

Resuscitation With a Hemoglobin-Based Oxygen Carrier After Traumatic Brain Injury

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Background: Traumatic brain injury (TBI) remains an exclusionary criterion in nearly every clinical trial involving hemoglobin-based oxygen carriers (HBOCs) for traumatic hemorrhage. Furthermore, most HBOCs are vasoactive, and use of pressors in the setting of hemorrhagic shock is generally contraindicated. The purpose of this investigation was to test the hypothesis that low-volume resuscitation with a vasoactive HBOC (hemoglobin glutamer-200 [bovine], HBOC-301; Oxyglobin, BioPure, Inc., Cambridge, MA) would improve outcomes after severe TBI and hemorrhagic shock.

Methods: In Part 1, anesthetized swine received TBI and hemorrhage (30 ± 2 mL/kg, $n = 15$). After 30 minutes, lactated Ringer's (LR) solution ($n = 5$), HBOC ($n = 5$), or 10 mL/kg of LR + HBOC ($n = 5$) was titrated to restore systolic blood pressure to ≥ 100 mm Hg and heart rate (HR) to ≤ 100 beats/min. After 60 minutes, fluid was given to maintain mean arterial pressure (MAP) at ≥ 70 mm Hg and heterologous whole blood (red blood cells [RBCs], 10 mL/kg) was transfused for hemoglobin at ≤ 5 g/dL. After 90 minutes, mannitol (MAN, 1 g/kg) was given for intra-

cranial pressure ≥ 20 mm Hg, LR solution was given to maintain cerebral perfusion pressure at ≥ 70 mm Hg, and RBCs were given for hemoglobin of ≤ 5 g/dL. In Part 2, after similar TBI and resuscitation with either LR + MAN + RBCs ($n = 3$) or HBOC alone ($n = 3$), animals underwent attempted weaning, extubation, and monitoring for 72 hours.

Results: In Part 1, relative to resuscitation with LR + MAN + RBCs, LR + HBOC attenuated intracranial pressure (12 ± 1 mm Hg vs. 33 ± 6 mm Hg), improved cerebral perfusion pressure in the initial 4 hours (89 ± 6 mm Hg vs. 60 ± 3 mm Hg), and improved brain tissue PO_2 (34.2 ± 3.6 mm Hg vs. 16.1 ± 1.6 mm Hg; all $p < 0.05$). Cerebrovascular reactivity and intracranial compliance were improved with LR + HBOC ($p < 0.05$) and fluid requirements were reduced (30 ± 12 vs. 280 ± 40 mL/kg; $p < 0.05$). Lactate and base excess corrected faster with LR + HBOC despite a 40% reduction in cardiac index. With HBOC alone and LR + HBOC, MAP and HR rapidly corrected and remained normal during observation; however, with HBOC alone, lactate clearance was slower and systemic oxygen extraction was transiently in-

creased. In Part 2, resuscitation with HBOC alone allowed all animals to wean and extubate, whereas none in the LR + MAN + RBCs group was able to wean and extubate. At 72 hours, no HBOC animal had detectible neurologic deficits and all had normal hemodynamics.

Conclusion: The use of HBOC-301 supplemented by a crystalloid bolus was clearly superior to the standard of care (LR + MAN + RBCs) after TBI. This may represent a new indication for HBOCs. Use of HBOC eliminated the need for RBC transfusions and mannitol. The inherent vasopressor effect of HBOCs, especially when used alone, may misguide initial resuscitation, leading to transient poor global tissue perfusion despite restoration of MAP and HR. This suggests that MAP and HR are inadequate endpoints with HBOC resuscitation. HBOC use alone after TBI permitted early extubation and excellent 72-hour outcomes.

Key Words: Colloid, hemoglobin-based oxygen carrier (HBOC), Intracranial pressure, Oxygenation, Swine.

J Trauma. 2005;59:553–562.

The brain is exquisitely sensitive to hypoxia, and this tissue may stand to benefit the greatest from early rapid restoration of normoxia. Stability at room temperature and ready availability in the far-forward battlefield or prehospital environment are characteristics that make hemoglobin-based oxygen carriers (HBOCs) attractive candidate flu-

ids for restoring cerebral tissue oxygenation after injury. The potential benefit from early restoration of oxygenation may be invaluable in any circumstance where a blood transfusion would be beneficial but is generally not available (e.g., the prehospital setting and the battlefield environment). Currently, traumatic brain injury (TBI) represents a relative exclusion criterion into clinical trials evaluating the effect of HBOCs in trauma, presumably because of a lack of data regarding this specific condition.

HBOCs have been evaluated in the setting of hemorrhagic shock and acute blood loss.^{1–9} These reports generally show favorable effects and improved outcomes after resuscitation with HBOCs. In one series with hemorrhage and resuscitation, brain tissue oxygenation rapidly improved with HBOC use⁴; however, this experiment did not incorporate any particular intracranial injury. Finally, one set of experiments with dapsirin cross-linked hemoglobin, which is no longer available, showed mixed results after TBI and hemorrhagic shock.¹⁰

Submitted for publication December 3, 2004.

Accepted for publication May 27, 2005.

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Supported by Office of Naval Research grants N000140210339 and N000140210035 and National Institutes of Health General Medical Sciences grant T32 GM08749-0.

Presented at the 63rd Annual Meeting of the American Association for the Surgery of Trauma, September 29–October 2, 2004, Maui, Hawaii.

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DOI: 10.1097/01.ta.0000177711.52191.62

The purpose of this investigation was to examine the impact of low-volume resuscitation with a vasoactive HBOC (hemoglobin glutamer-200 [bovine], HBOC-301; Oxyglobin, BioPure Inc., Cambridge, MA) after TBI and hemorrhagic shock. With the exception of a few previous studies using diaspirin cross-linked hemoglobin (which has since been withdrawn by the manufacturer), the use of a new-generation HBOC after TBI has never been investigated. We hypothesized that immediate resuscitation with an HBOC would improve outcomes after TBI and hemorrhagic shock. This investigation was divided into two parts. Part 1 examined short-term benefits of HBOC resuscitation after TBI, and Part 2 examined the effect of low-volume HBOC on 72-hour outcome.

MATERIALS AND METHODS

Animals were housed in a facility that was approved by the American Association of Laboratory Animal Care, with veterinarians available at all times. All procedures were performed according to National Institutes of Health guidelines for use of laboratory animals and were preapproved by our institutional animal care and use committee.

General Instrumentation

Farm-raised, cross-bred, fasted swine of both sexes (35–60 kg) were sedated with an intramuscular injection of 30 mg/kg ketamine and 3.5 mg/kg xylazine. The animals underwent orotracheal intubation and mechanical ventilation (Impact Portable Adult Ventilator Model 754, Impact Systems, West Caldwell, NJ) with tidal volumes of 12 mL/kg and rates between 8 and 12 breaths/min. Arterial blood gas analysis was used to maintain P_{CO_2} at 40 ± 5 mm Hg. The F_{IO_2} was 0.4 except where otherwise noted.

Anesthesia was maintained with continuous intravenous infusions of 10 mg/kg/h ketamine, 0.5 mg/kg/h xylazine, and 50 μ g/kg/h fentanyl. Pulse oximetry (Nellcor Pulse Oximeter, Hayward, CA) and electrocardiograms were continuously monitored. Catheters were placed in the femoral artery for continuous arterial blood pressure monitoring and in the external jugular vein for intravenous fluid administration. In Part 1 only, additional catheters were placed in the urinary bladder to measure urine output and in the pulmonary artery for continuous mixed venous pulmonary artery oxygen saturation and cardiac output monitoring (Abbott Critical Care Systems, Abbott Laboratories, North Chicago, IL).

In Part 1, cerebral tissue oxygenation and intracranial pressure (ICP) were continuously monitored by means of an intraparenchymal oxygen electrode and fiberoptic pressure transducer placed through a small frontal craniotomy (LICOX MCB Oxygen Monitor, Integra Neurosciences, San Diego, CA). During Part 2, only the fiberoptic pressure transducer was placed. Another craniotomy was centered 1 cm left lateral to the midline and 1 cm rostral to the bregma, and a hollow bolt was attached flush with the unbroken surface of the dura. This bolt was used to create the brain injury described later.

During this instrumentation period, 10 mL/kg of lactated Ringer's (LR) solution was given to normalize hemodynamics. After instrumentation, there was a 60-minute stabilization period before collecting baseline measurements.

Test of Cerebrovascular Reactivity and Compliance

This technique has been previously described in detail.^{10–14} Briefly, inhaled carbon dioxide (F_{ICO_2}) was titrated to an end-tidal CO_2 of 70 mm Hg for 10 minutes during baseline conditions and at various time points after TBI. The magnitude of the CO_2 -evoked ICP and tissue PO_2 changes vary with cerebral compliance and vascular reactivity in patients¹³ and animals.^{10–12,14} This test of cerebrovascular reactivity is a measure of global central nervous system autoregulation and is an indirect indicator of the state of health or disease of the brain.

Brain Trauma

This technique has been previously described in detail^{10–12,14} by our laboratory. Briefly, a standardized 8-atm fluid percussion injury was delivered through the left frontoparietal craniotomy bolt, followed immediately by an arterial hemorrhage. The magnitude of the fluid percussion injury is a physical constant based on the mass and height of the pendulum used to create the injury and the resistance of the coupling device. Each of these factors was controlled, identical, and reproducible for each animal.

Experimental Design, Part 1 (Nonsurvivor Protocol)

Immediately after TBI (time [t] = 0 minutes), blood was rapidly withdrawn (5 mL/kg/min) from the arterial catheter to maintain a mean arterial pressure (MAP) of 30 mm Hg until t = 30. During this period, breathing was spontaneous on room air through the endotracheal tube. At t = 30, F_{IO_2} was returned to 0.4, mechanical ventilation was restored, and fluid resuscitation with one of three fluid paradigms was randomly assigned: unlimited warmed LR solution, 10 mL/kg LR solution and then unlimited HBOC, or unlimited HBOC alone. Fluid was infused to restore systolic blood pressure to > 100 mm Hg and a heart rate below 100 beats/min until t = 60. This simulated care in the prehospital setting.

After t = 60, unlimited fluid (according to assigned group, respectively) was given to maintain MAP \geq 70 mm Hg and heterologous whole blood (red blood cells [RBCs], 10 mL/kg) was transfused for hemoglobin (Hb) \leq 5 g/dL. This simulated care in the emergency department.

After t = 90, a single one-time dose of mannitol (MAN, 1 g/kg administered intravenously) was given if ICP exceeded 20 mm Hg, additional LR solution was given to maintain cerebral perfusion pressure (CPP) \geq 70 mm Hg, and additional RBCs were given if Hb \leq 5 g/dL. This protocol was maintained for the remainder of the experiment (t = 360), simulating the intensive care unit setting.

The experimental design resulted in three treatment groups: LR + MAN + RBCs, LR + HBOC, and HBOC

Table 1 Veterinary Coma Scale

Score	Motor	Score	Eyes	Score	Respirations
6	Normal movement	4	Open	3	Normal
5	Mildly drowsy with spontaneous, purposeful movements	3	Open to stimulus	2	Ataxic
4	Lethargic, unable to stand but maintains sternal recumbency	2	Normal eyelid reflexes	1	Apneic
3	Lethargic, withdraws to pinch, and lifts head with attention to visual stimuli; no sternal recumbency	1	No eyelid response to stimuli		
2	Withdraws or pedals to pinch, or spontaneous pedaling behavior				
1	Flaccid posture or extensor posturing (spontaneous or to stimuli)				

Adapted from King et al.¹⁴

alone. Veterinary technicians titrated the fluid administration to the resuscitation targets and were blinded to the treatment group. Animals were evaluated with serial CO₂ challenges at bihourly intervals. At $t = 360$, the surviving animals were killed. Six animals were randomly selected to undergo post-mortem examination of the brain to rule out presence of a surgical lesion.

Experimental Design, Part 2 (Survivor Protocol)

The instrumentation and the method for delivering the TBI and hemorrhage were the same as the nonsurvivor protocol with modifications as indicated. Antibiotic prophylaxis (2 g of intravenous cefazolin) was administered at induction and before extubation (800 mg of intravenous clindamycin and 2 g of intravenous cefazolin). Inhaled carbon dioxide challenges were eliminated to avoid any potential confounding effects this may have on weaning and extubation.

After injury, animals were randomized to either unlimited LR solution resuscitation or unlimited HBOC alone under the same algorithm as in Part 1. This resulted in two treatment groups: LR + MAN + RBCs or HBOC.

The observation period ended once hemodynamic stability was achieved for 1 uninterrupted hour without fluid administration, up to a maximum of 6 hours. All catheters were removed and the wounds aseptically closed. Anesthesia was discontinued and the animals underwent attempted weaning from mechanical ventilation, extubation, and transfer to the vivarium for observation. For acute pain, 1.5 mg of intramuscular buprenorphine was administered and a 50- μ g/h transdermal fentanyl patch was placed.

Using a standardized veterinary coma scale (Table 1), a neurologic examination was performed each day by veterinarians who were blinded to the treatment group. After 72 hours, animals were anesthetized, reinstrumented, and evaluated for any signs of infection (which was considered exclusionary). Animals were then killed. Postmortem examina-

tion of the brain was conducted to rule out the presence of a surgical lesion created by the initial TBI.

Statistical Analysis

Commercially available StatView software was used. Between-group analysis was conducted with analysis of variance and post hoc nonparametric tests. Within-group analysis was conducted with the paired t test. All findings were considered statistically significant at the 95% confidence interval ($p < 0.05$). All results are expressed as mean values for each group.

RESULTS

Part 1

There was a total of 21 animals, with 6 early deaths before randomization. These deaths occurred after TBI and hemorrhage but before randomization, and were attributed to primary injury or irreversible shock. Randomization produced the following distribution: LR + MAN + RBCs, $n = 5$; LR + HBOC, $n = 5$; and HBOC alone, $n = 5$.

There were no differences between groups in baseline conditions (Table 2) or after TBI and hemorrhage, before randomization (Table 3). Other measured variables, including cardiac output, blood electrolytes, glucose, hematocrit, white blood cell count, and urine output, were similar before and after injury (data not shown).

Total intravenous fluid (IVF) (total volume of all infused substances) required to meet resuscitation endpoints is illustrated in Figure 1. After initial rapid fluid resuscitation in the first hour, the LR + MAN + RBC group had a persistent ongoing fluid requirement ($p < 0.05$ relative to other groups). In contrast, LR + HBOC and HBOC alone required very little fluid after the initial resuscitation period. Transfusion requirement for the LR + MAN + RBC group was 20 ± 5 mL/kg. No transfusions were required with LR + HBOC or HBOC alone.

Table 2 Baseline Variables in Part 1

Variable	LR + MAN + RBC	LR + HBOC	HBOC
Heart rate (beats/min)	69 ± 7	67 ± 7	72 ± 6
MAP (mm Hg)	83 ± 4	86 ± 6	85 ± 4
ICP (mm Hg)	7 ± 1	8 ± 1	6 ± 1
Svo ₂ (%)	73 ± 4	69 ± 4	74 ± 3
Pco ₂ (mm Hg)	41 ± 2	42 ± 1	40 ± 1
Hemoglobin (g/dL)	9.0 ± 0.5	8.4 ± 0.2	9.0 ± 0.6
Lactate (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.2

There were no differences between groups.

LR + MAN + RBC, lactated Ringer's solution + mannitol + red blood cells; LR + HBOC, 10 mL/kg lactated Ringer's solution + hemoglobin-based oxygen carrier; HBOC, hemoglobin-based oxygen carrier alone; Svo₂, mixed venous oxygen-hemoglobin saturation.

ICP is illustrated in Figure 2. Resuscitation with LR + MAN + RBCs resulted in a steady rise in ICP, resulting in severe intracranial hypertension. LR + HBOC and HBOC alone both prevented pathologic changes in ICP. Because of this, no animal in either the LR + HBOC or HBOC alone group required administration of MAN. The CO₂ challenges evoked ICP changes that are a measure of intracranial compliance (Fig. 2). With LR + MAN + RBCs, compliance ($\Delta P_{CO_2}/\Delta ICP$) deteriorated over time from 12 under baseline conditions (normal), to 1.6 at the end of the experiment ($p < 0.05$). Both the LR + HBOC and HBOC alone groups had normal compliance under baseline conditions (18 and 18, respectively). By the end of the experiment, intracranial compliance had decreased to 4.6 with LR + HBOC and 4.4 with HBOC alone (all $p > 0.05$).

Intraparenchymal brain tissue Po₂ was preserved with LR + HBOC and HBOC alone throughout the experiment (Fig. 3) but steadily declined with LR + MAN + RBCs ($p < 0.05$). Cerebrovascular reactivity ($\Delta Po_2/\Delta P_{CO_2}$, graphically represented by the serial peaks on Fig. 3), with LR + MAN + RBCs deteriorated from 2.1 at baseline to 0.72 ($p < 0.05$). With LR + HBOC and HBOC alone, cerebrovascular reactivity was preserved throughout the experiment (1.8 to 1.7

Table 3 Postinjury at Randomization, Part 1

Variable	LR + MAN + RBC	LR + HBOC	HBOC
Heart rate (beats/min)	130 ± 10	158 ± 15	140 ± 22
MAP (mm Hg)	31 ± 1	30 ± 0	30 ± 0
ICP (mm Hg)	4 ± 2	4 ± 1	4 ± 1
Svo ₂ (%)	26 ± 2	22 ± 2	28 ± 5
Lactate (mmol/L)	6.5 ± 0.7	7.3 ± 0.4	6.9 ± 0.9
Hemorrhage (mL/kg)	32 ± 4	32 ± 3	31 ± 1

There were no differences between groups.

LR + MAN + RBC, lactated Ringer's solution + mannitol + red blood cells; LR + HBOC, 10 mL/kg lactated Ringer's solution + hemoglobin-based oxygen carrier; HBOC, hemoglobin-based oxygen carrier alone; Svo₂, pulmonary artery mixed venous oxygen-hemoglobin saturation.

Supplemental crystalloid

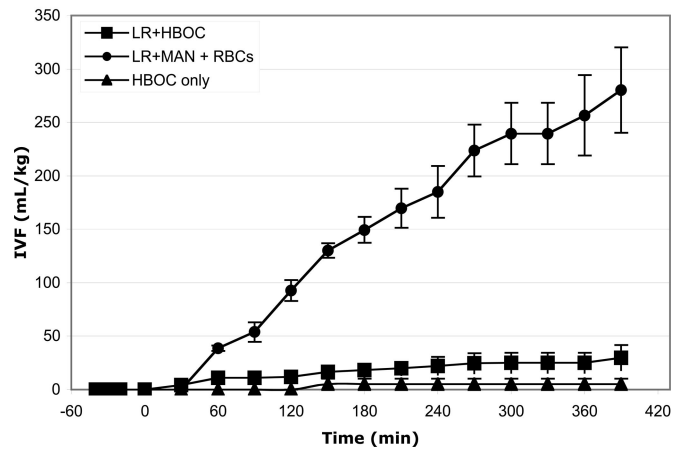


Fig. 1. Total IVF requirement to meet resuscitation endpoints in Part 1. Resuscitation endpoints are described in the text. Resuscitation with the “standard of care” LR + MAN + RBCs resulted in an ongoing IVF requirement. IVF, intravenous fluid; LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

with LR + HBOC and 0.99 to 1.6 with HBOC alone, all $p > 0.05$).

CPP is illustrated in Figure 4. Despite administration of over 250 mL/kg of fluid, as well as MAN to reduce ICP, the LR + MAN + RBC group had difficulty reaching the target CPP of 70 mm Hg ($p < 0.05$, within group). The LR + HBOC and HBOC alone groups met and maintained this CPP goal ($p > 0.05$, within group). Notably, resuscitation with LR + HBOC and HBOC alone resulted in rapid restoration of CPP to 70 mm Hg within 30 minutes, whereas LR + MAN

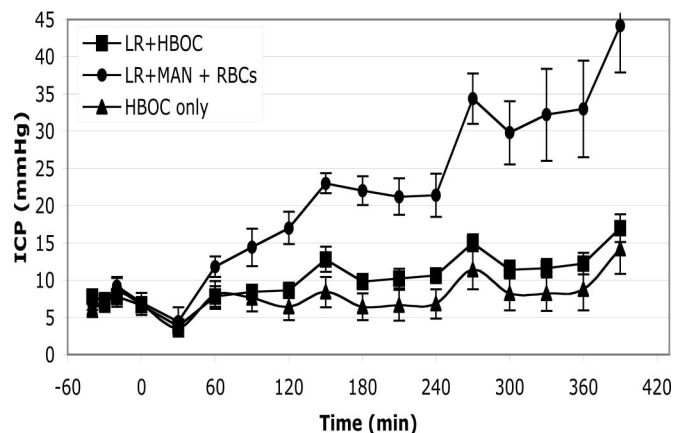


Fig. 2. ICP changes versus time in Part 1. Resuscitation with LR + HBOC and HBOC alone prevented intracranial hypertension. ICP, intracranial pressure; LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

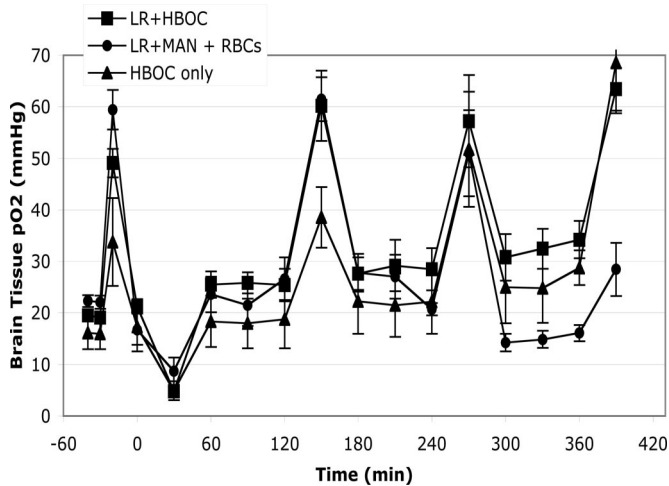


Fig. 3. Intraparenchymal brain tissue Po_2 changes versus time in Part 1. Normal brain tissue oxygenation was maintained with LR + HBOC and HBOC alone, whereas standard-of-care LR + MAN + RBCs resulted in a decline in oxygenation. LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

+ RBCs took over 3 hours to reach this goal. The small dips in Figure 4 represent inhaled CO_2 challenges to evaluate intracranial compliance and cerebrovascular reactivity.

Figure 5 illustrates mixed venous oxyhemoglobin saturation. Resuscitation with HBOC alone resulted in a persistently high O_2 extraction throughout the experiment ($p < 0.05$). LR + MAN + RBCs and LR + HBOC did not show this pattern and corrected toward normal by 6 hours ($p > 0.05$). In addition, arterial lactate clearance (Fig. 6) was

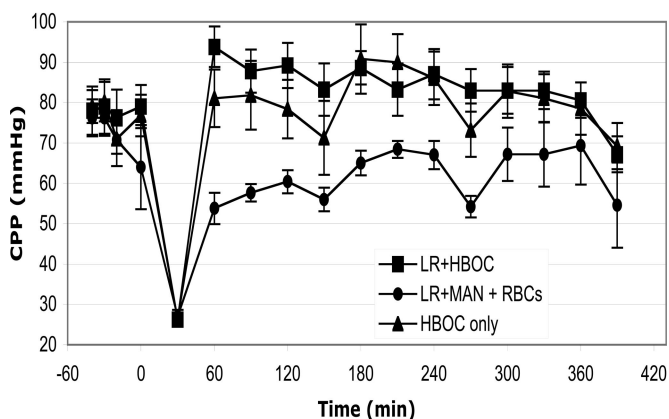


Fig. 4. CPP changes versus time in Part 1. CPP was maintained with LR + HBOC and HBOC alone, whereas standard-of-care LR + MAN + RBCs resulted in a reduction of CPP despite infusion of large amounts of resuscitation fluid. CPP, cerebral perfusion pressure; LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

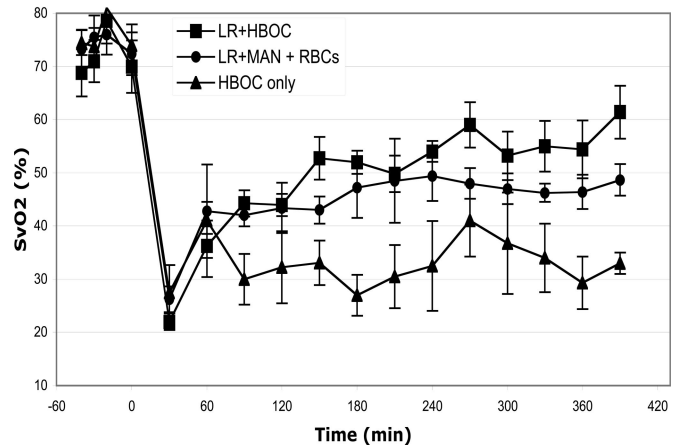


Fig. 5. Svo_2 changes versus time in Part 1. Resuscitation with HBOC alone resulted in an ongoing high systemic O_2 extraction. Svo_2 , mixed venous oxyhemoglobin saturation; LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

initially impaired with HBOC alone; however, all groups corrected appropriately and converged on the same point by the end of the experiment.

Part 2

There were eight animals total, with two prerandomization deaths. Randomization resulted in three animals in the LR + MAN + RBCs group and three animals that were administered HBOC alone. Data for the acute resuscitation period were similar to those in Part 1 and are not shown.

Those animals treated with HBOC alone weaned from mechanical ventilation and were returned to the vivarium. All LR + MAN + RBC-treated animals failed to wean within 6 hours and were killed. Motor scores for the HBOC alone group on postoperative days 2 and 3 were 4.3 ± 0.5 , 5.6 ± 0.3 , and 6.0 ± 0 .

On day 3, animals were brought back to the operating room and reinstrumented. All animals had normal hemodynamics. No metabolic abnormalities were identified. ICP was 6 ± 3 mm Hg, and lactate had corrected to 1.1 ± 0.3 mmol/L.

DISCUSSION

The results showed that, relative to LR + MAN + RBCs (our so-called standard of care), HBOC use, with or without supplemental LR solution, reduced the IVF required to maintain MAP, heart rate, and CPP; attenuated the rise in ICP; and improved brain tissue oxygenation, intracranial compliance, and cerebrovascular reactivity. The use of this HBOC also allowed us to avoid RBC transfusion and mannitol administration. To our knowledge, this is the first experimental series testing this new HBOC in the setting of TBI.

The major new findings are that, in addition to its hemodynamic effects, HBOC resuscitation also resulted in ex-

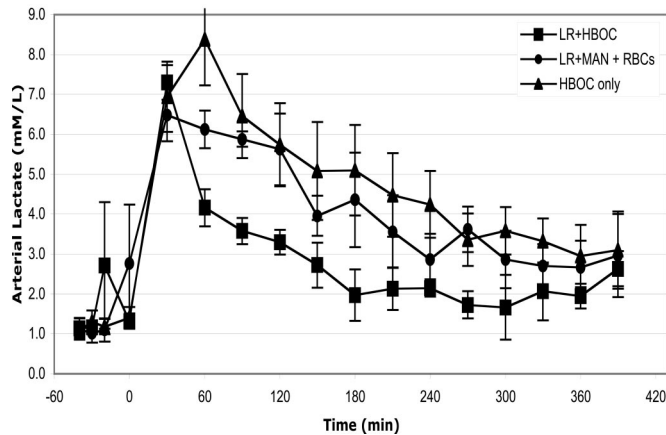


Fig. 6. Arterial lactate in part 1. Lactate clearance was initially fastest with standard-of-care LR + MAN + RBCs. Lactate clearance was initially impaired with HBOC alone. All groups converged on the same point by 6 hours. LR + HBOC, lactated Ringer's solution plus hemoglobin-based oxygen carrier; LR + MAN + RBCs, lactated Ringer's solution plus mannitol plus red blood cells; HBOC only, hemoglobin-based oxygen carrier alone.

cellent neurologic outcome despite the absence of critical care while animals were in the vivarium. Animals in the LR + MAN + RBC group were only followed for 6 hours before they were killed; therefore, it is not known whether these animals would have recovered at some later point in time. It is clear, however, that HBOC use permitted early hemodynamic stabilization and afforded a degree of neuroprotection with regard to respiratory function. This allowed for early extubation and return to independent function. This may represent a significant logistic advantage (no mechanical ventilation required, dramatic reduction in IVF, avoidance of RBC transfusion, avoidance of mannitol administration) and manpower (no critical care nursing or physician staff required) savings in any setting where resources are limited. This may be of profound importance in combat casualty care, such as occurs in the austere battlefield environment or during emergency medical transport.

Although the exact mechanism producing these advantages is unknown, we suspect there are at least three. First, this HBOC is known to have a "gentle" pressor action.^{8,15} Although many traumatologists consider administration of a pressor contraindicated in the setting of hypovolemic hypotension, there is an emerging body of evidence that may necessitate revisiting this long-held belief.^{16,17} Indeed, early pressor use may be particularly useful in the setting of TBI.^{10,11} The mild pressor effect of this HBOC resulted in rapid correction of CPP very early after injury. This may limit the total ischemic time the brain is experiencing after TBI and shock, at a time when additional minutes of cerebral ischemia probably have profound effects on neuronal function and outcome.

The second mechanism may be early restoration of oxygen-carrying capacity to the intravascular space and there-

fore to the ischemic brain. Although the actual oxygen-carrying capacity restored by HBOC infusion was small (mean, 2.46 mL/dL O₂) it is possible this minimal extra oxygen-carrying capacity may have been critically important because it is likely that oxygen consumption was supply dependent. Supplementation with this seemingly trivial oxygen-carrying capacity may have been enough to correct the VO₂/DO₂ curve just past the critical point. The benefits of this supplementation could be amplified because this was given very early after injury, compared with the standard of care. Other authors have demonstrated rapid correction of brain tissue oxygenation with a similar HBOC after hemorrhage.¹⁸

Third, use of the HBOC dramatically limited the total crystalloid required for resuscitation and avoided mannitol use. This prevents large fluxes of free water across the disrupted blood-brain barrier and is consistent with our previous work.¹⁴ In addition, some authors have shown exacerbation of intracranial hypertension after mannitol administration caused by free mannitol collecting in the brain parenchyma and drawing in free water.^{19,20} Indeed, any resuscitation strategy that limits crystalloid administration while rapidly restoring hemodynamics may be beneficial in the setting of TBI. Crystalloid itself, although isotonic on initial administration, rapidly leaves the intravascular space and becomes free water. This flux of free water can cause cerebral edema²⁰ and intracranial hypertension.

Despite the advantages demonstrated by HBOC use, the optimal dosing and administration guidelines remain to be elucidated. HBOC use alone resulted in increased systemic oxygen extraction, a transient increase in lactate, and slower lactate clearance. Similar observations have been reported previously.^{8,11} Preadministration of 10 mL/kg of LR solution appears to partially ameliorate these worrisome changes; however, it is unknown whether varying the crystalloid preload would produce any added benefits. The significance of the observed changes are questionable because these parameters converge by the end of the observation period, so this issue requires additional systematic investigation.

Altogether, these data are consistent with the conclusion that HBOC is safe and effective as a low-volume oxygen-carrying resuscitation fluid after TBI and hemorrhagic shock. This experiment was designed to be as clinically relevant as possible, but our efforts at relevance resulted in at least six limitations of the experimental design, which are considered below.

Critique

First, for ethical reasons, all animals were anesthetized at the time of injury. This anesthetic combination could have conferred neuroprotection or, alternatively, exacerbated the tissue damage, so the results may not directly apply to any situation when these drugs would be absent. Although the possibility of an anesthetic artifact cannot be ruled out, these conditions were imposed equally on all groups.

Second, the percussive injury and subsequent hemorrhage produced elevated ICP because of ischemic neuronal damage. The ischemia is reflected by immunoprecipitation of amyloid precursor protein within 6 hours¹¹ and histologic evidence of diffuse axonal injury, routinely visible with hematoxylin and eosin staining by 72 hours.¹² It should be emphasized that a percussive injury may not directly relate to elevated ICP secondary to a focal contusion, surgical lesion, or other intracranial process, as may occur in the human trauma condition.

Third, urine output was not replaced after mannitol administration. This was intentionally done to optimize the putative benefits of mannitol treatment relative to the HBOC groups that received no mannitol. There is evidence that replacing urinary fluid losses contributes to the “rebound effect” after administration of mannitol.^{19,20} The rate at which the ICP returns to, or even overshoots, the pretreatment value, is related to the volume of IVF replacement rather than to the absolute dose per se.

Fourth, there was no critical care in the vivarium. Because secondary injury after TBI depends on avoiding hypoxia or hypotension, this aspect of the experimental design clearly lacks clinical relevance. Nevertheless, we demonstrated a rapid return to normal function even under these extreme austere circumstances. It is reasonable to expect that results would be no worse in more favorable conditions. This approach to “worst-case” minimal care after trauma and recovery has been previously described.¹⁷

Fifth, the neurologic scoring system is subjective. It is only a semiquantitative method that is not capable of detecting subtle changes in neurologic function. It should be noted that only motor scores were relevant to this investigation because, in pilot experiments, the magnitude of TBI was adjusted so that recovery in the vivarium with no critical care or mechanical ventilation was possible. According to the scoring system (Table 1), normal eye opening and respiratory patterns were essential for weaning from mechanical ventilation.

Sixth, in Part 2, animals in the LR + MAN + RBC group were only followed for 6 hours after injury before being killed. It is unknown whether these animals would have stabilized with return of respiratory drive if followed indefinitely with intensive neurologic critical care, as would be done with human patients. Nevertheless, we demonstrated that HBOC use allowed rapid extubation and return to function compared with the standard of care.

Comparison to Other Work

Several studies have investigated the role of similar HBOCs in isolated hemorrhagic shock, with promising results.¹⁻⁹ There are even reports of successful use of a very similar substance in humans.²¹ Other investigators have shown that HBOC use in the setting of extreme hemodilution can maintain cerebral oxygenation; however, this model did not incorporate any TBI.²² In addition, others have shown

rapid restoration of brain tissue oxygenation after isolated hemorrhagic shock.⁴ With the notable exception of our previous work with diaspirin cross-linked hemoglobin,¹⁰ no other studies have evaluated an HBOC after TBI and hemorrhagic shock.

CONCLUSION

In this experimental model with TBI and hemorrhagic shock, HBOC is an effective low-volume alternative to resuscitation with crystalloid plus mannitol plus heterologous blood transfusions. HBOC use results in significant logistic and manpower savings. Additional studies should be conducted to definitively determine whether TBI represents a specific indication for early HBOC use.

ACKNOWLEDGMENTS

We thank Laura K. Parke, Jennifer E. Zuccarelli, Evan S. Jacobs, and Ashvin K. Reddy for technical assistance; George Beck of Impact Instrumentation (West Caldwell, NJ) for providing the ventilators; Concetta Gorski, RN, BS, CCRA, Integra LifeSciences Corporation (Plainsboro, NJ) for providing the Camino monitors and LiCOX probes; and Terry Shirey, PhD, of Nova Biomedical (Waltham, MA) for providing the Stat Ultra Blood Gas and Electrolyte Analyzer.

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DISCUSSION

Dr. Steven R. Shackford (Burlington, VT): Dr. Sakamoto, Dr. Cornwell, Dr. Fabian, members and guests, resuscitation of the rain injured patient with ongoing hemorrhage presents a significant clinical challenge, and it is incumbent upon us to devise a resuscitation strategy that prevents secondary brain injury, and does not increase hemorrhage and maintains vital organ function. Dr. King and his colleagues are to be commended for attempting to evaluate a hemoglobin-based oxygen carrier in a model of fluid percussion brain injury and hemorrhage.

I have several comments and questions. I am glad to see that the presentation had the addition of a hypothesis because that was lacking in the manuscript. It's very difficult to tell, based on what you told us, that the hypothesis was to improve

outcome. Is this an efficacy study or an effectiveness study? Please comment on that.

I think that scientific inquiry should be driven by a hypothesis because the hypothesis allows us to identify the variable of interest, and then enables us to determine what the sample size is and then perform a focused, statistical analysis. As I read the manuscript and I looked at the presentation, I'm a little unsure of exactly what the hypothesis is here, because if it is an effectiveness study, I'd like to see a power calculation on an N of three to validate these conclusions.

Secondly, the method section lacks a description of the magnitude of the hit. I agree with you that the fluid percussion model is well described, but the magnitude of the hit can vary from less than 1 atm up to 10 atm so what was the magnitude of the hit that you used in these animals? Third, the degree of brain injury associated with fluid percussion, as I am sure you are aware, can vary, even when the magnitude of the hit is controlled. I noticed that no postmortem examinations were done in any of the animals, so how do you know that there was a uniformity in the degree of brain injury in each of the groups?

It is quite possible that the animals randomized for a C-Ringer's lactate may have had a more severe brain injury than the animals randomized to receive the HBOC. This is particularly relevant in your 72-hour study, and I think that all the animals in that, both the survivors and the nonsurvivors, should have undergone a postmortem examination, particularly now that I find out that there was hemodynamic instability in the Ringer's group. As you know, the fluid percussion can cause torque on the brain stem right in the vasomotor center, and that is where we see this hemodynamic instability.

Finally, I think the manuscript did suffer a bit from a thorough review of the literature. For example, the use of diaspirin cross-linked hemoglobin in a head injury was investigated a full 10 years ago, and demonstrated that there was improvement in the cerebral perfusion pressure, the intracranial pressure, and a reduction in the size of the standardized cryogenic model. Now, those results were attributed to the vasoconstrictive properties of the hemoglobin-based oxygen carrier. I think that the vasoconstrictive properties, which really we observed as well, decreased cardiac index and accumulation of lactate and base deficit is due to an increase in the after load. But I think that those vasoactive properties are what make hemoglobin-based oxygen carriers so attractive in brain injury because it reduces the cerebral blood volume.

Fourth, I think vasoactive or vasogenic brain edema begins immediately after brain injury, and is maximal about 18 hours after injury. It is entirely possible that the HBOC animals, if they had been followed long enough, would have developed the same ICP changes that you observed in your Ringer's group. In fact, I saw that at around age plus 360 at 6 hours, your ICPs were, in fact, trending up, which looks like

you have some ongoing vasogenic edema in those animals, so please comment on the length of your study paradigm.

In conclusion, I commend the authors for attempting to address an important clinical problem in trauma, but for the reasons I've listed, I'm not sure that this particular study brings us any closer to an answer. For example, I would have liked to have seen a comparison of DCLHB, and I know Ken has access to that and your biopure, to truly try to isolate that because biopure has less of a presser effect than DCLHB to compare those two in your model to see if there is an effect. Again, I commend you for this work and I encourage you to continue your fine attempts to unlock this vexing problem.

Dr. John R. Hall (Kingsport, TN): I noticed you used Oxiglobin, which is the animal product instead of Hemopure, the human product. There are some differences in the oncotic pressure, osmotic pressure, and tetramers and vasogenic properties of the two. Did you have any usage of the latter?

Dr. Naoki Aikawa (Tokyo, Japan): I am wondering if you have determined a urine histology, and if there is any pathohistological study on that model, too. My simple question is that is the swine model most appropriate for this kind of study? Thank you.

Dr. Ajai Malhotra (Richmond, VA): I am a bit perturbed as to why you blindly used mannitol. While it's true that it is used in treating head injury, it's not just blindly given to people. Secondly, you comment that the amount of fluid required for the standard of care group was much, much more, but could it not be just that the mannitol was causing a severe diarrheses, and that's why you were causing it?

Lastly, again, on mannitol, I'm sorry, interference with it, does the product not interfere with the saturation that you measure because you showed that mixed venous saturation in this product really measures, and similarly, does it not interfere with the tissue brain, brain tissue oxygen that you have put forward? Once again, I enjoyed the paper very much.

Dr. David R. King (Closing): I'll try to address these questions in a systematic fashion. This study was designed with a specific aim in mind; that is most clinical trials that are being designed to evaluate the use of hemoglobin-based oxygen carriers in the human population all exclude traumatic brain injury, probably because of the universally poor outcomes associated with that. We also think that this is the group that may stand to benefit the most from the use of a hemoglobin-based oxygen carrier, especially one that may have some presser action. So, our goal was to set out to see if there were any short-term benefits from using this substance in this setting—that is brain injury and combined hemorrhagic shock.

That led to the first series, just looking at secondary measures of outcome. Well, of course, we looked at mortality, but there were no mortality differences between those groups except secondary outcomes like intracranial pressure and brain tissue po_2 . The survivor series, you are correct, is not necessarily powered to detect mortality differences for sure. It was merely to assure ourselves that there wasn't some

horrid disaster lurking behind a transient increase in lactate and increase in O_2 extraction that we saw in the first series. In fact, this issue does have to be investigated further in a much larger animal population.

Next, you asked about the magnitude of the hit. We agree. In fact, in our experience and our lab has a lot of experience with the fluid percussion brain injury, there are significant variables that can exist, especially with regard to the device itself. Most of the variability of the hit, we found, can be eliminated by careful maintenance of the device and by particular attention to how the fittings are connected and so on. In our hands, the device delivers a relatively reliable—about 9 atm—hit in a very reproducible fashion. In terms of postmortem exams, we used to perform postmortem exams on every single animal, every single experiment.

In this series we performed posts on all the survivors, and posts on only a sampling of the nonsurvivor series. The reason for this is because in our experience doing hundreds and hundreds of these animals the results are relatively reproducible, and so we can usually detect when there is something wrong with the way the hit has gone. In terms of the vasoconstrictive properties of the HBOC, indeed, we think that's probably one of the main mechanisms to explain the benefits of this substance. It's hard to believe that restoring the minimal amount of oxygen carrying capacity we infused by using Oxiglobin resulted in any significant increase in O_2 delivery. In fact, we didn't detect that, but it's possible, although we don't think that's significant. In fact, we only restored about 2.5 mm of O_2 per dL of total body blood of oxygen-carrying capacity, which is relatively insignificant in the grand scheme. So we agree. We think that the benefits are probably the vasoconstrictive effects as well as the crystalloid sparing effects. In our mind and one of our proposed mechanisms of action, although not verified scientifically, is that probably Oxiglobin isn't so good, it's the fact that our so-called "standard of care" is probably not that great. In terms of intracranial pressure, by the end of our 6-hour observation period, intracranial pressure was rising. In fact, it wasn't as bad as the standard of care arm, up in the range of 40, but it was on the rise.

Of course, you have to draw the line in the sand somewhere. We don't have unlimited resources in personnel. Obviously, the ideal thing to do would be to follow these animals nearly indefinitely or until complete recovery in the ICU. That's just not possible. However, in the survivor series, for the animals that did go to the vavarium and returned on Day 3, their intracranial pressure in that group was 6 ± 2 mm Hg. So, it's possible that while the animals are not receiving neurocritical care in the vavarium that their ICP went very high and then recovered. We just don't know that for sure. However, in our experience, animals that have an ICP over about 25 are unable to wean and be extubated. In fact, they are unable to have organized respiration. Those animals all die in the cage. The fact that the animals lived 3 days without neurocritical care is some indirect clinical evidence that ICP

did not spike up into the range of 30 or 40, although we just don't know that for a fact.

Dr. Hall asked about the Hemopure substance versus the Oxiglobin substance. In our communication with the manufacturer we did not have access to Hemopure. It would have been nice to use that, because as you said there are some small differences between those substances but access to that was not granted to us, and we used the animal product called Oxiglobin.

The next question was about histology, and if swine were an appropriate model for this. We think that a large animal is an excellent model to study this in, although all models are faced with certain limitations, and regardless of what model you choose, there are certain criticisms and limitations that are incumbent and associated with that model.

In terms of histology, no, we did not look at histologic outcomes in this series. In terms of mannitol, we did not use mannitol indiscriminately. Our criterion for administration of

mannitol was whenever ICP was greater than 20. We tried to pick a criterion that we thought was clinically relevant, so this is just what we decided on.

The next question was about urine output and whether or not the dosing of high dose mannitol, because we gave a gram per kilogram, resulted in a dramatic diarrhesis. No, that didn't happen. In fact, urine output between these groups was not significantly different. Neither of the groups need a lot of urine. And bullous administration of a dose of mannitol appeared not to change that.

Finally, with respect to instrumentation, you're correct. The manufacturer even has warnings on their packaging regarding how Oxiglobin and free globular hemoglobin may affect laboratory measurements. The devices that we used, the Abbott swans and the cooximeter we used in our lab, have all been verified to be accurate in the presence of these molecules. I hope that addresses all the questions. Again, I want to thank the association for allowing me to present this.