Prehospital HBOC-201 After Traumatic Brain Injury and Hemorrhagic Shock in Swine

Mayur B. Patel, MD, Ara J. Feinstein, MD, MPH, Alvaro D. Saenz, MD, Matthias Majetschak, MD, PhD, and Kenneth G. Proctor, PhD

Background: **Data are limited on the actions of hemoglobin based oxygen carriers (HBOCs) after traumatic brain injury (TBI). This study evaluates neurotoxicity, vasoactivity, cardiac toxicity, and inflammatory activity of HBOC-201 (Biopure, Cambridge, Mass.) resuscitation in a TBI model.**

Methods: **Swine received TBI and hemorrhage. After 30 minutes, resuscitation was initiated with 10 mL/kg normal saline (NS), followed by either HBOC-201 (6 mL/kg, n = 10) or NS control (n = 10). Supplemental NS was administered to both groups to maintain mean arterial pressure (MAP) >60 mm Hg until 60 minutes, and to maintain cerebral perfusion pressure (CPP) >70 mm Hg from 60 to 300 minutes. The control group received mannitol (1 g/kg) and blood (10 mL/kg) at 90 minutes and half (n = 5) received CPP directed phenylephrine (PE) therapy after 120 minutes. Serum cytokines were measured with ELISA and coagulation was evaluated with thromboelastography. Brains were harvested for neuropathology.**

Results: **With HBOC administration, MAP, CPP, and brain tissue Po_2 were restored within 30 minutes and maintained until 300 minutes. Clot strength and fibrin formation were maintained and 9/10 successfully extubated. In contrast, with control, MAP and brain tissue Po_2 did not correct until 120 minutes, after mannitol, transfusion and 40% more crystalloid. Furthermore, without PE, CPP did not reach target and 0/5 could be extubated. Lactate, heart rate, cardiac output, mixed venous oxygenation, muscle oxygenation, serum cytokines, and histology did not differ between groups.**

Conclusions: **After TBI, a single HBOC-201 bolus with minimal supplements provided rapid resuscitation, while maintaining CPP and improving brain oxygenation, without causing cardiac dysfunction, coagulopathy, cytokine release, or brain structural changes.**

Key Words: **Brain injury, Swine, Blood substitute, HBOC, Hemoglobin based oxygen carrier.**

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Hemoglobin based oxygen carriers (HBOCs) are currently being tested in US Food and Drug Administration (FDA) approved phase III clinical trials in several patient populations, including urban trauma patients with major blood loss.[1–5] Efficacy endpoints, such as mortality, transfusion avoidance, or organ perfusion, are key issues in the design and interpretation of all of these trials.[6] However, safety remains the major obstacle to FDA approval, with specific concerns related to neurotoxicity, vasoactivity, cardiac toxicity, and proinflammatory activity.[7]

Current HBOC trials exclude patients sustaining severe TBI, likely because of limited preclinical and animal data. Interestingly, this is the population that stands to substantially benefit with HBOC prehospital resuscitation, as rapid reversal of hypotension and cerebral ischemia represents the cornerstone of therapy against secondary brain injury.[8] After fluid percussion TBI and hemorrhagic shock in swine, our group showed that DCLHb (Baxter Healthcare Corp., Round Lake, Ill.) improved cerebral perfusion pressure (CPP) and cerebral oxygen delivery, but had undesired vasoactivity.[9,10] We also showed that HBOC-301 (Oxyglobin, Biopure Inc., Cambridge, Mass.) improved cerebrovascular and neurologic function.[11] Others have shown Hemolink (Hemosol, Toronto, Ontario) maintains cerebral oxygenation in a model of severe isovolemic hemodilution.[12,13] However, DCLHb and Hemolink are no longer available and Oxyglobin has not been developed for use in humans. HBOC-201 or Hemopure (Biopure Inc., Cambridge, Mass.) is in advanced stages of development and it rapidly corrects brain tissue oxygenation ($PbtO_2$) in a non-TBI hemorrhagic swine model.[14,15] However, neither HBOC-201 nor any current generation HBOC in phase III clinical trials has been thoroughly characterized after TBI.

To fill this gap, we evaluated the neurotoxicity, vasoactivity, cardiac toxicity, and pro-inflammatory actions after resuscitation with HBOC-201 in a simulated "prehospital setting" relative to a control group, which incorporated standard neurotrauma care, including mean arterial pressure (MAP) and CPP management, blood transfusion, mannitol, and ± phenylephrine (PE) pressor therapy. Utilizing a well characterized and clinically relevant swine model of TBI and

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From the Divisions of Trauma and Surgical Critical Care (M.B.P., A.J.F., M.M., K.G.P.), DeWitt Daughtry Family Department of Surgery; Department of Pathology (A.D.S.), University of Miami Miller School of Medicine, Miami, Florida.
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Address for reprints: Kenneth G. Proctor, PhD, Divisions of Trauma and Surgical Critical Care, Daughtry Family Department of Surgery, University of Miami School of Medicine, Ryder Trauma Center, 1800 NW 10th Ave. Miami, FL 33136; email: kproctor@miami.edu.

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controlled hemorrhagic shock, the hypothesis was that the safety of prehospital HBOC resuscitation was equivalent, or superior, to standard neurotrauma care.

MATERIALS AND METHODS

Animal Preparation and Physiologic Endpoints

This protocol was approved by the University of Miami Animal Care and Use Committee. All animals in this study were handled according to the *Guide of the Care and Use of Laboratory Animals.*

Twenty-six farm-raised cross-bred swine of both genders (25–32 kg) were fasted overnight. Anesthesia was induced with 10 mg/kg intramuscular ketamine and 1 mg/kg intramuscular xylazine. After orotracheal intubation, anesthesia was maintained with 10 mg/kg/hr, 0.25 mg/kg/hr, and 50 μg/kg/hr of intravenous ketamine, xylazine, and fentanyl, respectively. Mechanical ventilation (Portable Adult Ventilator Model 754; Impact Systems, West Caldwell, N.J.) consisted of tidal volumes of 10 mL/kg, rates adjusted to maintain $Paco_2$ of 40 mm Hg, fraction of inspired oxygen (FiO_2) at 0.4, unless otherwise specified, and positive end expiratory pressure of 3 cm H_2O. All surgical sites were cleansed with betadine, and subsequently closed with a continuous stitch. Bilateral external jugular veins were accessed with 8-Fr introducer catheters for fluid administration and pulmonary artery catheter placement, respectively. An 8-Fr introducer catheter was placed in the right femoral artery for continuous arterial pressure monitoring and blood sampling. Cardiorespiratory status, pulse oximetry, capnography, MAP, and pulmonary artery pressure (PAP) were continuously monitored (Zoll M-Series Defibrillator Monitor Pacemaker, Chelmsford, Mass.). Cardiac output and mixed venous oxygen saturation (SVO_2) were continuously monitored (Baxter CCO Swan-Ganz catheter/Vigilance Monitor; Edwards Lifesciences, Irvine, Calif.). Muscle tissue oxygen saturation was measured using a near infrared spectroscopy probe (InSpectra Tissue Spectrometer; Hutchinson Technologies, Inc., Hutchinson, Minn.) placed on the surface of the left hind limb, as previously described.[16] A suprapubic cystostomy was made for Foley catheter placement and monitoring urine output. A heating blanket was used intermittently to maintain core temperature between 36°C and 39°C throughout the experiments.

A 1-cm craniotomy was made 1 cm left lateral of the bregma on the coronal suture. A hollow bolt was attached flush with the unbroken surface of the dura for subsequent fluid percussion delivery. Two cm anterior to the hollow bolt, on the contralateral side, another small craniotomy was made to introduce a Camino bolt for introduction of a fiberoptic intracranial pressure (ICP) transducer, a temperature electrode, and an intraparenchymal $PbtO_2$ electrode (all products; Integra Neurosciences, Kiel-Mielkendorf, Germany).

Experimental Design

After a 60 minutes postsurgery stabilization period, baseline data were collected for 30 minutes before injury. FiO_2 was reduced to 0.21 for 15 minutes before injury and remained at this level until 30 minutes after injury. At time zero, the injury was delivered by standardized fluid percussion TBI (~10 msec pulse at 6–8 atm) followed immediately by an arterial hemorrhage to a MAP of 20 mm Hg. The shed blood was stored in sterile plastic bags (CPDA-1 whole blood collection bag; Baxter Healthcare Corp.).

After 30 minutes, FiO_2 was increased to 0.40 and 10 mL/kg bolus of normal saline (NS) was administered to simulate initial prehospital care. All groups then underwent several sequential resuscitation phases: "prehospital" (t = 30–40 minutes), "emergency room" (ER, t = 40–60 minutes), and "intensive care unit" (ICU, t = 60–300 minutes).

The experimental design is outlined in Figure 1. In the prehospital and ER periods, the goal MAP was >60 mm Hg. In the ICU period, the goal CPP was >70 mm Hg.

At 300 minutes, if ICP <20, there was a 60 minutes extubation trial. During the extubation period, anesthesia and mechanical ventilation were discontinued sequentially, if tolerated. Those animals maintaining arterial oxygen saturation >90% were extubated.

After the initial bolus of 10 mL/kg NS, the test group (n = 10) received a prehospital bolus HBOC-201 (6 mL/kg) followed by supplemental NS to maintain MAP >60 mm Hg until 90 minutes and CPP >70 mm Hg thereafter. This HBOC-201 dose was shown to be effective in other prehospital models of resuscitation.[14,15]

After the initial bolus of 10 mL/kg NS, the control group (n = 10) received NS during prehospital care, ER, and ICU periods to the same MAP and CPP targets. Within the ICU phase (t = 90 minutes), this group received a 10 mL/kg transfusion of autologous shed blood, as well as 1 g/kg intravenous bolus of mannitol. After 120 minutes, half of this group (n = 5), received phenylephrine (PE, 0.1 mg/mL) therapy titrated to maintain CPP >70 mm Hg. Supplemental NS was withheld, unless central venous pressure <10 mm Hg. The HBOC group did not receive autologous shed blood, mannitol, or PE.

Cerebrovascular reactivity to CO_2 was evaluated at baseline, 125, 185, and 245 minutes post TBI. This technique has been previously described in detail.[17–19] During the CO_2 challenges, inhaled CO_2 was maintained at 10% for 10 minutes, while FiO_2 was maintained at 0.40. The magnitude of the CO_2-evoked ICP and $PbtO_2$ changes varies with cerebral compliance and vascular reactivity in animals[9,11,17–19] and patients.[20]

Other Outcome Variables

In addition to the physiologic data described above, arterial blood gases and electrolytes (Nova Stat Ultra; Nova Biomedical Corp., Waltham, Mass.) and complete leukocyte counts (Abbott Cell Dyne; Abbott Laboratories, Abbott Park, Ill.) were serially measured.

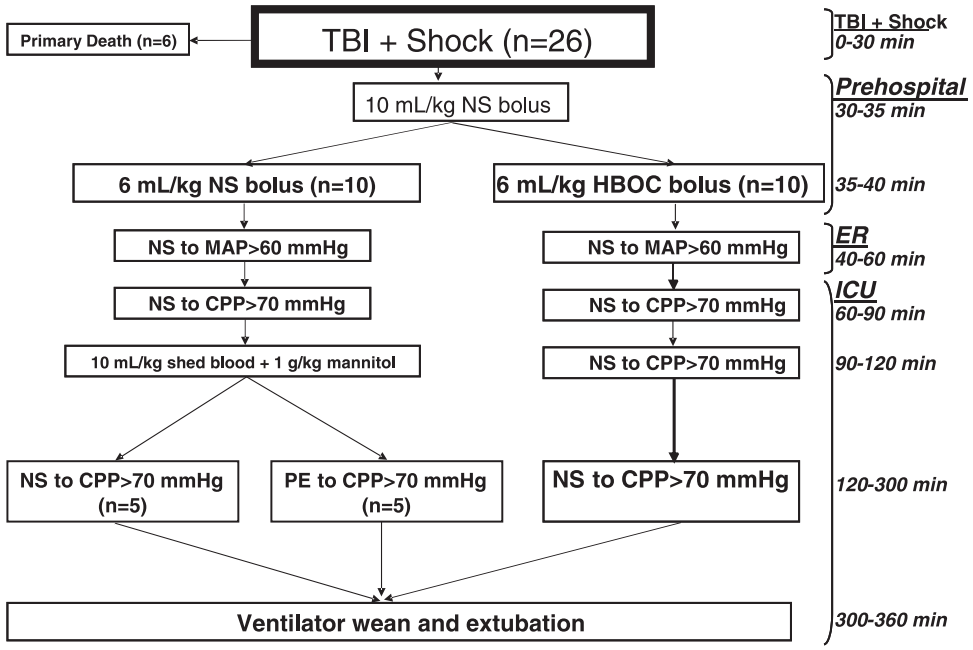

Fig. 1. *Resuscitation scheme after TBI and hemorrhage (shock) in swine. NS, normal saline; PE, phenylephrine; HBOC, HBOC-201; ICP, intracranial pressure; CPP, cerebral perfusion pressure. Resuscitation phases are prehospital, emergency room (ER), and intensive care unit (ICU).*

Cytokine assays were performed by collecting serum at baseline, 30, 90, 150, 240, and 300 minutes. Serum TNF-α and IL-6 were quantified using porcine ELISA kits (R&D systems, Minneapolis, Minn.).

Blood coagulation parameters were evaluated by thrombelastography (TEG Analyzer and TEG Analytical Software, Hemoscope Corp., Skokie, Ill.), as we previously described using native whole blood samples.[19] Briefly, the R time is the period of time from initiation of the test to initial fibrin formation. The K time is measured from the beginning of clot formation until the amplitude of the TEG reaches 20 mm, and represents the dynamics of clot formation. The alpha angle represents the kinetics of fibrin build up and cross-linking. The maximum amplitude (MA) reflects clot strength.

Finally, at the end of observation, brains were fixed in situ with 10% formalin via retrograde perfusion through bilateral internal jugular veins, and subsequently stored in 10% formalin. Gross pathology was noted before sectioning and staining with hemotoxylin and eosin with luxol fast blue. The sections of the brain specimens of each animal were screened by an experienced pathologist unaware of the identity and fate of each animal. A qualitative analysis of ischemic changes in uninjured and injured frontal-parietal region was completed.

Statistics

Data are expressed as mean ± SEM. Between-group comparisons were conducted with ANOVA and post hoc protected least significant difference analyses calculated with SPSS for Windows release 13.0 (Chicago, Ill.). All findings were considered statistically significant at the 95% confidence interval ($p <$ 0.05, two-tailed). Categorical variables were analyzed using the χ^2 test.

RESULTS

In baseline preinjury conditions, all physiologic variables were similar between the groups. Before resuscitation group assignment, there were six deaths during the 30 minutes shock period (Fig. 1). The hemorrhage volume to maintain a MAP of 20 mm Hg for 30 minutes (858 ± 39 and 895 ± 36 mm Hg) and the resultant lactate levels (9.4 ± 0.8 and 8.8 ± 0.8 mmol/L) were similar for the control and HBOC groups, respectively. There were no between group differences for any measured variable until the time of resuscitation.

Neurotoxicity

Upon resuscitation with HBOC, CPP was maintained at target levels throughout the observation period, except during the CO_2 challenges. In contrast, in the control group, it was impossible to meet the CPP target with NS (max. flow rate ≈4 L/h), mannitol (1 g/kg), and autologous blood transfusion (10 mL/kg), because of significantly increased ICP. After 120 minutes, in those that received PE, NS requirements ceased and ICP decreased to HBOC group levels, while CPP was maintained (Fig. 2, top and middle).

With HBOC, $PbtO_2$ was increased to baseline levels within 30 minutes after TBI. In contrast, control group $PbtO_2$ did not approach baseline levels until 120 minutes after TBI, with restoration to baseline only after PE administration. At 300 minutes, $PbtO_2$ remained significantly higher with HBOC than with NS alone. During the CO_2 challenges, PE increased

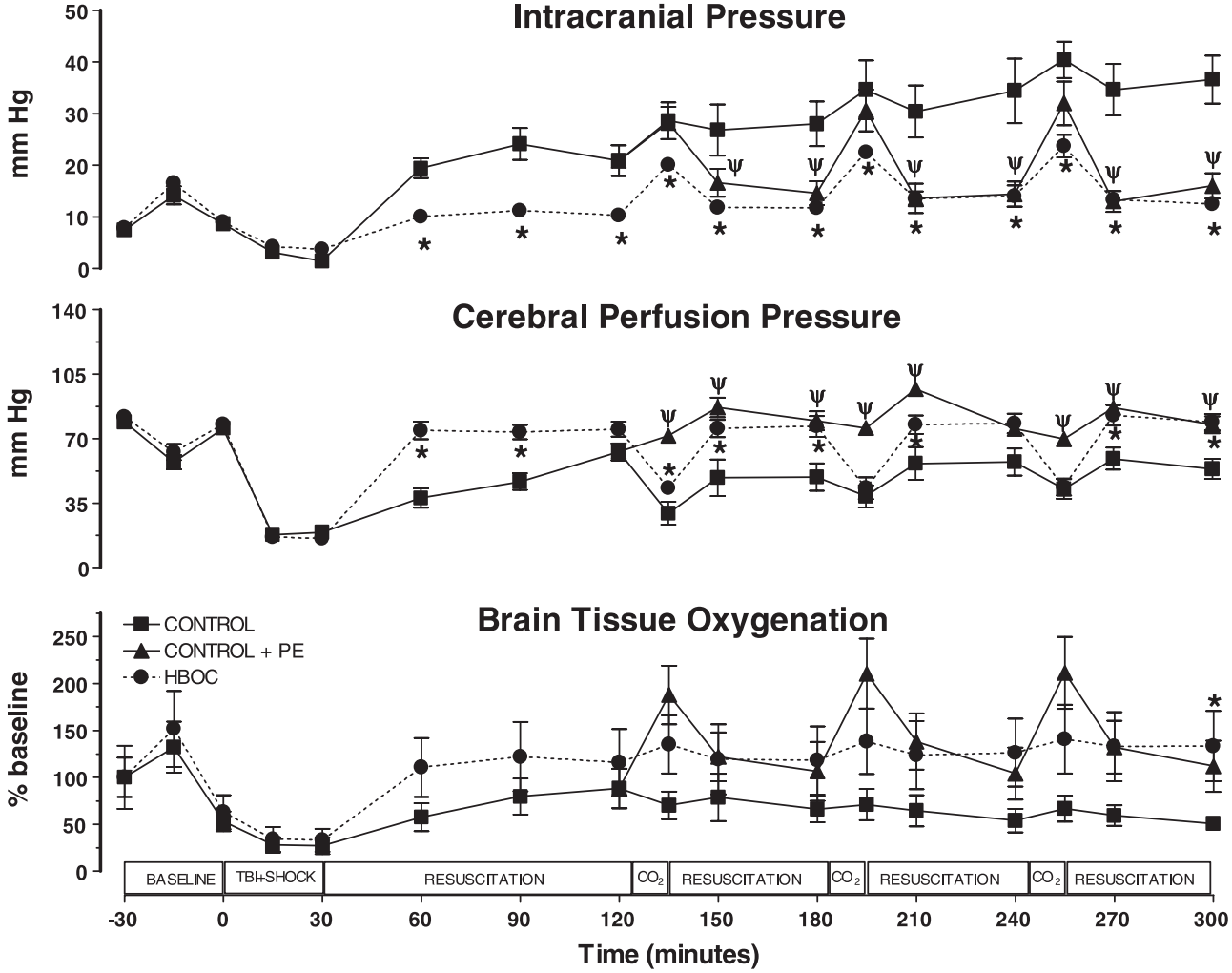

Fig. 2. *Intracranial pressure (ICP, top); cerebral perfusion pressure (CPP, middle); brain tissue oxygenation ($PbtO_2$, bottom). CONTROL, normal saline + mannitol + blood group; CONTROL + PE, normal saline + mannitol + blood + phenylephrine group; HBOC, HBOC-201 group; CO_2 represent 10% carbon dioxide challenges assessing cerebrovascular reactivity. In HBOC animals, CPP and brain tissue oxygenation corrected within 30 minutes. ICP, CPP, and brain tissue oxygenation were similar between HBOC and CONTROL + PE at 300 minutes. Cerebrovascular reactivity was higher in CONTROL + PE during all challenges.* *$p < 0.05$ *HBOC versus CONTROL,* $\psi p < 0.05$ *CONTROL versus CONTROL + PE,* $\zeta p < 0.05$ *CONTROL + PE versus HBOC.*

cerebrovascular reactivity compared with the other groups (Fig. 2, bottom).

At 330 minutes, 0/5 control animals met criteria to extubate, while 9/10 HBOC and 5/5 control + PE animals had ICP <20 mm Hg. These animals breathed spontaneously on room air, maintained oxygen saturation >90%, and maintained their airway postextubation ($p < 0.001$, χ^2).

Gross pathologic changes included subarachnoid hemorrhage. Histopathological changes included focal bilateral cortical ischemic changes, but there were no obvious treatment related differences at the site of injury or in the cortex (Fig. 3).

Vasoactivity

With HBOC administration, MAP corrected to baseline levels within 30 minutes of resuscitation (60 minutes post-TBI). During this time, MAP was significantly higher with HBOC than in the control group (Fig. 4, top). Furthermore, less total fluid was required to reach this goal (Fig. 4, bottom). In contrast, baseline MAP was not achieved in the control group until 120 minutes post-TBI, even though more NS and a blood transfusion were administered.

Cardiopulmonary Toxicity

By the end of the observation period, there were no differences in arterial blood gases or electrolytes (data not shown), however pH decreased in the control group (Table 1). Other physiologic variables, such as heart rate, cardiac output, calculated stroke volume, and SVO_2 did not differ significantly between groups. But, central venous pressure was lower in the HBOC and control + PE group. All animals maintained urine output, but there was an osmotic diuresis in the two control groups secondary to the bolus of mannitol at

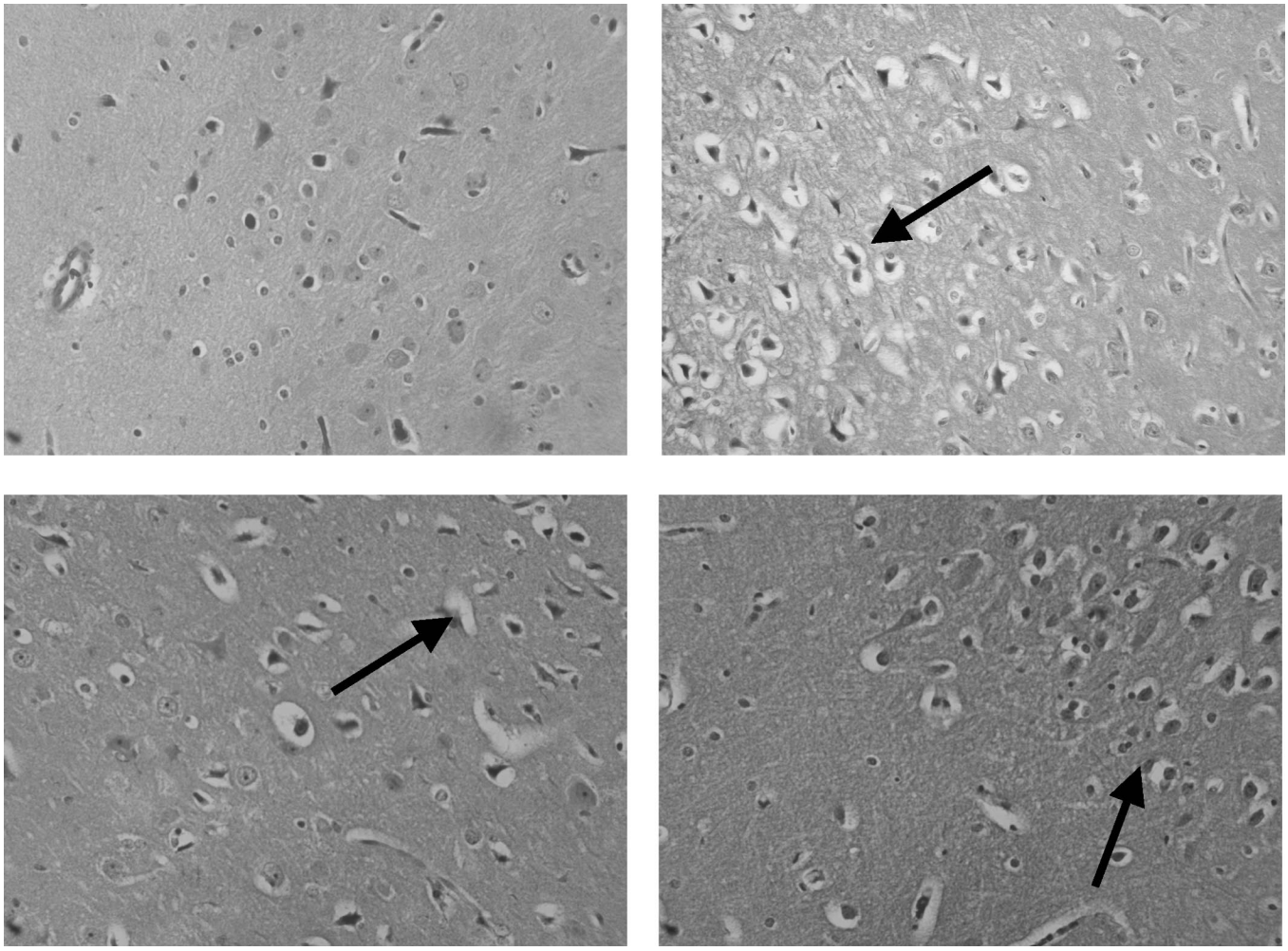

Fig. 3. *Neuropathology of brain cortex near site of fluid percussion injury (200× magnification). Normal porcine brain (upper left), HBOC animal (upper right), control (lower left), and control + phenylephrine (lower right). Compared with normal porcine brain, all groups had focal areas of acute ischemic changes including neurons with eosinophilic cytoplasm and nuclear pyknosis (arrows). No differences were noted between groups.*

90 minutes. End organ perfusion as assessed muscle tissue oxygenation and lactate clearance was similar between groups (Table 1).

By 180 minutes, peak inspiratory pressure was significantly increased in the control group, compared with the control + PE and HBOC groups. Lung function continued to deteriorate in the control group and by 300 minutes, there was a corresponding decrease in arterial Po_2 (Fig. 5).

Proinflammatory Toxicities

In baseline conditions, TEG ranges were as follows: R time, 11.6 to 28.1 minute; K, 2.3 to 18.8 minutes; alpha angle, 14.3 to 54.4 degrees; MA, 41.3 to 73.3 mm. There were no significant differences between groups. Figure 6 shows these coagulation parameters, expressed as % baseline. By 60 minutes post-TBI, time to fibrin formation, as reflected by R time, declined >20% in all groups. At 300 minutes, this R time decrease was sustained and significant with HBOC, compared with control, though not with control + PE. Clot strength, as reflected by MA, was significantly depressed 60 minutes post TBI in the two control groups, and remained depressed at 300 minutes post-TBI without pressor. Control + PE restored MA toward baseline levels. With HBOC, there was no change in MA at any time-point. In all groups, K decreased by 25% postinjury and remained depressed, while α-angle increased.

In baseline conditions serum TNF-α ranged from 0 to 978 pg/mL and IL-6 ranged from 0 to 173 pg/mL. TNF-α and IL-6 (expressed as % baseline) showed no differences between groups over time (Fig. 7).

White blood cell counts and platelet levels did not differ between groups at any time point before or after injury (Table 2).

DISCUSSION

No previous studies have compared the neurotoxicity, vasoactivity, cardiac toxicity, and proinflammatory actions of

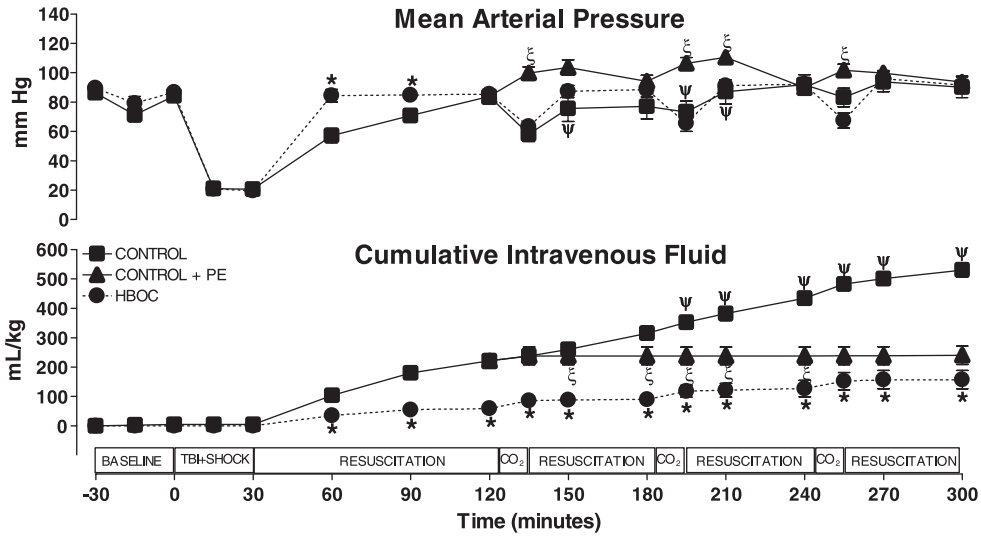

Fig. 4. *MAP, top. Cumulative intravenous fluid (IVF) requirements, bottom. CONTROL, normal saline + mannitol + blood group; CONTROL + PE, normal saline + mannitol + blood + phenylephrine group; HBOC, HBOC-201 group. MAP was rapidly restored in HBOC group by 60 minutes, while cumulative IVF requirements were lowest. After 120 minutes, all groups maintained MAP >60 mm Hg (except during CO_2 challenges).* *p $<$ *0.05 HBOC versus CONTROL,* ψp $<$ *0.05 CONTROL versus CONTROL + PE,* ζp $<$ *0.05 CONTROL + PE versus HBOC.*

HBOC-201 versus a control group, which incorporated standard neurotrauma care, including MAP and CPP management, blood transfusion, mannitol, and ± pressor therapy. There are several new findings from this study.

First, there was no evidence of HBOC-associated neurotoxicity. This is based on the observations that an initial bolus of HBOC-201 in simulated prehospital conditions provided early rapid correction of CPP and $PbtO_2$ while limiting ICP rise (Fig 2). Early extubation was possible without any obvious gross or histologic evidence of pathology, relative to control (Fig. 3).

Second, there was no evidence of HBOC-associated vasotoxicity or functional cardiac toxicity. MAP corrected more rapidly with HBOC, but there was no evidence that this early pressor action was harmful. Cardiac output and heart rate were similar between groups (Table 1), which suggests there was no cardiac toxicity associated with HBOC, per se. It should be emphasized that in the control group, without pressor, there were progressive rises in preload and right ventricular afterload, without any increases in stroke volume; this indicates that cardiac performance was compromised within that group (Table 1 and Fig. 5). This is noteworthy because the same pattern was not observed with HBOC or after pressor use in the control animals.

Third, there was no evidence for an HBOC-evoked proinflammatory condition. As reflected by changes in coagulation parameters, platelet levels, serum cytokine levels, or leukocyte counts. Fibrin formation was quicker in all groups immediately after injury, indicating a trauma-induced hypercoagulable state. With HBOC-201 and pressor use in controls, this heightened activity was maintained postinjury. Clot strength was maintained in HBOC animals (Fig. 6), and returned to baseline levels in pressor treated animals. No significant changes in serum cytokine levels, platelet levels or leukocyte counts were noted between groups (Fig. 7 and Table 2).

Overall, these data suggest that HBOC was both safe and effective when used for the early resuscitation after TBI, at least in these unique model conditions. It provided equivalent outcome compared with a standard of care group that included maximal crystalloid, mannitol, transfusion, and pressor therapy. Obviously in field situations and during transport, a single bolus of HBOC would offer a major logistic advantage, because these unlimited resources would not be available. Even with unlimited resources, HBOC use after TBI could decrease manpower and/or intensive care requirements.

Table 1 **Physiological Variables at 300 minutes**

Variable (mean ± SEM)	CONTROL	CONTROL + PE	HBOC
Heart rate, beats/min	86 ± 4	88 ± 16	80 ± 8
Cardiac output, L/min	2.3 ± 0.4	2.6 ± 0.5	2.0 ± 0.2
Stroke volume, mL/kg/beat	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
Mixed venous oxygen saturation, %	62 ± 7	64 ± 8	65 ± 7
Central venous pressure, mm Hg	31 ± 4	13 ± 1*	14 ± 1*
Muscle tissue oxygen saturation, %	54 ± 9	55 ± 16	62 ± 7
pH	7.19 ± 0.05	7.35 ± 0.03*	7.42 ± 0.02*
Lactate, mmol/L	0.8 ± 0.3	0.7 ± 0.1	1.1 ± 0.2
Urine Output, mL/kg/hr	19 ± 2	10 ± 3*	4 ± 1*

Critique

There are at least five elements of the experimental design that might limit the practical application of these observations to

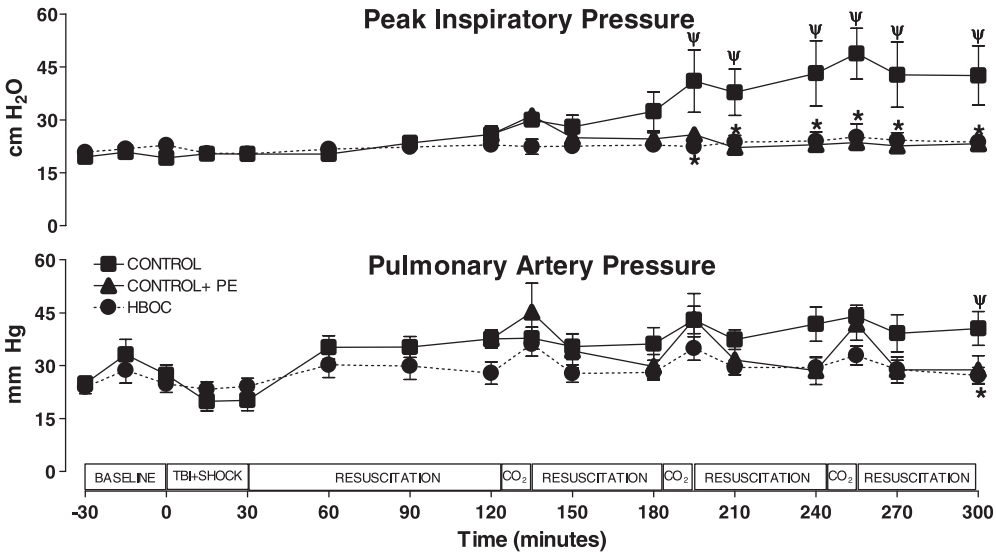

Fig. 5. *Peak inspiratory pressure, top, and pulmonary artery pressure, bottom. CONTROL, normal saline + mannitol + blood group; CONTROL + PE, normal saline + mannitol + blood + phenylephrine group; HBOC, HBOC-201 group. The CONTROL group experienced high PIP and PAP by 300 minutes, while HBOC and CONTROL + PE group PIPs and PAPs were similar at the experiment's conclusion.* *p $<$ *0.05 HBOC versus CONTROL,* ψp $<$ *0.05 CONTROL versus CONTROL + PE,* ζp $<$ *0.05 CONTROL + PE versus HBOC.*

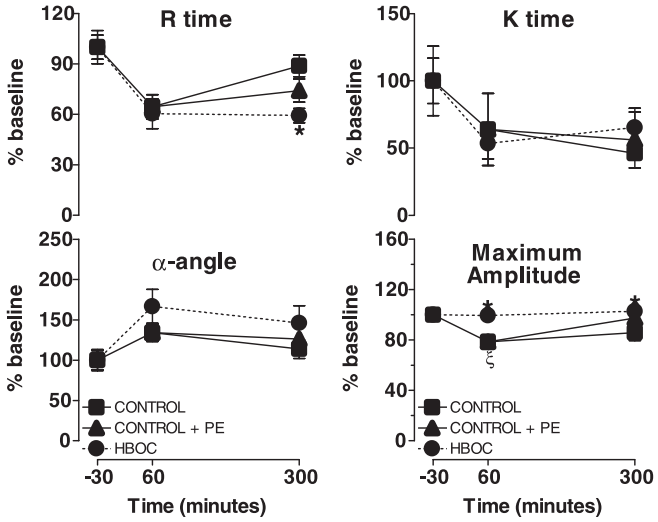

Fig. 6. *Coagulation parameters measured by thromboelastography at baseline and 60 and 300 minutes post-TBI. R time is the period from initiation of the test to the initial fibrin formation; K time is the period that represents the dynamics of clot formation; α-angle represents the kinetics of fibrin buildup and cross-linking; maximum amplitude (MA) reflects clot strength; CONTROL, normal saline + mannitol + blood group; CONTROL + PE, normal saline + mannitol + blood + phenylephrine group; HBOC, HBOC-201 group. Fibrin formation was quicker in all groups immediately after injury; however, HBOC-201 treated animals maintained this heightened activity postinjury. Clot strength was maintained in HBOC animals.* *p $<$ *0.05 HBOC versus CONTROL,* ψp $<$ *0.05 CONTROL versus CONTROL + PE,* ζp $<$ *0.05 CONTROL + PE versus HBOC.*

the clinical situation. First, a single dose of HBOC was administered at a fixed time after a well-defined injury in an anesthetized, mechanically ventilated animal. The outcomes might have differed with various model manipulations, such as providing an HBOC only resuscitation, changing HBOC dose, using uncontrolled hemorrhage with TBI, using a different multisystem trauma model, and/or altering anesthesia (ketamine contraindicated in head injury). Furthermore, in these model conditions, the physiologic changes in the control group may have been somewhat fluid dependent. Immediately upon resuscitation, the control group entered an inescapable cycle where hypotension prompted a flood of fluid administration that resulted in increased third spacing and increases in peak inspiratory pressure and ICP.[17–19] By 120 minutes, when MAP was finally restored to baseline levels in this group, ICP had been elevated so much that the CPP goal could never be achieved with fluid alone, plus an autologous blood transfusion, plus mannitol. So, to add a level of clinical relevance, we added pressor management after 120 minutes, which resulted in meeting the CPP goal and in decreasing ICP by limiting fluid requirements.[17] The bottom line is that the treatment differences observed in this present study could be model specific.

Second, the observation period was relatively short and the neurologic endpoints were primitive. One criterion was extubation at 6 hour, as a surrogate for neurologic outcome. More than likely, this reflected a difference in fluid shifts between pulmonary, neurologic, and other compartments, allowing early extubation. An extended observation period would allow more definitive conclusions regarding the neurologic benefit of any one therapy. Another neurologic criteria was brain oxygenation, which was higher during the immediate postinjury period with HBOC-201, however, it is recognized that a single point mea-

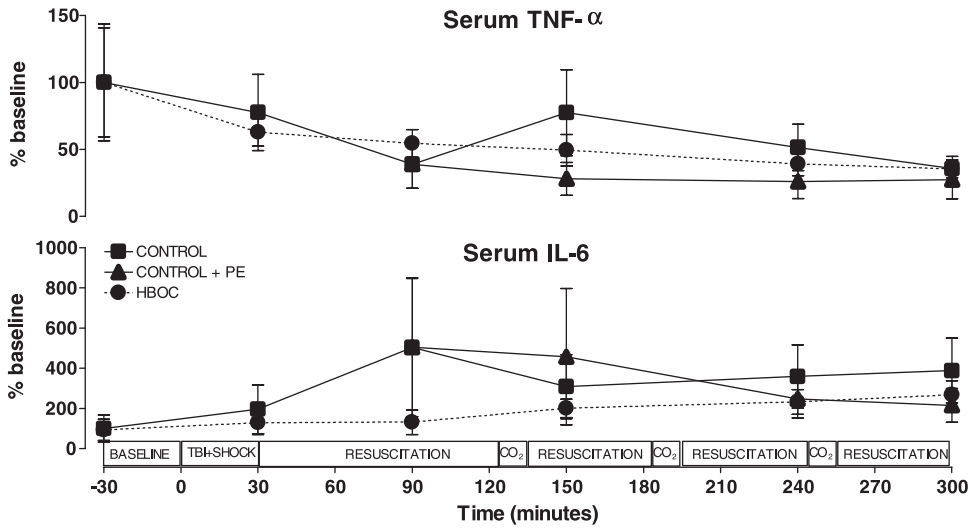

Fig. 7. *Serum TNF-α and IL-6 levels, expressed as percent baseline. CONTROL, normal saline + mannitol + blood group; CONTROL + PE, normal saline + mannitol + blood + phenylephrine group; HBOC, HBOC-201 group. There were no differences between groups.*

surement of $PbtO_2$ reflects local, but not regional or global oxygenation. Long term studies addressing neurologic outcome, as well as injured, penumbral, and global cerebral blood flow, are necessary to make more definitive statements about the potential benefits of HBOC after TBI.

Third, this study provides no new information on the mechanism of action of HBOC after trauma. The rapid increase in MAP after HBOC was probably partly because of its vasoactivity,[21] which is likely NO mediated,[22] though other mechanisms may exist.[23] No adverse vasoactivity and/or functional cardiac toxicity related to HBOC-201 was observed in this model, but this may be a dose- or species-related phenomenon. In this study, preload, afterload, stroke volume, and heart rate, were measured to assess myocardial performance. Echocardiography would have provided more functional data on cardiac toxicity.

Fourth, a proinflammatory condition was assessed with changes in coagulation, cytokine levels, and complete leukocyte counts. None of these measurements are specific. Furthermore, TEG is not routinely clinically used, as are standard tests of coagulation, such as prothrombin time, partial thromboplastin time, fibrinogen, and d-dimer. The cytokine levels were highly variable, so there is a possibility that a proinflammatory condition was present, but was not detected with these measurements.

Table 2 White Blood Cell and Platelet Counts

Parameters (mean ± SEM)	Control	Control + PE	HBOC
White blood cell count (K/μL) at baseline	10.2 ± 0.7	—	12.7 ± 0.7
White blood cell count (K/μL) at 300 min	11.4 ± 2.5	18.6 ± 5.1	16.3 ± 1.4
Platelet count (K/μL) at baseline	434 ± 11	—	416 ± 72
Platelet count (K/μL) at 300 min	171 ± 14	284 ± 23	189 ± 31

Fifth, the large volume of NS, especially, in the control and control + PE groups, likely caused a hyperchloremic metabolic acidosis, although we did not measure chloride ions. This difference in pH could have altered every parameter we measured, including all neurologic, cardiopulmonary, inflammatory, and coagulation measurements. However, this lower pH in the control groups would have left-shifted the oxyhemoglobin dissociation curve, possibly artificially elevating $PbtO_2$ in the controls.

Despite these major limitations in the experimental design, the basic observations are consistent with the idea that HBOCs are not inert oxygen carriers, but rather pharmacologic agents with systemic effects that can ameliorate certain patho-physiologic conditions.

Comparison to Previous Studies

HBOCs have variable results in models of neurologic injury, such as cerebral ischemia,[24] subarachnoid hemorrhage,[25,26] and TBI.[9–11,27] In general local $PbtO_2$ is improved in non-TBI models, as mentioned previously.[13–15] Also, Ortegon demonstrated HBOC-201 to be nontoxic to neuronal cells in culture.[28] Our model indicates that HBOC-201 rapidly increases and sustains $PbtO_2$ at supraphysiologic levels without histopathologic alterations.

Despite the contraindication of pressors during resuscitation after hemorrhagic shock, there is recent data suggesting pressor utility in this scenario.[29,30] Furthermore, vasoactive HBOCs may be particularly useful after TBI, where continued episodes of hypotension and hypoxia could lead to secondary brain injury. Our group has demonstrated pressor utility for resuscitation after TBI and hemorrhagic shock in swine, especially if combined with moderate fluid therapy.[19,31] After resuscitation from chest trauma, we evaluated the actions of four different HBOCs, all of which reduced fluid requirements and increased right and left ventricular afterload against saline. These actions compromised an already marginal cardiac perfor-

mance. All of these compounds were eventually removed from commercial development.[32]

The unacceptable pressor action of early, first generation HBOCs probably depended in part on the dose and route of delivery, as well as toxic effects of the 64 kDa fraction of hemoglobin, and/or various antioxidants that were added to the HBOC solution. Based on these early lessons, the 64 kDa fraction is filtered and the antioxidants are not used in the current generation of HBOCs. Most importantly, HBOCs are no longer considered pharmacologically inert, so the dose and route of administration are key factors in profiling HBOC side effects. In this present study, a bolus of 10 mL/kg NS was first administered over 5 minutes, followed by 6 mL/kg HBOC-201 over 5 minutes. In these conditions, the relatively mild pressor action of HBOC-201 allowed rapid resuscitation postinjury, and there was no delay in lactate clearance, as seen by other investigators,[23,33] or any other toxic effect on several other measured variables.

Uptake of free hemoglobin or iron based debris by macrophages may increase cytokine release, such as IL-8 and/or TNF-α.[34,35] HBOC-201 activates human neutrophils, as measured by CD11b and oxidative burst activity in vitro, and IgG specific anti-HBOC-201 antibodies confirm prior exposure in canines.[36] However, in a recent study, resuscitation with HBOC-201 had no significant adverse or beneficial effect on immune function, as measured by lymphocyte counts, differentials, T-lymphocyte subset analysis, or cytokine (TNF-α, IL-6) release.[37] Also, polymerized human hemoglobin (Polyheme, Northfield Laboratories, Evanston, Ill.) does not prime neutrophils,[38] avoids increases in IL-8 gene expression in neutrophils or total leukocytes,[39] and reduces levels of circulating IL-6, IL-8, and IL-10.[40] In this study, HBOC-201 did not cause alterations of TNF-α and IL-6 compared with other groups. However, it is known that a multitude of inflammatory and noninflammatory mediators exist not only in the serum, but also the CSF,[41–44] and these intertwined systems may be altered.

Coagulation analyzers for standard tests, such as PT, PTT, and fibrinogen may be affected by HBOCs,[45] whereas mechanical detection methods may be less affected.[46] HBOC-201 prolongs bleeding times and decreases arterial thrombus formation in a rabbit model of carotid arterial thrombosis.[47] HBOC-201 and lactated Ringers have similar effects on TEG values in vitro at clinically relevant concentrations.[48] Recently, Arnaud showed in a controlled model of hemorrhage that R time increased in HBOC-201 treated animals early and late after resuscitation.[49] On the other hand, there are coagulation changes specific to TBI.[18] Early after TBI, R time is depressed indicating a hypercoagulable state, and is independent of treatment. However, R time depression was maintained in the HBOC group at 300 minutes post-TBI. Clot strength (MA) was normal throughout the experiment.

Perspective

At the time of this writing, HBOC-201 is approved in South Africa for treating adult surgical patients who are acutely anemic and for eliminating, reducing or delaying the need for allogenic red blood cell transfusion in these patients; however no peer reviewed publications exist on this experience. The United States Navy's RESUS (Restore Effective Survival in Shock) trial involving HBOC-201 in trauma patients is currently on hold by the FDA, primarily because of the agency's concerns about the risk-benefit profile in this patient population. Concurrently, a pivotal FDA approved phase III study is ongoing and designed to evaluate the safety and efficacy of prehospital PolyHeme for treatment of patients in traumatic hemorrhagic shock.

Conclusions

After TBI and controlled hemorrhage, a single HBOC-201 bolus provided swift and adequate resuscitation, while maintaining CPP, limiting ICP rise, limiting intravenous fluid requirements, causing no functional coagulopathy or cytokine release, in the absence of a blood transfusion, mannitol, or pressor therapy. Thus, HBOC-201 may provide a safe and effective low volume resuscitation bridge until definitive neurotrauma care is available.

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On behalf of the American Association for the Surgery of Trauma, I am proud to announce the appointment of the new Executive Director. Sharon Gautschy joins us with the opening of the central office of the AAST in Chicago. This office will be located in the building occupied by the American College of Surgeons and house Ms. Gautschy and eventually other staff. The central office will work to direct and coordinate most of the activities of the AAST, its officers and committees. We welcome Sharon to our organization and look forward to her bringing her vast experience, pleasant personality and can-do attitude to our organization. Please welcome Sharon as our new executive director.

C. William Schwab, M.D.
President
American Association for the Surgery of Trauma

SHARON L. GAUTSCHY
EXECUTIVE DIRECTOR
AMERICAN ASSOCIATION FOR THE SURGERY OF TRAUMA (AAST)

Sharon Gautschy has over 16 years experience in Association Management where she started her career at the largest association management company in the United States, Smith Bucklin & Associates. After leaving Smith Bucklin in 1991, Ms. Gautschy worked for the next five years at the American Marketing Association where she was promoted several times and held numerous positions. In 1996, she returned to the National Association on Quick Printers (NAQP) as their Chapter Relations and Group Purchasing Manager. She worked on increasing supplier partnerships and was responsible for two of their four special interest groups.

In 1998, Ms. Gautschy was recruited to American Association of Women Dentists (AAWD) where she was the Executive Vice President for two and a half years. As the chief staff person she was responsible for: accounting, membership, annual meeting content, logistics and exhibits, tradeshows, marketing, newsletter content and production and dealing with all outside consultants and vendors. While at AAWD, she designed and coordinated the corporate literature, developed a web presence, enhanced their annual meeting with exhibitors and content, developed a marketing strategy and industry wide presence, developed a sponsorship program and increased their membership. Ms. Gautschy was also the Program Manager for their Smiles for Success Foundation (SFS).

After leaving AAWD, Ms. Gautschy was hired as the Marketing Director for American Medical Technologists (AMT) – a certification and professional membership association. She was promoted after two years to Director of Marketing, Membership and Operations. She had 15 staff members reporting to her and was responsible for the certification departments, office services, member services, and accounts receivable. Besides the operations duties, she was in charge of all membership and corporate marketing, developing a yearly advertising campaign, public relations, tradeshows, redesigning the website, content management of the website and liaison to 38 state societies. In the four years she was employed, membership increased by 10,000 members or 40%, and the annual income budget increased by 40%.

Sharon graduated from Governor's State University in University Park, IL in 1990 and in 2004 she received a graduate certificate in Non-Profit Management from North Park University in Chicago, IL. In November 2005, she was a co-presenter at an educational session for the National Organization for Competency Assurances' (NOCA) annual meeting.

Sharon was born and raised in Chicago. In fact, she lives just over one mile from the AAST office. She enjoys traveling, dining out, reading, sporting events, theater and all the amenities Chicago has to offer.