Resuscitation From Hemorrhagic Shock Comparing Standard Hemoglobin-Based Oxygen Carrier (HBOC)-201 Versus 7.5% Hypertonic HBOC-201

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Background: **Hemoglobin-based oxygen carrier (HBOC) resuscitation has been associated with increased systemic and pulmonary vascular resistances (SVR, PVR), which may result in reduced blood flow and severe pulmonary hypertension. The physiologic and immunologic properties of 7.5% hypertonic saline solution (HTS), such as reduction of SVR and PVR, as well as inhibition of neutrophil and endothelial activation may be beneficial in reducing some of these undesirable effects of HBOCs. The aim of this study was to evaluate the hemodynamic effects of the HBOC and HBOC-201 suspended in 7.5% hypertonic saline solution (HT-HBOC) when compared with standard HBOC resuscitation.**

Methods: **Thirty-two domestic crossbred pigs (50–60 kg) were hemorrhaged to a mean arterial pressure (MAP) of 35 mm Hg ± 5 mm Hg for 45 minutes and resuscitated to a baseline mean arterial pressure using the following groups: (1) sham, no hemorrhage; (2) shed blood + lactated Ringer's solution; (3) standard HBOC-201; (4) hypertonic saline 7.5%; (5) hypertonic 7.5% HBOC-201. After resuscitation, observation was continued for 4 hours. Hemodynamic variables, oxygen consumption, and arterial blood gases were monitored continuously. Data were analyzed using analysis of variance.**

Results: **SVR ($p = 0.001$), PVR ($p = 0.001$), and MPAP ($p = 0.01$) were significantly reduced in the HT-HBOC group compared with the standard HBOC group.**

Conclusion: **In this model of hemorrhagic shock, hypertonic HBOC-201-resuscitated pigs had significantly reduced SVR and PVR, as well as mean pulmonary artery pressure (MPAP) and increased cardiac output. HT-HBOC may be beneficial in reducing the undesirable effects of standard HBOC-201. The mechanisms of these beneficial effects need to be investigated.**

Key Words: **HBOC-201, Small volume, Resuscitation, Hypertonic solution, Hemorrhagic shock, Hemoglobin-based oxygen carriers.**

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Hypertonic saline (HTS) solutions have been studied for several years. Data from human and animal studies have shown HTS to be safe and effective low-volume resuscitation fluids.[1–6] Some of the beneficial properties of HTS include rapid volume expansion,[2,7,8] restoration of microcirculatory blood flow by capillary reopening,[9,10] increased cardiac output (CO),[11,12] correction of acid-base disturbances,[13,14] and decreased systemic vascular resistances (SVR).[15] In addition, data suggest that HTS has some immunomodulating properties.[16] For example, HTS resuscitation prevents endothelial vascular dysfunction and the generation of reactive oxygen species,[17,18] as well as abrogation of polymorphonuclear leukocytes (PMN) activation.[17,19,20] However, because HTS does not carry oxygen, increased oxygen delivery is limited by improvements in CO. Thus, HTS by itself, particularly in the setting of trauma and blood loss, may not achieve or maintain adequate oxygen delivery to the compromised tissues.

Recently, significant clinical and laboratory information has accumulated regarding the efficacy of low-volume resuscitation fluids as therapeutic agents for hemorrhagic shock.[21,22] Experimental data obtained by several research groups have suggested that among low-volume strategies, hemoglobin-based oxygen carriers (HBOCs)[23,24] seem to have significant potential. Along with HTS, HBOCs have been proposed as an effective low-volume resuscitation solution for hemorrhagic shock.[23,25,26] The bovine polymerized HBOC-201 has been shown to be more effective in restoring mean arterial pressure (MAP) to normal values,[27,28] while requiring significantly less fluid volume when compared with other low-volume and standard resuscitation fluids.[29–31] Moreover, HBOC-201 resuscitation increased survival in animal models of controlled and uncontrolled hemorrhage, and provided better tissue oxygenation for survival with no long-term organ dysfunction.[24,29–34] Additionally, clinical studies have shown that HBOC-201 reduces transfusion requirements in 35% to 40% of adult surgical patients.[35]

However, one of the main concerns that have limited the clinical use of HBOCs is the associated vasoconstriction/

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hypertension and its limited plasma expansion capacity. The vasoconstrictive effect is reflected by increases in systemic and pulmonary vascular resistance, which results in decreased CO and pulmonary hypertension. Studies have shown that the HBOC-induced hypertension is directly correlated to vasoconstriction of the vascular arterioles.[36] The mechanisms by which this vasoconstriction occurs remain unclear.

Based on this evidence, both HTS and HBOC-201 have significant potential benefits and limitations as low-volume resuscitative solutions for clinical use. Interestingly, HTS restores microcirculatory blood flow by capillary reopening, whereas HBOC-201 causes vasoconstriction. Thus, the combination of both solutions may prove beneficial. For example, the plasma-expanding and vasodilator capacity of HTS 7.5% may attenuate the vasoconstrictive effect of HBOC, and the oxygen-carrying capacity of HBOC may improve and prolong the hemodynamic effects of HTS. To our knowledge, the combination of HBOC-201 with HTS 7.5% has not been investigated. The aim of this study was to evaluate the physiologic and hemodynamic effects of HBOC-201 in a hypertonic saline 7.5% carrier (HT-HBOC), and compare it with standard HBOC-201 and HTS 7.5% alone. We hypothesized that the prevention of endothelial dysfunction and the vasodilator and cardiac effects of HTS 7.5% combined with the oxygen-carrying capacity of HBOC-201 may work synergistically and be a more effective small-volume resuscitative fluid.

MATERIALS AND METHODS

Animal Preparation

This study was approved by the University of Texas Southwestern Medical Center at Dallas Institutional Animal Care and Use Committee. All animals used in this study were cared for and handled according to the Guide for the Care and Use of Laboratory Animals. Thirty-two domestic crossbred pigs (50–60 kg) obtained from a single provider source were used in this experiment. Animals were fasted overnight before surgery and only received water ad libitum. Sedation was induced with an intramuscular injection of Telazol (Fort Dodge Animal Health, Fort Dodge, Iowa) (4 mg/kg) and atropine (0.04–0.4 mg/kg). General anesthesia was induced with mask ventilation using isoflurane (2.5%–3.0%) in 40% oxygen.

Maintenance Fluids

Before, and during anesthesia induction and preparation surgical procedures, an 18-gauge intravenous catheter was placed in an ear vein, and intravenous fluids [lactated Ringer's (LR) solution] were administered to maintain MAP greater than 80 mm Hg, heart rate less than 120 bpm. The average maintenance fluid rate was 6 mL $\cdot$ kg^{-1} $\cdot$ hr^{-1} to 8 mL $\cdot$ kg^{-1} $\cdot$ hr^{-1} during the experiment.

Each animal underwent endotracheal intubation followed by mechanical ventilation. The following parameters were used: respiratory rate, 10 to 12 bpm; tidal volume, 7 mL/kg to 10 mL/kg; FIO_2, 50%. Tidal volume was adjusted to maintain a Pco_2 of approximately 40 mm Hg and maintained at that volume for the rest of the experiment. Electrocardiogram leads, pulse oxymeter, and an esophageal temperature probe were placed. Body temperature was maintained at 37.5°C ± 1.0°C by using a forced air warmer (BAIR Hugger, Augustine Medical, Eden Prairie, MN). A Foley catheter was inserted into the urinary bladder, and urine output was recorded hourly.

Surgical Procedures

A small incision was made on the left lower extremity and a 5F catheter introduced into a superficial branch of the femoral artery, threaded proximally into the iliac artery, secured in position, and used for continuous arterial pressure. While supine, the neck was prepped, an incision was made, and a 9F vascular introducer was placed into the right internal jugular vein. A Swan-Ganz catheter (SW) was inserted and flow directed into the pulmonary artery for hemodynamic monitoring and physiologic evaluation (Vigilance Monitor, Edwards Lifesciences, Irving, CA). A catheter was positioned in the carotid artery and was used for controlled hemorrhage and blood draws. Animals were allowed to stabilize for 15 minutes, and baseline measurements of hemodynamic and physiologic variables were taken, and values repeated throughout the experimental protocol (Marquette model 8000 mol/L, GE/Marquette, Milwaukee, WI). Animals were maintained under general anesthesia for the duration of the protocol.

Hemorrhage and Resuscitation Procedures

After the stabilization period, the animals were rapidly hemorrhaged via the carotid artery for 5 minutes to 10 minutes until the MAP was decreased to 35 mm Hg ± 5 mm Hg (Fig. 1). Animals were maintained hypotensive with a MAP of 35 mm Hg ± 5 mm Hg for a total of 45 minutes (including the 5 minutes to 10 minutes rapid hemorrhage period) by continued intermittent hemorrhage (Fig. 1). No intervention was made if the MAP decreased below 35 mm Hg during the shock period. Shed blood was collected in standard donor bags containing citrate phosphate dextrose (Baxter Corp., Deerfield, IL) and kept at room temperature until used for reinfusion in the LR/shed blood group. Net weight of the donor blood bags was used to calculate volume of hemorrhage.

At the end of the 45-minute shock period, animals were randomized to receive one of four resuscitation fluids. A separate Sham group (n = 3) underwent surgical procedures but was not hemorrhaged.

Experimental Groups

The groups were as follows: group 1 (LR)—standard resuscitation of LR solution (33 mL/kg), followed by shed blood, infused rapidly; group 2 (HBOC)—standard HBOC-201 (6 mL $\cdot$ kg^{-1} $\cdot$ 5 min^{-1}); group 3 (HTS)—hypertonic saline

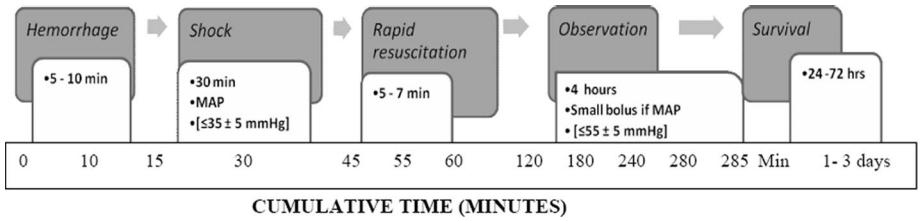

Fig. 1. *Schematic diagram of experimental timeline in swine undergoing hemorrhage and resuscitation.*

7.5% (4–5 mL · kg^{-1} · 5 min^{-1}); group 4 (HT-HBOC)—hypertonic 7.5% HBOC-201 (4–5 mL · kg^{-1} · 5 min^{-1}).

The HT-HBOC solutions was prepared in our laboratory by removing 80 mL from the 250 mL bag of HBOC-201 (13 g/dL of hemoglobin) and replacing this volume with pharmaceutical-grade stock solution of 23% NaCl (Baxter Corp.) to a final concentration of 7.5%-hypertonicity HBOC-201 (10.4 g/dL of hemoglobin). The HTS solution (250 mL) was similarly made by diluting the 23% stock saline solution to 7.5% with sterile water.

The LR group was included to provide a familiar control reference and the HTS 7.5% to have a hypertonic control. An observation period of 4 hours followed the initial resuscitation. Maintenance fluids were resumed, and additional boluses of 5 mL/kg were given at 30 minutes, 60 minutes, 120 minutes, and 180 minutes, if MAP was <60 mm Hg. At the end of the observation (4 hours) tubes were removed, incisions closed, and animals were allowed to awake from the anesthetic before taken to their cages.

Animals were followed for survival for 24 hours or 72 hours. At these time points, half of each group of pigs underwent the same previously mentioned procedures for monitoring; a Swan-Ganz catheter was reintroduced and physiologic and hemodynamic data, as well as blood and tissue samples, were obtained. Animals were then killed with an overdose of barbiturate.

Blood Samples

Blood was taken serially during the experiment at the following time points: at baseline; at 30 minutes and 45 minutes (completion) of shock; and at 30 minutes, 60 minutes, 120 minutes, 180 minutes, and 240 minutes of resuscitation. Arterial and mixed venous blood samples were drawn from the external carotid artery and the distal port of the Swan-Ganz catheter, respectively, for blood gas and chemistry analysis. Blood gas analysis was performed on an ABL 725 (Radiometer, Copenhagen, Denmark). Serum chemistry measurements were performed on an OSM3 (Radiometer).

Physiologic and Hemodynamic Measurements

Pulmonary and arterial pressures, body temperature, oxygen saturation, and vital signs were measured continuously throughout the experiment. Cardiac and oxygenation profiles were measured at the following time points: at baseline; at 15 minutes, 30 minutes, and 45 minutes (completion of shock); and at 30 minutes, 60 minutes, 90 minutes, 120 minutes, 180 minutes, and 240 minutes of resuscitation. Analysis and calculations were made using a Marquette monitor 8000 mol/L (GE/Marquette).

Statistical Analysis

Data are reported as mean ± SE for each group. Statistical analysis was performed using SPSS for Windows version 12.0 (SPSS, Inc., Chicago, IL). Comparisons included repeated measures and one-way analysis of variance followed by post hoc analysis (Tukey's multiple comparison test) where appropriate. Significance was defined as $p < 0.05$.

RESULTS

There were no differences in the groups with respect to weight, estimated blood volume, hemorrhage volume, and percentage of estimated blood volume hemorrhaged (Table 1).

Hemodynamic Parameters

There were no significant hemodynamic changes in the sham animals. Statistical comparisons included only the LR, HBOC, HTS, and HT-HBOC groups. The sham group data are included in the graphics to present a reference for comparison. Because the aim of this study was to compare the standard HBOC-201 versus HT-HBOC-201 and HTS 7.5%, the statistical analysis is between these groups.

Baseline measurements and physiologic responses to hemorrhage were comparable in all groups. Hemorrhage decreased MAP and CO pulmonary capillary wedge pressure (PCWP) and increased heart rate equally in all groups.

The following differences were found during the resuscitation and observation periods: the HT-HBOC- and HBOC-resuscitated animals required significantly less fluid volume (Fig. 2).

Table 1 Group Characteristics (Mean ± SD)

Experimental Group	Weight (kg)	Hemorrhage Volume (mL)	Estimated Blood Volume (%)
Sham (n = 3)	56.5 ± 1.7	NA	NA
LR (n = 6)	56.1 ± 1.3	2,130 ± 400	38.2 ± 7.7
HBOC (n = 8)	56.6 ± 0.7	2,198 ± 308	39.1 ± 1.3
HTS 7.5% (n = 6)	56.3 ± 2.3	2,125 ± 343	38.4 ± 3.3
HT-HBOC (n = 8)	56.2 ± 7.3	2,268 ± 399	40.06 ± 0.5
p	0.999	0.719	0.753

NA, not assayed.

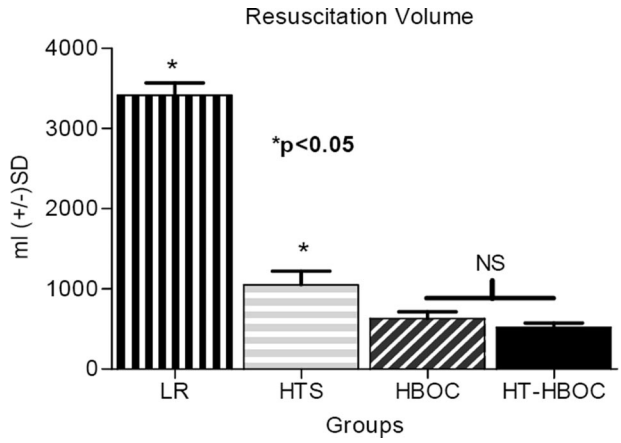

Fig. 2. *Resuscitation fluids; the LR group required significantly more fluids when compared with the rest of the groups (*p < 0.001). Similarly, the HTS group required more fluids when compared with the HBOC and HT-HBOC groups (*p < 0.001). There were no differences between the HBOC and HT-HBOC groups (>0.05).*

Mean Arterial Pressure

By the end of the observation period (4 hours), MAP returned to near baseline in the HBOC and HT-HBOC groups, whereas it remained low in the HTS and LR groups. The HBOC and HT-HBOC groups had a more sustainable MAP response (Fig. 3A).

Cardiac Output

CO returned to near baseline in the LR and HT-HBOC groups, remained significantly lower in the HBOC group, and was not sustained in the HTS group (Fig. 3B).

Pulmonary Capillary Wedge Pressure

PCWP was slightly lower in the HBOC group during the observation period, but this did not reach statistical significance. However, by the end of the 4-hour observation period, PCWP was similar in all groups. Heart rate returned to near-baseline levels in all groups by the end of the observation period (Fig. 3C).

Systemic and Pulmonary Vascular Resistances (SVR/PVR)

Hemorrhage did not cause significant changes in SVR. However, resuscitation with standard HBOC significantly increased SVR when compared with the other groups. PVR was similarly increased in all groups at the end of hemorrhage. Upon resuscitation, PVR returned to near-baseline levels in the HT-HBOC and HTS groups, and remained

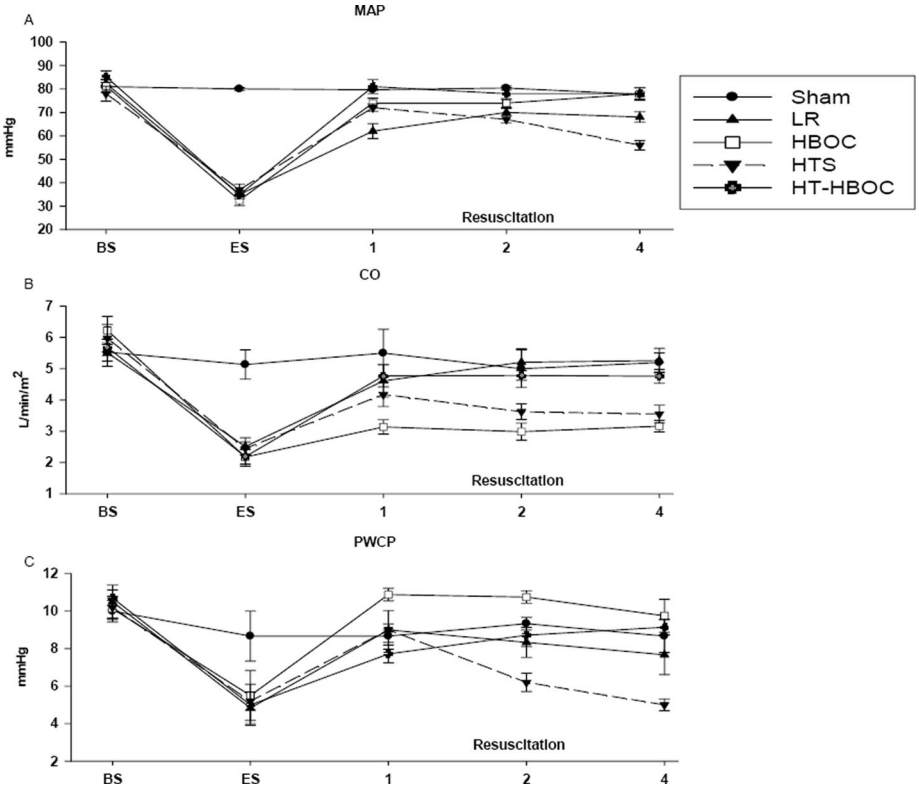

Fig. 3. (A) *MAP recovered faster (~10–20 minutes) in the HTS, HT-HBOC, and HBOC groups, when compared with LR. However, the only significant difference was at 4 hours when the MAP in the HTS group was lower than that in all other groups* (p = 0.02). (B) *CO was significantly lower in HBOC-resuscitated pigs at 1 hour, 2 hours, and 4 hours when compared with LR and HT-HBOC* (p = 0.001). *This was not significantly different from HTS.* (C) *PCWP was similar in all groups after resuscitation, but PCWP was significantly lower in the HTS group when compared with the rest of the groups from 2 hours to 72 hours* (p = 0.03).

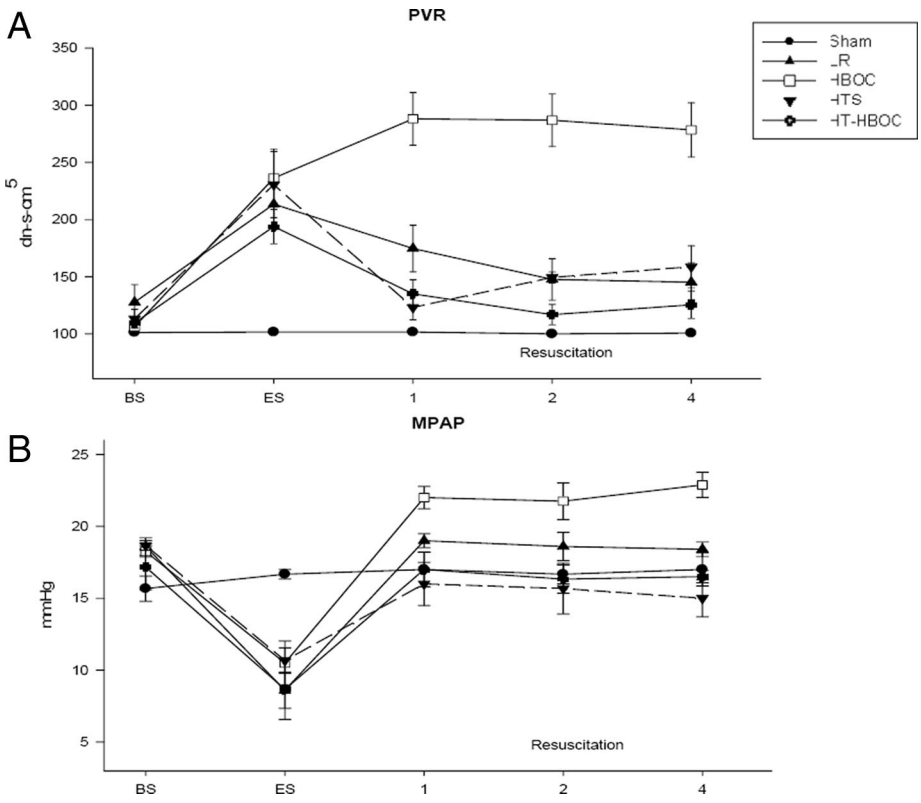

Fig. 4. *(A) Resuscitation with HBOC significantly increased PVR greater than that in the other groups (*$p < 0.001$*). (B) MPAP was significantly increased in the HBOC group at all time points (*$p < 0.01$*).*

slightly elevated in the LR group. Resuscitation with standard HBOC further increased PVR and it remained significantly elevated throughout the duration of the observation period when compared with the other experimental groups (Fig. 4A).

Mean Pulmonary Artery Pressure

By the end of hemorrhage, MPAP was decreased in all groups. Upon resuscitation, MPAP gradually began to increase, and by the end of the observation period, was slightly below baseline levels in both hypertonic groups, above baseline in the LR group, and significantly elevated in the HBOC group (Fig. 4B).

Mixed Venous Oxygen Saturation

Mixed venous oxygen saturation (Svo_2) decreased in response to hemorrhage and gradually increased during the beginning of resuscitation in the HT-HBOC, LR, and HTS groups. By the end of the observation phase, Svo_2 was significantly decreased in the HTS-resuscitated animals. Standard HBOC resuscitation did not restore Svo_2, and throughout the observation period Svo_2 values remained significantly lower as compared with the HT-HBOC and LR groups (Fig. 5).

Metabolic Markers

Lactate increased during hemorrhage in all groups. Resuscitation with HT-HBOC and LR, similarly, returned lactate levels to baseline. Lactate levels remained slightly elevated in the HBOC and HTS groups. However, the differences were not significant. Sodium levels increased upon resuscitation in the HBOC, HT-HBOC, and HTS groups. By the end of the observation period, sodium levels returned to baseline in the HBOC-resuscitated animals, and remained slightly elevated in both hypertonic groups.

DISCUSSION

In this model of controlled hemorrhage, we demonstrated for the first time that the HBOC-201 in a hypertonic saline carrier (HT-HBOC) improved hemodynamic parameters when compared with HTS alone. This combination also attenuated the vasoconstrictive effects observed with standard HBOC-201 resuscitation.

Administration of 250 mL of HT-HBOC-201 significantly improved CO and restored mixed venous oxygen saturation (Svo_2), when compared with administration of an equivalent volume of standard HBOC-201 or HTS 7.5%. Additionally, HT-HBOC maintained hemodynamic parameters better than the HTS 7.5% and LR groups.

Furthermore, HT-HBOC-201 attenuated the increases in SVR and PVR as well as the pulmonary hypertension observed with standard HBOC-201 resuscitation. The vasoconstrictive effect of HBOC solutions is one of the potential limitations for its clinical use. Although several studies have reported this effect, the exact mechanism remains undefined. Nitric oxide scavenging by polymerized hemoglobin has been

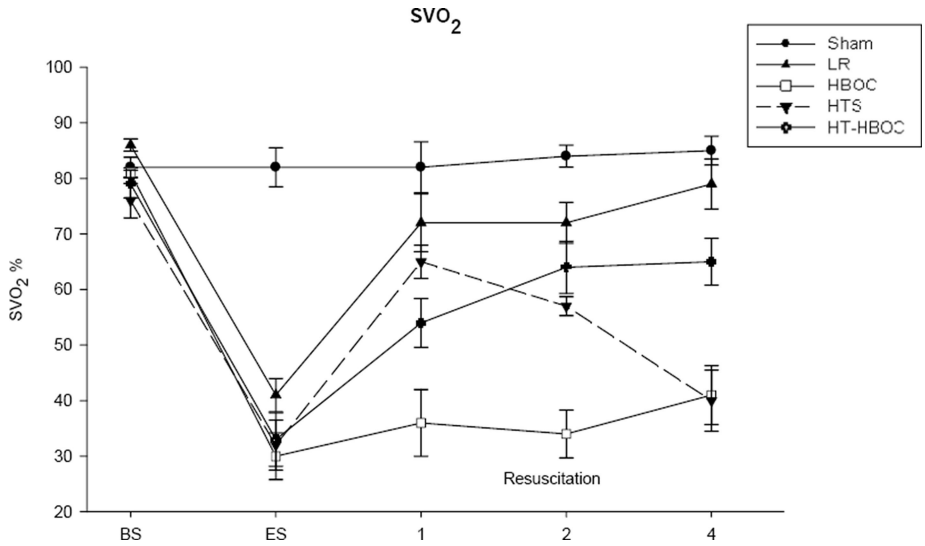

Fig. 5. *Mixed venous oxygen saturation (Svo_2) was significantly decreased in the HBOC pigs when compared with all the other groups at all time points postresuscitation except HTS at 4 hours (*$p = 0.03$*).*

suggested as the most probable mechanism. It has been previously shown that vasoconstriction was independent of nitric oxide. Alternative mechanisms for HBOC-induced vasoconstriction are those secondary to the interaction of free hemoglobin with endothelium-derived factors, resulting in endothelial dysfunction, and the subsequent production of reactive oxygen species, enhanced endothelin-1 production, neutrophil activation, and increased permeability.

In fact, several studies have previously demonstrated the effects of hypertonic saline in the mechanisms mentioned above.[16] For instance, HTS resuscitation prevents leukocyte adhesion to the endothelium, abrogation of PMN activation[17,19,20] as well as decreased generation of reactive oxygen species.[17–19]

Additionally, the cardiac effects of HTS resuscitation such as increases in heart rate, cardiac contractility, venous return (preload), and the reductions in peripheral and pulmonary vascular resistance (after load)[10–12,15] may explain the increases in CO and the restored mixed venous oxygen saturation (Svo_2).

The effects of hypertonicity such as increased blood flow[12] in the micro and peripheral circulation by arteriolar vasodilatation and the rapid induced reduction in blood viscosity by hemodilution[10,22] might also contributed to the reduction in PVR and SVR observed in the HT-HBOC group.

Resuscitation with HTS 7.5% also has its limitations. For example, if administered rapidly, HTS 7.5% may cause hypotension and arrhythmia, secondary to hypertonicity-induced vasodilatation and reduction in peripheral vascular resistances. There are concerns that these effects could overcome homeostatic mechanisms, such as vasoconstriction. Thus, there is potential risk for increased internal bleeding. Interestingly, the vasoconstrictive capacity of HBOC-201 may help to antagonize these effects. In addition, the inability of HTS to maintain hemodynamics could be secondary to the fact that HTS solution does not have oxygen-carrying capacity. Thus, the oxygen-carrying capacity of HBOC-201 contributed to the sustained hemodynamic parameters in the HT-HBOC group.

Another concern is the increased sodium blood concentration caused by HTS. However, the increases were only transient and within clinically acceptable levels. We did not observe hyperchloremic acidosis, which has been associated with HTS.

It is clear that the observed benefits in this study in the HT-HBOC cannot be attributed to only one solution. Therefore, we hypothesized that the beneficial effects observed with HT-HBOC are synergistic.

However, one of the major the limitations of this study is the controlled hemorrhagic shock model. Although our fixed-pressure model is good to study the acute and long-term effects of hypotension, on inflammatory responses as well as the effects of our resuscitation strategies on organ function and outcome, this model does not actually resemble the uncontrolled hemorrhage situation observed in trauma patients. For example, studies using uncontrolled shock animal models have shown that raising MAP to normal or near-normal values resulted in delayed hemostasis, increased blood loss, and higher acute mortality from washout anemia. Therefore, further studies investigating the use of HT-HBOC-201 in a more clinically relevant model of uncontrolled hemorrhage as well as the investigation of the cellular and molecular mechanisms should be performed. Nevertheless, these findings suggest a potential benefit of this combination to be considered for the initial management of hemorrhage.

CONCLUSION

In conclusion, this study demonstrates for the first time that the combination of HBOC-201 with hypertonic saline produces physiologic benefits. Both standard HBOC-201 and

HT-HBOC-201 were superior to LR and HTS 7.5% in restoring and maintaining hemodynamics. However, the combination of HBOC-201 with hypertonic saline further improved hemodynamics and oxygenation, as indicated by restoration of CO and higher Svo_2, respectively. More importantly, this combination attenuated the increases in pulmonary and systemic vascular resistance as well as pulmonary hypertension, observed with standard HBOC-201.

REFERENCES

1. Dubick MA, Wade CE. A review of the efficacy and safety of 7.5% NaCl/6% dextran 70 in experimental animals and in humans. *J Trauma.* 1994;36:323–330.
2. Kramer GC. Hypertonic resuscitation: physiologic mechanisms and recommendations for trauma care. *J Trauma.* 2003;54: S89–S99.
3. Terajima K, Aneman A, Haljamae H. Haemodynamic effects of volume resuscitation by hypertonic saline-dextran (HSD) in porcine acute cardiac tamponade. *Acta Anaesthesiol Scand.* 2004; 48:46–54.
4. Wade C, Grady J, Kramer G. Efficacy of hypertonic saline dextran (HSD) in patients with traumatic hypotension: meta-analysis of individual patient data. *Acta Anaesthesiol Scand Suppl.* 1997;110: 77–79.
5. Wade CE, Kramer GC, Grady JJ, Fabian TC, Younes RN. Efficacy of hypertonic 7.5% saline and 6% dextran-70 in treating trauma: a meta-analysis of controlled clinical studies. *Surgery.* 1997;122:609–616.
6. Wade CE, Grady JJ, Kramer GC. Efficacy of hypertonic saline dextran fluid resuscitation for patients with hypotension from penetrating trauma. *J Trauma.* 2003;54:S144–S148.
7. Mazzoni MC, Borgstrom P, Arfors KE, Intaglietta M. Dynamic fluid redistribution in hyperosmotic resuscitation of hypovolemic hemorrhage. *Am J Physiol.* 1988;255:H629–H637.
8. Mazzoni MC, Borgstrom P, Arfors KE, Intaglietta M. The efficacy of iso- and hyperosmotic fluids as volume expanders in fixed-volume and uncontrolled hemorrhage. *Ann Emerg Med.* 1990; 19:350–358.
9. Kreimeier U, Frey L, Dentz J, Herbel T, Messmer K. Hypertonic saline dextran resuscitation during the initial phase of acute endotoxemia: effect on regional blood flow. *Crit Care Med.* 1991; 19:801–809.
10. Rocha-e-Silva M, Negraes GA, Soares AM, Pontieri V, Loppnow L. Hypertonic resuscitation from severe hemorrhagic shock: patterns of regional circulation. *Circ Shock.* 1986;19:165–175.
11. Constable PD, Muir WW III, Binkley PF. Effect of hypertonic saline solution on left ventricular afterload in normovolumic dogs. *Am J Vet Res.* 1995;56:1513–1521.
12. Crystal GJ, Gurevicius J, Kim SJ, Eckel PK, Ismail EF, Salem MR. Effects of hypertonic saline solutions in the coronary circulation. *Circ Shock.* 1994;42:27–38.
13. Hannon JP, Wade CE, Bossone CA, Hunt MM, Coppes RI, Loveday JA. Blood gas and acid-base status of conscious pigs subjected to fixed-volume hemorrhage and resuscitated with hypertonic saline dextran. *Circ Shock.* 1990;32:19–29.
14. Kolsen-Petersen JA, Nielsen JO, Tonnesen E. Acid base and electrolyte changes after hypertonic saline (7.5%) infusion: a randomized controlled clinical trial. *Scand J Clin Lab Invest.* 2005;65:13–22.
15. Marshall RJ, Shepherd JT. Effect of injections of hypertonic solutions on blood flow through the femoral artery of the dog. *Am J Physiol.* 1959;197:951–954.
16. Staudenmayer KL, Maier RV, Jelacic S, Bulger EM. Hypertonic saline modulates innate immunity in a model of systemic inflammation. *Shock.* 2005;23:459–463.
17. Angle N, Hoyt DB, Cabello-Passini R, Herdon-Remelius C, Loomis W, Junger WG. Hypertonic saline resuscitation reduces neutrophil margination by suppressing neutrophil L selectin expression. *J Trauma.* 1998;45:7–12.
18. Pascual JL, Ferri LE, Seely AJ, et al. Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage. *Ann Surg.* 2002; 236:634–642.
19. Angle N, Hoyt DB, Coimbra R, et al. Hypertonic saline resuscitation diminishes lung injury by suppressing neutrophil activation after hemorrhagic shock. *Shock.* 1998;9:164–170.
20. Rizoli SB, Kapus A, Parodo J, Rotstein OD. Hypertonicity prevents lipopolysaccharide-stimulated CD11b/CD18 expression in human neutrophils in vitro: role for p38 inhibition. *J Trauma.* 1999;46: 794–798.
21. Dubick MA, Atkins JL. Small-volume fluid resuscitation for the far-forward combat environment: current concepts. *J Trauma.* 2003; 54:S43–S45.
22. Rocha-e-Silva M, Poli de Figueiredo LF. Small volume hypertonic resuscitation of circulatory shock. *Clinics.* 2005;60:159–172.
23. Anbari KK, Garino JP, Mackenzie CF. Hemoglobin substitutes. *Eur Spine J.* 2004;13(Suppl 1):S76–S82.
24. Arnoldo BD, Minei JP. Potential of hemoglobin-based oxygen carriers in trauma patients. *Curr Opin Crit Care.* 2001;7:431–436.
25. Standl T. Haemoglobin-based erythrocyte transfusion substitutes. *Expert Opin Biol Ther.* 2001;1:831–843.
26. Waschke KF, Frietsch T, Quintel M, Lenz C, Rebel A. Hemoglobin solutions. *Acta Anaesthesiol Scand Suppl.* 1997;111:259–263.
27. Lee SK, Morabito D, Hemphill JC, et al. Small-volume resuscitation with HBOC-201: effects on cardiovascular parameters and brain tissue oxygen tension in an out-of-hospital model of hemorrhage in swine. *Acad Emerg Med.* 2002;9:969–976.
28. Boura C, Caron A, Longrois D, Mertes PM, Labrude P, Menu P. Volume expansion with modified hemoglobin solution, colloids, or crystalloid after hemorrhagic shock in rabbits: effects in skeletal muscle oxygen pressure and use versus arterial blood velocity and resistance. *Shock.* 2003;19:176–182.
29. Kasper SM, Walter M, Grune F, Bischoff A, Erasmi H, Buzello W. Effects of a hemoglobin-based oxygen carrier (HBOC-201) on homodynamic and oxygen transport in patients undergoing preoperative hem dilution for elective abdominal aortic surgery. *Anesth Analg.* 1996;83:921–927.
30. Katz LM, Manning JE, McCurdy S, et al. HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. *Resuscitation.* 2002;54:77–87.
31. Gurney J, Philbin N, Rice J, et al. A hemoglobin based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus Hetastarch (HEX) in an uncontrolled liver injury hemorrhagic shock swine model with delayed evacuation. *J Trauma.* 2004;57:726–738.
32. Freitag M, Standl TG, Gottschalk A, et al. Enhanced central organ oxygenation after application of bovine cell-free hemoglobin HBOC-201. *Can J Anaesth.* 2005;52:904–914.
33. Standl T, Freitag M, Burmeister MA, Horn EP, Wilhelm S, Am Esch JS. Hemoglobin-based oxygen carrier HBOC-201 provides higher and faster increase in oxygen tension in skeletal muscle of anemic dogs than do stored red blood cells. *J Vasc Surg.* 2003;37:859–865.
34. Hughes GS Jr, Yancey EP, Albrecht R, et al. Hemoglobin-based oxygen carrier preserves submaximal exercise capacity in humans. *Clin Pharmacol Ther.* 1995;58:434–443.
35. LaMuraglia GM, O'Hara PJ, Baker WH, et al. The reduction of the allogenic transfusion requirement in aortic surgery with a hemoglobin-based solution. *J Vasc Surg.* 2000;31:299–308.
36. Kavdia M, Tsoukias NM, Popel AS. Model of nitric oxide diffusion in an arteriole: impact of hemoglobin-based blood substitutes. *Am J Physiol Heart Circ Physiol.* 2002;282:H2245–H2253.