorbance at 460 nm was measured with a spectrophotometer (Molecular Devices Corp, Sunnyvale, Calif) to determine myeloperoxidase (MPO) activity.

Lung permeability. After the 2-hour resuscitation period, the animals were injected with 30 mg/kg of Evans blue dye through the femoral vein; 6 hours later, 1.0 mL of blood was drawn from the femoral artery and centrifuged at 400*g* at 4°C for 15 minutes. The resultant plasma was serially diluted to form a standard curve. The animals were subsequently sacrificed and bronchoalveolar lavage performed. Five milliliters of NS was injected and aspirated 3 times, and collected. The lavage was performed 3 times, and the combined recovery of bronchoalveolar lavage fluid (BALF) was consistently greater then 12 mL. The BALF was centrifuged at 400*g* at 4°C for 15 minutes to remove cells; the supernatant was then assayed spectrophotometrically at 620 nm for Evans blue dye concentration.

Statistical analysis. Data are reported as mean ± SEM and were compared by analysis of variance using the Fisher protected least significant difference procedure for post hoc comparisons. A P value of <.05 is considered statistically significant.

RESULTS

Hemodynamic response to resuscitation. Circulating total Hb levels at the end of resuscitation were 10.5 ± 0.2 g/dL in the SB groups and 10.3 ± 0.3 g/dL in the HBOC groups. The mean arterial pressure (MAP) of the study groups were equivalent at baseline and the end of shock (Table I). At the end of the Prehospital Phase and the Inhospital Phase, the Prehospital HBOC group had increased MAP; during the Inhospital Phase, the MAP from the Inhospital HBOC group was increased, compared with the Prehospital SB group.

Physiologic response to resuscitation. Tissue oxygenation was measured in the hind limb by NIRS. Tissue oxygenation (tissue oxygen saturation) was restored sooner in the Prehospital HBOC cohort, and this differential was sustained into the mid-Inhospital Phase (Fig 2). Furthermore, the Inhospital HBOC group had a higher tissue oxygenation, compared with both SB groups during the early Inhospital Phase. Arterial BD measurement appeared consistent with these findings (Fig 3). The BD (mmol/L) was attenuated in the Prehospital HBOC group at the end of the Prehospital Phase (6.0 ± 1.5 vs 13.7 ± 0.9, 12.7 ± 0.9, and 13.3 ± 0.7); this difference was maintained throughout both segments of resuscitation during the Inhospital Phase (3.7 ± 0.3 vs 7.7 ± 0.9, 5.7 ± 0.3, 7.3 ± 0.3 and 2.7 ± 0.3 vs 7.7 ± 0.7, 3.7 ± 0.7, 7.0 ± 0.6). The Inhospital HBOC group also had

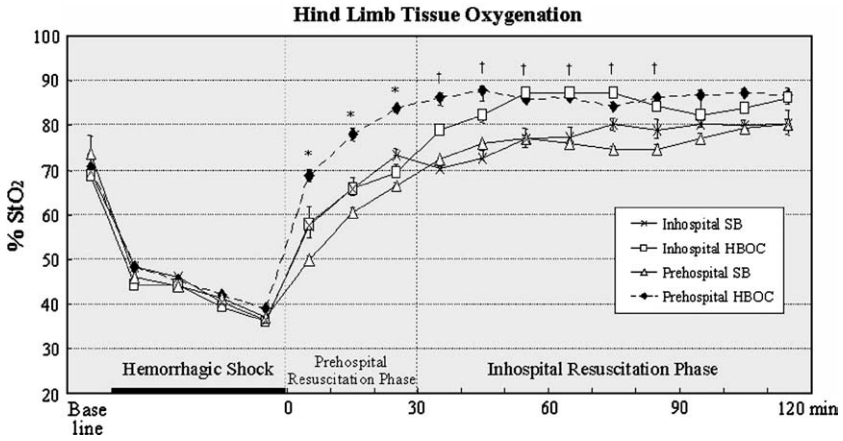

Fig 2. Tissue oxygenation was monitored continuously with an NIRS device placed on the hind limb. **P* < .05 vs other groups; †*P* < .05 Prehospital HBOC and Inhospital HBOC vs Prehospital SB and Inhospital SB. *StO_2,* Tissue oxygen saturation; *SB,* shed blood; *HBOC,* hemoglobin-based oxygen carrier.

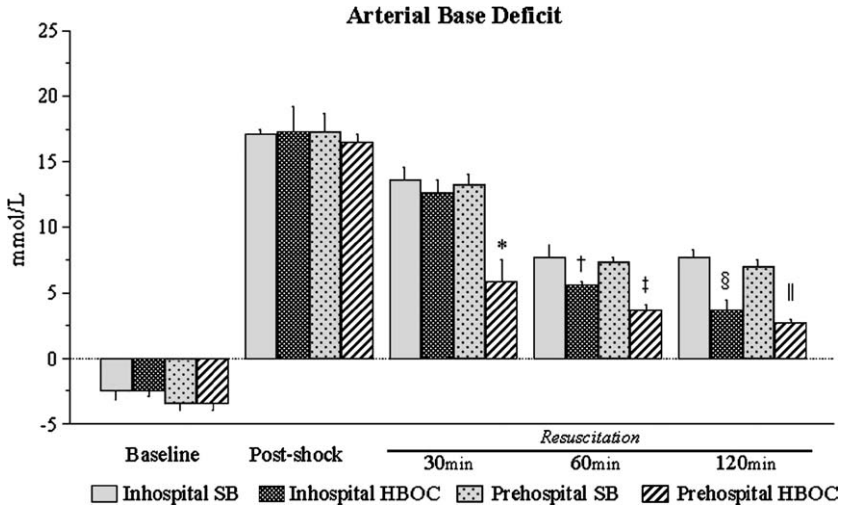

Fig 3. Arterial base deficit was measured at the end of shock, and at 30-minute, 60-minute, and 120-minute resuscitation periods. **P* < .05 vs other groups; †*P* < .05 vs Inhospital SB and Prehospital SB, ‡*P* < .05 vs other groups; §‖ *P* < .05 vs Inhospital SB and Prehospital SB. *SB,* Shed blood; *HBOC,* hemoglobin-based oxygen carrier.

improved metabolic recovery in the late resuscitation phase. On the basis of these results, we performed additional experiments to determine if the improved tissue oxygenation and BD by Prehospital HBOC resuscitation could be accounted for by the initial volume difference in HBOC and SB. However, despite correcting for this volume differential, the improved tissue oxygenation and BD by Prehospital HBOC resuscitation persisted (83.5 ± 0.5 vs 72.0 ± 2.0 and 6.0 ± 1.5 vs 11.3 ± 0.9, *P* <.05).

Acute lung injury. Acknowledging the central role of PMNs in the pathogenesis of postinjury acute lung injury (ALI), we determined the accumulation of PMNs by the MPO assay (Fig 4). PMN accumulation was prominent in all study groups at the end of Shock/Resuscitation (first event) but was reduced in the Prehospital HBOC group. Moreover, the Prehospital HBOC group continued to have less PMN accumulation in the lungs at the end of the experiment after the second event (Shock/Resuscitation + LPS). The In hospital HBOC group also had less PMN accumulation after the second event. The primary study endpoint was ALI, determined by Evans blue extravasation into the alveolar space at 8 hours postinsult (Fig 5). The sham control (0.41 ± 0.10), Shock/Resuscitation alone (1.05 ± 0.19), and LPS

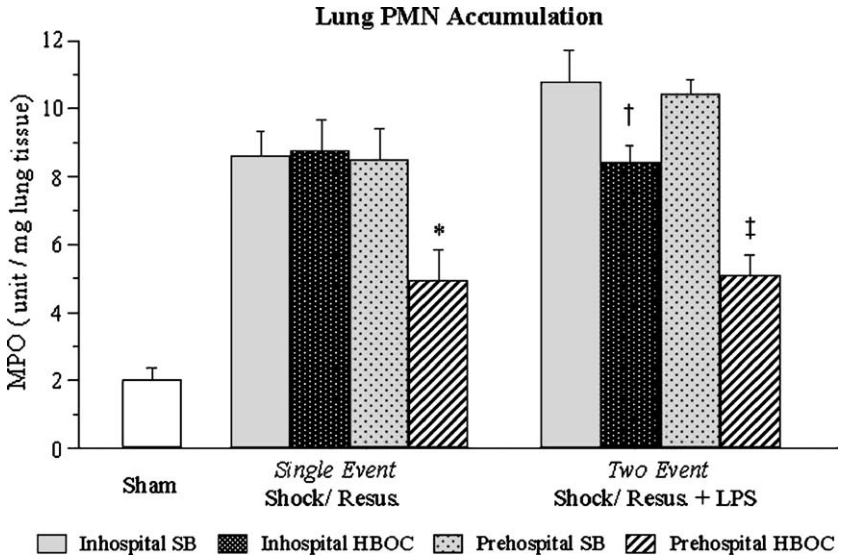

Fig 4. Lung PMN accumulation, determined by the MPO assay, was measured at the end of the first event (Shock/Resuscitation) and at the end of the study after the second event (Shock/Resuscitation + LPS). *$P < .05$ vs other groups; †$P < .05$ vs Inhospital SB and Prehospital SB; ‡$P < .01$ vs other groups. *PMN,* Polymorphonuclear neutrophil; *MPO,* myeloperoxidase; *LPS,* lipopolysaccharide; *SB,* shed blood; *HBOC,* hemoglobin-based oxygen carrier.

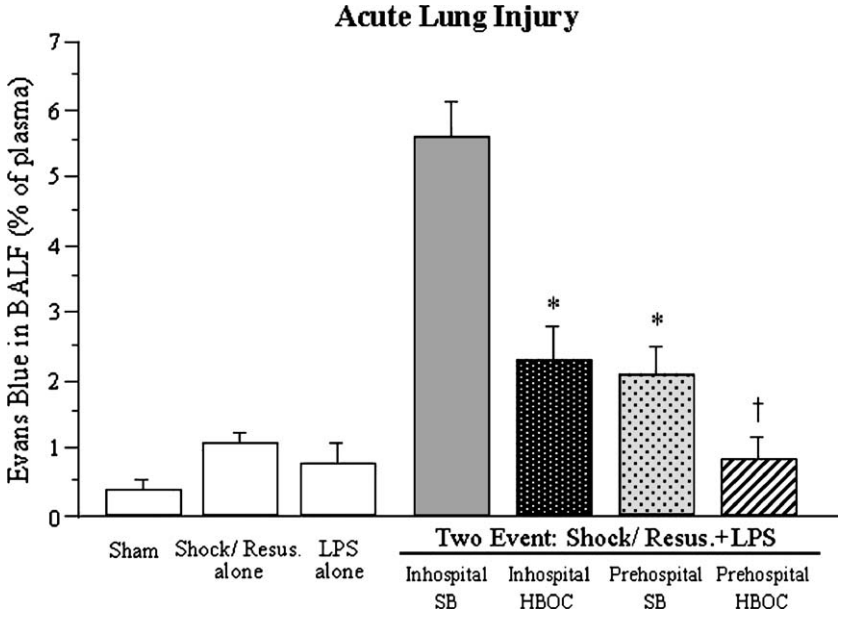

Fig 5. Acute lung injury, determined by Evans blue alveolar extravasation, was evaluated at the end of the study (8 hours postinsult). *$P < .05$ vs Inhospital SB and Prehospital HBOC; †$P < .05$ vs other Two Event groups. *BALF,* Bronchoalveolar lavage fluid; *LPS,* lipopolysaccharide; *SB,* shed blood; *HBOC,* hemoglobin-based oxygen carrier.

alone (0.80 ± 0.27) were not different. Consistent with our previous work, the 2-event (Shock/Resuscitation + LPS) construct provoked marked lung permeability (5.60 ± 0.48).

The pulmonary leak was attenuated with simulated Inhospital HBOC (2.27 ± 0.54) and Prehospital SB (2.08 ± 0.45) but was virtually eliminated by Prehospital HBOC (0.84 ± 0.39).

DISCUSSION

The optimal resuscitation fluid for acute blood loss remains unclear, and the practical options for

prehospital care have been limited to expansion of the circulating blood volume. The issue is magnified in the combat scenario where access to blood transfusion is further delayed.[11] Resurgent interest in field resuscitation has challenged the long-standing practice of unbridled crystalloid loading[3] because of the potential risk of exacerbating hemorrhage via dislodging hemostatic clots[12] and diluting plasma coagulation factors. Conversely, the magnitude of oxygen debt after hemorrhagic shock correlates directly with adverse outcome.[5-7] The emerging availability of HBOCs[10] offers a new strategy for this clinical "catch 22." An FDA-approved Phase III US multicenter prehospital trial, comparing initial HBOC with standard crystalloid resuscitation, was initiated in January 2003. The purpose of the present in vivo study was to simulate this clinical trial to determine the effects in a controlled 2-event construct of postinjury MOF. Our primary objective was to contrast HBOC versus NS in the Prehospital Phase, but we expanded the study groups to encompass the possible availability of stored blood in the field[13] and our previous Inhospital Phase II clinical work with HBOC resuscitation. We selected ALI as our primary study endpoint because of our previous work suggesting a protective anti-inflammatory effect of HBOCs[10] and the practicality of a relatively short term in vivo model.

The results of the present in vivo controlled study demonstrate that the initial resuscitation with HBOC (Prehospital), compared with standard prehospital crystalloid, abrogates postshock ALI. Moreover, initial resuscitation with blood (Prehospital) or secondary resuscitation with HBOC (Inhospital) affords partial lung protection, compared with initial crystalloid. The precise mechanism for HBOC salvage remains to be elucidated, but several potential explanations are suggested by previous investigations with HBOCs.[10] Perhaps the most conspicuous interpretation is more-efficient restoration of systemic oxygen delivery, apparent in the NIRS monitoring of hind limb tissue oxygenation. Measurement of skeletal muscle oxyhemoglobin levels by NIRS is a valuable noninvasive method for assessing adequacy of resuscitation.[14] McKinley et al[15] evaluated deltoid muscle O_2 saturation during resuscitation of critically injured patients and confirmed strong correlations with systemic O_2 delivery, arterial BD, and serum lactate. In the present study, sequential determination of arterial BD corroborates improved systemic O_2 delivery. Work in other laboratories[16] has shown that metabolic recovery in ischemic muscle is more efficient with HBOCs, compared with packed red blood cells (PRBCs). Our results would appear to support this assertion as Prehospital HBOC improved tissue oxygenation and restored acid-base status more efficiently than SB resuscitation with an equivalent hemoglobin load or with an equal volume to HBOC. Presumably, the findings in the present study would be magnified if stored PRBCs were used rather than shed blood. However, maintaining functional viability in rodent-stored PRBCs has been challenging,[17] and the relevance of employing stored human red blood cells is questionable.[18] An alternative or supplemental mechanism for the apparent benefits of HBOC resuscitation may be via downregulation of the systemic hyperinflammation invoked in the pathogenesis of postinjury MOF.[19] Our previous work has shown that the use of this HBOC avoids stored PRBC priming of human PMNs in vitro as well as in clinical trials.[10] Furthermore, HBOC resuscitation eliminates the early postinjury elevation of interleukins 6, 8, and 10.[9] Beyond the salutary metabolic effects of HBOC resuscitation, initial prehospital blood transfusion reduced ALI in the present study. The explanation for this observation is not apparent in the available data. While crystalloid is the standard initial fluid resuscitation for acute blood loss in the United States, the potential physiologic superiority of initial stored blood administration for severe hemorrhagic shock remains debated.[20-22] The virtual epidemic of postshock compartment syndromes underscores the merit of this reassessment.

The study limitations in this set of experiments are common to most animal studies that attempt to model resuscitation of seriously injured patients. The threshold for critical anemia differs substantially in rats, compared with humans,[23] and the necessity for anesthesia adds another level of complexity. Moreover, despite our best intentions, controlling all the variables is challenging. For example, while the transient MAP elevations in the HBOC study groups may raise the question of vasoconstriction, we believe these elevations are more likely due to our study design to achieve equivalent Hb loading. Specifically, the Prehospital HBOC group was administered 7.0 ± 0.8 mL of HBOC, whereas the Prehospital SB group was given 5.6 ± 0.1 mL of SB during the initial 30 minutes. Our clinical experience has not confirmed vasoconstriction with the HBOC used in the present study.[24] In addition, we acknowledge the clinical relevance of uncontrolled hemorrhage in this arena. Recent animal studies[25] of such a design suggest that HBOCs may be ideally suited for contemporary combat situations in which high-

energy wounds produce life-threatening blood loss and evacuation to definite care is delayed.

CONCLUSION

On the basis of evaluating the responses to crystalloid, blood, and an HBOC, this work suggests that the optimal initial fluid resuscitation for severe postinjury shock is an HBOC. In this model, HBOC reversed oxygen debt more efficiently and prevented ALI. Potential application to the current military challenges in delivering care to our soldiers is compelling.

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