SHOCK, Vol. 27, No. 6, pp. 652–656, 2007

RESUSCITATION FROM HEMORRHAGIC SHOCK WITH HBOC-201 IN THE SETTING OF TRAUMATIC BRAIN INJURY

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Received 6 Sep 2006; first review completed 26 Sep 2006; accepted in final form 27 Sep 2006

ABSTRACT—Outcomes after mild or moderate head trauma are worsened with associated hypotension, and secondary brain injury can be reduced with timely resuscitation. This study was performed to investigate HBOC-201 as a resuscitation therapy in a combined hemorrhagic shock and brain injury model. Anesthetized rats sustained moderate brain injury using a controlled cortical impact device, followed by rapid hemorrhage to a mean arterial pressure of 30 mmHg. After 30 min of hypotension, animals were resuscitated with HBOC-201, autologous shed blood (SB), or lactated Ringer solution (LR). Brain injury was assessed by measurements of cerebral blood flow (CBF) and cerebral vasoreactivity to hypercapnia (CVH) using a laser Doppler flowmeter. Contusion volume was evaluated histologically, and cerebral edema was determined by total water content. The HBOC rats required significantly less resuscitation volume versus LR and SB. The CBF was significantly diminished at 60 min after resuscitation with HBOC (70.1% ± 3.8% baseline) compared with LR (105.8% ± 10.1% baseline; $P < 0.01$) and SB (96.8% ± 5% baseline; $P < 0.05$). The CVH was preserved in the HBOC and SB groups. The CVH was significantly diminished compared with baseline in the LR group at 30 min after resuscitation and showed a significant loss compared with HBOC at 60 min after resuscitation. The contusion volume for HBOC (45.1 mm^3) and SB (35.1 mm^3) was less than LR (63.5 mm^3, $P < 0.01$). Although CBF was diminished after resuscitation in the HBOC group, HBOC-treated animals maintained CVH and experienced significantly smaller contusion volume than those treated with LR. These results suggest that resuscitation with HBOC-201 protects autoregulatory mechanisms and may reduce secondary brain injury in traumatic brain injury.

KEYWORDS—Hemoglobin-based oxygen carriers, rat model, controlled cortical impact injury

INTRODUCTION

Head injury remains the leading cause of traumatic death in the United States (1, 2). Several investigators have noted that outcomes after head injury are severely worsened with associated hypotension (3–5), and that the secondary injury that results can be significantly reduced with early and aggressive resuscitation (6, 7). The ideal fluid for volume resuscitation in a head-injured patient is a low-volume resuscitative fluid that maintains effective mean arterial pressure (MAP) and cerebral blood flow (CBF) while keeping intracranial pressure (ICP) low. Significant advances have been made in the development of oxygen-carrying solutions based on cell-free hemoglobin preparations in recent years (reviewed in (8, 9)). The most promising of these seems to be polymerized hemoglobin-based oxygen-carrying (HBOC) solutions. The HBOC-201 (Hemopure; Biopure Corp, Cambridge, Mass), a polymerized hemoglobin oxygen carrier, has been shown in several preclinical studies to be an effective low-volume resuscitative agent (10–12). This solution requires no crossmatch and can be stored for prolonged periods (up to 3 years) without the need for refrigeration (13). This makes HBOC-201 ideal for use in the prehospital civilian or military environment where blood is not readily available. However, concerns have been raised about the potential for direct neurotoxicity of hemoglobin-containing fluids in the setting of traumatic brain injury (TBI). Although free hemoglobin has been shown to be directly toxic to neural cells in culture (14), *in vitro* studies evaluating HBOC-201 have shown that this agent is not directly neurotoxic (15). In addition, it is unclear what effect the vasoactive properties of these solutions will have on cerebral perfusion and secondary brain injury. This report outlines an initial *in vivo* study to evaluate the effects of HBOC-201 as a resuscitative fluid in a rat model of combined hemorrhagic shock and TBI.

MATERIALS AND METHODS

Animals

All experiments 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 University of Texas Institutional Animal Care and Use Committee, and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International. Sixty-four Male Sprague-Dawley rats (400–500 g) were used (Harlan Laboratories, Indianapolis, Ind). All animals were observed daily for general health, and all invasive procedures were performed under aseptic conditions. Rats were fasted 12 hours before surgery but received water *ad libitum*.

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This study was partially supported by Biopure Corporation via the donation of Hemopure (HBOC-201) and the Office of the United States Air Force Surgeon General. The opinions expressed here are solely those of the authors and do not represent the views of the United States Air Force, the United States Department of Defense, or the United States Government.

Data presented as a poster at the Society of Critical Care Medicine 34th Critical Care Congress, Phoenix, Arizona, January 15–19, 2005.

DOI: 10.1097/01.shk.0000248584.10400.dc

TABLE 1. **Treatment details**

Group	Weight (kg)	Hemorrhage (mL/kg)	Resuscitation (mL)
HBOC	0.421 ± 20	24.4 ± 0.8	3.9 ± 0.1
SB	0.420 ± 15	24.4 ± 0.7	10.2 ± 0.4
LR	0.406 ± 15	26.2 ± 0.9	45.9 ± 2.7
ANOVA *P*	0.767	0.191	<0.001

Animal preparation

General anesthesia was induced in a vented anesthesia gas chamber using 5.0% halothane in air. After induction anesthesia, animals had their airway secured using either an open tracheostomy tube placement (physiology experiments; short survival) or endotracheal intubation using a 14-gauge angiocath (24-hour survival). Open tracheostomy tube placement was necessary for physiology experiments to ensure that no air leak occurred during induced hypercapnea. After airway control, animals were mechanically ventilated using a time-cycled, pressure-limited small animal ventilator (Harvard Apparatus, Holliston, Mass). Halothane levels were decreased to 1% maintenance and titrated as needed, not exceeding 1.5%. The femoral vessels were then isolated bilaterally, and arterial and venous catheters were placed on one side to allow for controlled hemorrhage and resuscitation, and an arterial catheter was placed on the opposite side for blood pressure monitoring. Animals were then placed in a stereotactic frame (David Kopf Instruments, Tujunga, Calif).

Controlled cortical impact injury

Traumatic brain injury was produced with a controlled cortical impact (CCI) device as described previously (16). Briefly, after a midline incision, the scalp and temporal muscle were reflected, and an approximately 10-mm-diameter craniotomy was performed using a dental drill over the left temporoparietal skull. The dura mater was left intact. A stream of cool air was directed at the site during drilling to prevent thermal injury to the brain tissue. An additional craniotomy was performed on the ipsilateral side for later use in CBF monitoring. After craniotomy, the animal was allowed to equilibrate for 30 min before cortical injury. Injury was induced using a pneumatic cylinder with a 9.5-mm-diameter impact bullet tip connected to a pneumatic control box (Bioengineering Department, Medical College of Virginia, Richmond, Va). The impact velocity was adjusted to 7.9 ± 0.1 m/s with a duration of 50 ms, resulting in a 2.5-mm tissue deformation.

Hemorrhage

Immediately after the CCI injury, each animal was rapidly hemorrhaged through the femoral artery over approximately 7 min, until the MAP had decreased to 30 mmHg. All animals were kept hypotensive (MAP, 30 ± 5 mmHg) for 30 min by continued intermittent hemorrhage. The shed blood (SB) was collected into citrate phosphate dextrose and kept at room temperature until reinfusion. After the hypotensive period, the animals were resuscitated to baseline MAP with either: (1) lactated Ringer solution (LR, n = 9); (2) autologous SB (n = 9); or (3) Hemopure HBOC-201 (Biopure Corp; n = 9), all administered i.v. During the entire procedure, MAP was continuously monitored and recorded using a MacLab system (ADInstruments, Grand Junction, Colo). Core temperature was maintained at 37°C by using a heating lamp or a heating pad connected to a temperature controller coupled to a rectal probe (Dwyer Instruments Inc, Michigan City, Ind).

Cerebral blood flow

Cerebral blood flow on the same side of injury was continuously monitored through the previously placed craniotomy using a laser Doppler flowmeter as described previously (16). Briefly, a laser Doppler flowmeter (Vasamedics, St Paul, Minn) was connected to a 0.8-mm probe that was mounted on a stereotactic micromanipulator and placed approximately 0.1 to 0.2 mm above the dura mater. The probe position was moved until an area with a flow signal of less than 50 arbitrary units (tissue perfusion units [TPU]) was found, thereby avoiding large blood vessels, and this position remained unchanged for the rest of the experiment.

Hypercapnia vasodilation

The CBF responses to hypercapnia were tested at baseline, 30 min, and 60 min after resuscitation as described previously (16). Moderate hypercapnia (partial pressure of carbon dioxide [pCO_2] = 50–65 mmHg) was induced by adding CO_2 to the circuit of the ventilator and confirmed by monitoring arterial blood gas. The elevation in pCO_2 was maintained until the resulting increase in CBF reached a plateau state (approximately 7–8 min). After the end of the 60-min post-resuscitation measurements, animals were euthanized with a hypertonic potassium chloride solution given i.v.

Contusion volume and edema

A separate group of animals underwent CCI followed by hemorrhage and resuscitation and survived for an additional 24 h to assess the degree of contusion volume and brain edema formation. Animals were euthanized as previously described, and brain tissue was harvested. For contusion volumes (HBOC, n = 6; LR, n = 5; SB, n = 5), the forebrain, including the entire area of injury, was snap frozen, coronally sectioned with a cryostat in 30-μm segments and collected in 150-μm intervals onto microscope slides, then stained with thionine as described elsewhere (17). Briefly, the tissue was fixed in 10% formaldehyde, and then stained in thionine solution for 60 min. After staining, the slides were dehydrated through a series of ethyl alcohol washes, and slide covers were mounted. Digital images were then taken of all sections, and contusion volume was measured by blinded observers using Image-Pro (Media Cybernetics Inc., Silver Spring, Md) software (version 4.1 for Windows NT). The software was used to calculate the total contusion volume (mm^3) for each animal. For measurement of edema formation, animals (n = 7 for each group) were euthanized, and whole brains were harvested as described previously. The amount of edema in each brain was assessed by a previously described water content method (18). Briefly, excised brain tissue was weighed immediately after dissection and after 48 h of dehydration at 70°C. The water content was expressed as a percentage, calculated as ([wet weight − dry weight]/wet weight) × 100.

Statistics

Analysis of variance (ANOVA) was used to evaluate comparisons among multiple groups using Origin 5.0 software (Microcal Software, North Hampton, Mass). Pairwise comparisons were used to analyze differences between groups. $P <$ or = 0.05 (2-sided) were considered significant.

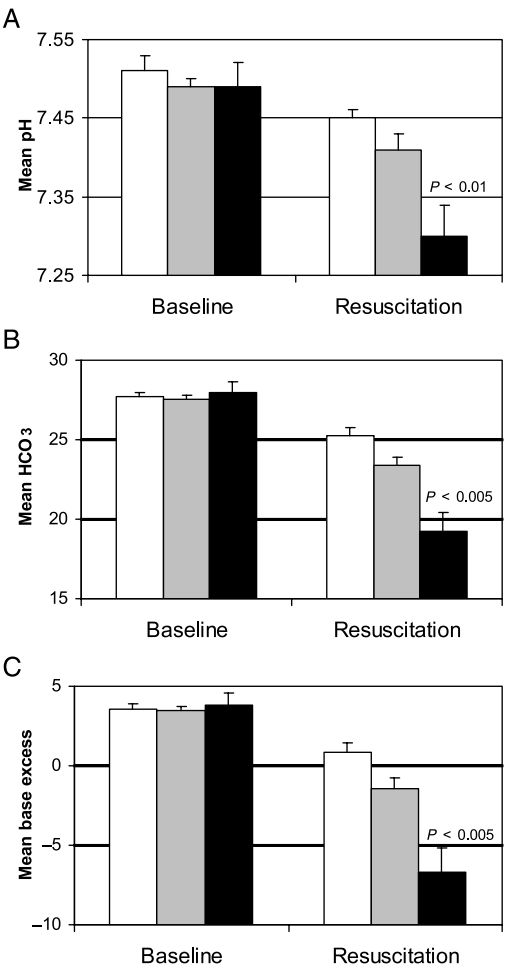

FIG. 1. **Metabolic indicators.** Bar graphs showing the mean (±SEM) metabolic indicators before hemorrhage and after resuscitation with HBOC (open bars), SB (gray bars), or LR (black bars). A, pH. B, Bicarbonate. C, Base excess.

RESULTS

Treatment details

All experimental groups were similar with regard to weight and blood volume hemorrhaged (Table 1). All animals survived the hemorrhage-resuscitation portion of the protocol. However, the total volume of resuscitation fluid that the animals received was significantly less in the HBOC group when compared with the LR group (3.9 ± 0.1 mL vs. 45.9 ± 2.7 mL; $P < 0.001$). Lower volumes of HBOC-201 were required when compared with SB, although the total amount of resuscitation did not reach statistical significance between these groups.

Metabolic indicators

All groups manifested a metabolic response to shock with acidosis. Arterial pH in all groups was equivalent at baseline (Fig. 1A). All groups showed a decrease in pH that persisted into resuscitation. However, the pH in the LR group was significantly lower than either the HBOC or SB groups (7.30 ± 0.04 vs. 7.45 ± 0.01 and 7.41 ± 0.02, respectively, $P < 0.01$). The bicarbonate and base excess levels paralleled the pH results, with the LR group remaining lower after resuscitation (Fig. 1, B and C).

Cerebral blood flow

All groups were similar with regard to percentage change from baseline at the end of the shock, although CBF was noted to be higher than baseline in the LR and SB groups in the postresuscitation period. Compared with LR, animals resuscitated with HBOC had significantly decreased CBF after resuscitation at 30 (74.8% ± 5.9% baseline vs. 129.2% ± 11.6% baseline; $P < 0.01$) and 60 min (70.1% ± 3.8% baseline vs. 105.8% ± 10.1% baseline; $P < 0.01$). In addition, HBOC-resuscitated animals had significantly decreased CBF when compared with SB at 60 min (96.8% ± 5.0% baseline; $P < 0.05$). Although the SB group demonstrated a lower overall CBF when compared with LR, these differences were not statistically significant. No significant differences were noted between groups with regard to systemic MAP at any of the time points analyzed (Fig. 2B).

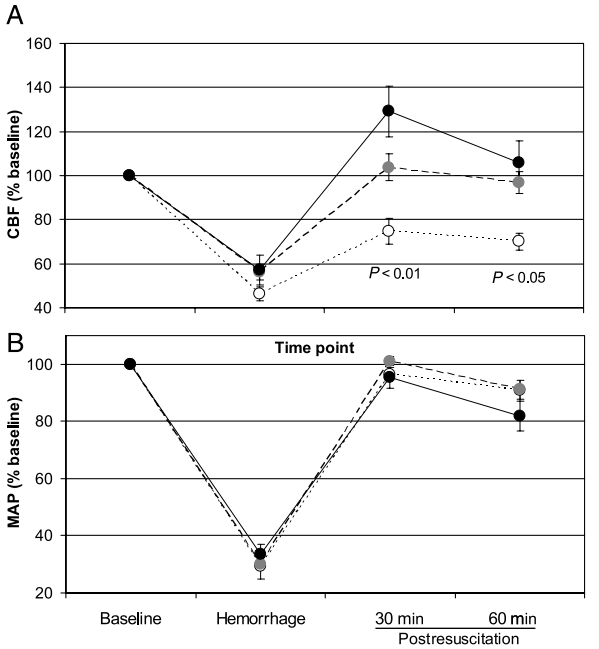

FIG. 2. **Cerebral blood flow and arterial blood pressure.** A, Mean (±SEM) percentage changes from baseline CBF determined using laser Doppler flowmeter before and after hemorrhage, and at 30 and 60 min after resuscitation with HBOC (open circles, baseline = 44.7 ± 3.0 TPU), SB (gray circles, baseline = 40.7 ± 2.2 TPU), or LR (black circles, baseline = 38.6 ± 2.8 TPU). B, Mean (±SEM) percentage changes in MAP before and after hemorrhage, and at 30 and 60 min after resuscitation with HBOC (open circles, baseline = 112 ± 2.0 mmHg), SB (gray circles, baseline = 112 ± 2.4 mmHg), or LR solution (black circles, baseline = 111 ± 2.0 mmHg). ANOVA *P* values for between-group significance are shown.

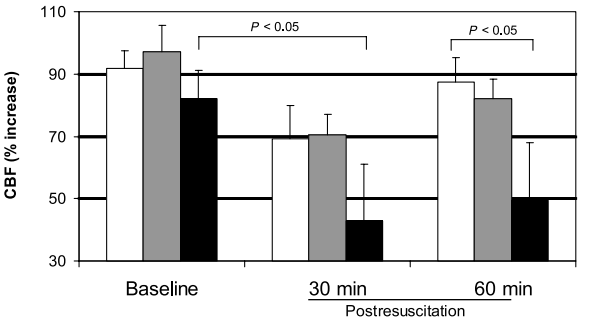

FIG. 3. **Cerebral blood flow reactivity to hypercapnia.** Mean (±SEM) percentage increases in CBF with moderate hypercapnea (pCO_2 = 50 – 65 mmHg) before hemorrhage (baseline), and at 30 and 60 min after resuscitation with HBOC (open bars), SB (gray bars), or LR (black bars). ANOVA *P* values for significance are shown.

CO_2 reactivity

No differences were noted between groups when comparing prehemorrhage percentage increase in CBF with induced hypercapnea (Fig. 3). The CO_2 reactivity was slightly diminished compared with prehemorrhage levels at 30 min after resuscitation in SB- and HBOC-resuscitated animals (P = not significant for both groups) and showed recovery to near prehemorrhage levels at 60 min. The LR response was significantly diminished at 30 min after resuscitation when compared with prehemorrhage (43.0% ± 18.1% vs. 82% ± 9.1%; $P < 0.05$) and was significantly diminished when compared with HBOC (49.4% ± 18.5% vs. 87.4% ± 7.8%; $P < 0.01$) at 60 min after resuscitation.

Brain edema and contusion volume

No differences were recorded in the amount of brain edema on either the ipsilateral or contralateral side of the injury between the treatment groups (Table 2). A difference was noted in the size of the lesions depending on which resuscitation fluid was used, with the SB (35.1 ± 2.6 mm^3)

TABLE 2. **Brain edema, expressed as water content, and contusion volumes measured after CCI followed by hemorrhage and then resuscitation with HBOC, SB, or LR**

Group	Edema (% water)	Contusion volume (mm^3)
HBOC	86.3 ± 0.8	45.1 ± 4.3
SB	86.4 ± 0.7	35.1 ± 2.6
LR	87.3 ± 1.2	63.5 ± 5.2
ANOVA *P*	0.904	$P < 0.01$

and HBOC (45.1 ± 4.3 mm^3) groups having significantly smaller contusion size when compared with the LR group (63.5 ± 5.2 mm^3; $P < 0.01$; Table 2).

DISCUSSION

The data from this study provide additional evidence to support further study in the use of HBOC-201 for the prehospital treatment of patients with combined hemorrhagic shock and TBI. To appropriately review these results within the context of the prehospital setting requires direct comparison of isotonic crystalloid resuscitation (the current standard of care) with HBOC-201. In this study, HBOC-201 was superior to LR with regard to contusion volume and preservation of CO_2 vascular reactivity. This model provides a highly reproducible and consistent degree of brain injury in the animals. Therefore, the difference in contusion volume size is related to the amount of secondary brain injury sustained by the animals. Human clinical studies have shown that between 50% and 90% of patients dying from TBI have evidence of secondary brain ischemia caused in part by hypotension and hypoxemia (19, 20). Patients sustaining episodes of hypoxemia or hypotension after TBI have twice the incidence of an adverse outcome (5, 21). The finding of significant decreases in contusion volume with HBOC-201 resuscitation suggests that outcome in patients with hemorrhagic shock and TBI may be improved with HBOC-201 resuscitation in the prehospital setting.

Additional studies in TBI have been performed using an earlier-generation HBOC, diaspirin cross-linked hemoglobin (DCLHb), as a resuscitation fluid. Studies conducted in a large animal model comparing standard prehospital resuscitation (LR) with DCLHb have shown increased cerebral perfusion pressure (CPP) and decreased ICP, with reduced, although not statistically significant, lesion volume (22). This study supports a previous preclinical study evaluating the effects of HBOC-201 after TBI and hemorrhage (23). The previous HBOC-201 study used a large animal model of TBI and shock and found that a single bolus of HBOC-201 given in a simulated prehospital dose improved CPP and brain oxygenation. Although CPP and ICP were not measured in our study, CBF was directly measured in an uninjured portion of the ipsilateral contused brain using laser Doppler flowmetry. All groups were similar with regard to baseline and hypotensive CBF. Although animals resuscitated with HBOC-201 showed significantly decreased CBF after resuscitation to baseline MAP compared with SB and LR groups, this did not have an adverse outcome on size of contusion volume, as LR animals had significantly larger contusion size. This may be partially explained by the high P_{50} and rheologic properties of HBOC-201 that favor oxygen delivery and off-loading at the tissue level. In a study using a large animal model of hemorrhage and resuscitation, small volumes of HBOC-201 were found to increase brain tissue oxygen tension levels by 66% more than baseline (24). Increased oxygen delivery around the area of injury would diminish the ischemic penumbra after TBI and could subsequently lead to a reduction in secondary brain injury. The maintenance of CO_2 reactivity in the HBOC-resuscitated animals provides additional evidence supporting adequate oxygen delivery. Although overall CBF was reduced in this study after HBOC-201, cerebral vascular autoregulatory mechanisms remained intact. If significant cerebral hypoxic conditions were present, these autoregulatory mechanisms would be significantly altered as seen in the LR group. Maintenance of CO_2 reactivity was also demonstrated in another study in which a less purified form of HBOC (Oxyglobin, Biopure Corp, Cambridge, Mass) was used to resuscitate animals after TBI and hemorrhage. This study also showed an improvement in the maintenance of this autoregulatory mechanism when compared with LR resuscitation (25).

Maintenance of CO_2 reactivity in the brain after resuscitation with HBOC-201 is interesting in light of the fact that HBOCs are thought to be potent nitric oxide (NO) scavengers (26). It is well established that NO plays a critical role in cerebral vascular reactivity to hypercapnia, and a baseline level of NO is required as a permissive factor for vascular dilatory action to occur (27). In the current study, we demonstrated that CO_2 reactivity was well preserved, thereby suggesting that NO scavenging has not occurred to a significant degree, and therefore local NO concentrations are adequate to allow vasodilation. This finding lends support to other studies reporting vasoconstriction without decrease in local NO levels after resuscitation from hemorrhagic shock with HBOC-201, thereby suggesting that other mechanisms besides NO scavenging may play an important role in the vasoconstrictive properties of these agents (28).

The persistent low cerebral perfusion after resuscitation is most likely secondary to the significantly smaller resuscitation volumes required by the HBOC animals. This is consistent with other studies showing HBOC-201 to be an effective low-volume resuscitation agent (10–12). Another study using DCLHb in a small animal model of brain injury without associated hemorrhagic shock showed that DCLHb, given in equivalent volumes as other agents, increased CPP, decreased ICP, and increased CBF (29). In addition, the previously mentioned large animal models using DCLHb used equivalent volumes of agent or LR in the initial resuscitation and showed improved CPP (22, 30, 31). Therefore, evaluating this study in the context of previously published work with other HBOCs, we believe that the decrease in CBF after resuscitation is most likely secondary to the significantly reduced amount of resuscitation fluid required by the HBOC group. Further studies using a volume end point to guide resuscitation will need to be conducted to confirm this hypothesis. However, as long as the cerebral vascular reactivity is intact, so that oxygen delivery can be adjusted to meet the metabolic demands of brain tissue, the low CBF in the anesthetized rat does not seem to affect the extent of brain injury as demonstrated in the current study.

In the prehospital environment where autologous blood is not readily available, these data provide compelling evidence that HBOC-201 may lead to improvements in neurological outcomes when compared with currently available crystalloid resuscitation fluids. The results of this study suggest that low-volume resuscitation with HBOC-201 prevents secondary brain injury and significantly reduces brain lesion size when

compared with LR. Further study is warranted with these agents in TBI to prove clinical efficacy.

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