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Vital Organ Tissue Oxygenation After Serial Normovolemic Exchange Transfusion With HBOC-201 in Anesthetized Swine

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Abstract

This study determined the effects of serial, normovolemic, stepwise exchange transfusions with either 6% human serum albumin (HSA) or the hemoglobin-based oxygen carrier, HBOC-201, on tissue oxygenation of the heart, brain and kidney in intact anaesthetized pigs. Exchange transfusions to 10%, 30% and 50% of the pigs total blood volume were completed at a withdrawal rate of 1.0 ml/kg/min followed by an infusion rate of 0.5 ml/kg/min of HBOC-201 or iso-oncologically matched 6% HSA. Measurements included invasive systemic hemodynamic (blood pressures, left ventricular end diastolic pressure [LVEDP]), hematologic (hemoglobin, hematocrit, methemoglobin), acid-base (pH, pCO₂) and biochemistry (serum lactate) measurements. Brain and kidney tissue oxygenation (tPO₂) were determined by electron paramagnetic resonance (EPR) and heart tPO₂ by O₂ sensitive fiber optic probe. The main results demonstrated that tPO₂ after HBOC-201 remained stable despite significant decreases in hematocrit and changing hemodynamics. *In vivo* tPO₂ measurements (heart tPO₂ average ≥ 22 mm Hg; brain tPO₂ average ≥ 8 mmHg; and kidney tPO₂ average ≥ 10 mmHg) were maintained in all groups at all times. Blood pressures were 20–30 mm Hg higher after HBOC-201 compared to HSA controls. Heart rate and LVEDP were not different among treatment groups. In conclusion, the administration of HBOC-201 maintained tPO₂ in three vital organs after profound hemodilution.

Keywords

Blood substitutes; HBOC-201; tissue oxygenation; isovolemic; resuscitation; brain; heart; kidney

Introduction

Hemoglobin- and perfluorocarbon-based oxygen carrying solutions have been developed with the goals of improving oxygen delivery, tissue oxygenation (tPO₂) and patient survival.¹

Hemoglobin (Hb) based oxygen carriers (HBOCs) are considered to be superior to

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perfluorocarbons because they carry more oxygen (O_2) and do not require elevated oxygen tension to increase oxygen delivery.¹ Most HBOCs possess physical characteristics (molecular structure, Hb concentration, P_{50} , viscosity, oncotic pressure) similar to blood.² The administration of a purified, polymerized, bovine Hb solution (HBOC-201), for example, improves tPO_2 in skeletal muscle, liver and central organs following profound isovolemic hemodilution, improves tPO_2 in the brain after traumatic brain injury and hemorrhagic shock, reverses anaerobic metabolism and improves survival in rats, hamsters, dogs and pigs following experimental anemia, hemorrhage or trauma.^{3–13}

Questions remain, however, concerning the mechanism and result of vasoconstrictive effects of HBOCs, the development of systemic and pulmonary hypertension and the potential for oxidative stress in human patients thereby limiting potential benefits afforded by increases in blood oxygen carrying capacity.^{14–17} HBOC-induced vasoconstriction has been attributed to nitric oxide scavenging and distortion of the nitric oxide diffusion field by plasma and extravascular molecular Hb, and an oversupply of arteriolar O_2 .^{18, 19} Furthermore, *in vitro* and *in vivo* studies suggest that the molecular size and O_2 affinity of most Hb solutions in conjunction with their relatively low viscosity may predispose to autoregulatory vasoconstriction.^{22–23} Reports of arteriolar vasoconstriction and reduced cardiac output have led to the consensus opinion that conventional HBOCs may predispose to spatial heterogeneity in microcirculatory blood flow and a decrease in functional capillary density (FCD) and tPO_2 regardless of increases in the oxygen carrying capacity of blood.^{16, 24, 25} Others, however, suggest that optimization of a low O_2 -affinity acellular Hb concentrations (Oxyglobin®) increases tissue oxygenation without substantial vasoconstriction.^{13, 26} Indices of tissue oxygenation and tPO_2 are currently considered the most specific and reliable quantitative markers of tissue perfusion.²⁷ The objective of this study was to determine the effects of HBOC-201 on tPO_2 in heart, brain and kidney after extreme isovolemic hemodilution in a large animal model, anesthetized pigs. We hypothesized that extreme isovolemic hemodilution with HBOC-201 would not compromise tPO_2 in vital organs.

Materials and Methods

The study was approved and conducted in accordance with the guidelines established by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University. A veterinarian was available 24 hours a day 7 days a week to provide care if required.

Animals

The study included forty 12–15 kg pigs purchased from Genetiporc USA, Alexandria, MN 56308. The pigs were identified by ear notch, group housed in climate controlled, indoor pens and offered Mazuri mini pig active feed. All pigs underwent a seven-day acclimation period before beginning the study. Prestudy laboratory evaluation included a physical examination, blood chemistry profile and complete blood count to verify the health of the pigs. Food, but not water, was withheld after midnight on the morning of the procedure.

Experimental Design

The pigs were randomly assigned into one of three groups. Group one (Control) included four pigs that served as time controls and were anesthetized but not subjected to test procedures. The remaining 36 pigs underwent a 1:1 stepped isovolemic exchange transfusion representing 10%, 30% and 50% of their total blood volume (70 ml/kg). Eighteen pigs (Group 2, HSA, $n=6$ /tissue for heart, brain and kidney tPO_2) were administered oncologically matched, HSA (6 g/dL; Baxter Healthcare Corporation, Westlake Village, CA) and eighteen pigs (Group 3, HBOC, $n=6$ /tissue for heart, brain and kidney

tPO₂) were administered HBOC-201 (Biopure Corporation, Cambridge, MA): mean molecular weight 250 KD, Hb 13 g/dl, P₅₀ 40 mmHg. Blood was removed from a femoral arterial catheter followed by infusion of HSA or HBOC-201 through a jugular vein catheter. The initial volume exchange was 8 ml/kg (10% of estimated blood volume) followed by two 15 ml/kg (second exchange, 30%; then third exchange, 50%) exchanges (Figure 1). The withdrawal rate was 1 ml/kg/min and infusion rate was 0.5 ml/kg/min.

Animal Preparation

Each pig was immobilized with ketamine IM (15–20 mg/kg) and atropine IM (0.1 mg/kg) at least three hours before beginning the study. Lactated Ringers solution (2–4 ml/kg/hr) was administered via an ear vein. General anesthesia was produced and maintained by administering sodium pentobarbital, 15 mg/kg, IV followed by 3–10 mg/kg/hr. All pigs were oral-tracheally intubated, placed in a supine position and ventilated with room air (21% O₂) in order to maintain PaCO₂ between 35–45 mmHg and PaO₂ > 80 mmHg (ABL 725; Radiometer America, Westlake, OH). A dual tipped pressure sensing catheter (Millar Instruments, Houston, Texas) was inserted into the surgically exposed left carotid artery and advanced into the left ventricle (LV) for measurement of left ventricular end diastolic pressure (LVEDP; distal tip) and proximal aortic blood pressure (proximal tip). A polyethylene (PE) 160 catheter was inserted into the surgically exposed right femoral artery and advanced to the level of the diaphragm and used for blood withdrawal during exchange transfusion. A PE 160 catheter was inserted into the surgically exposed left jugular vein and advanced into the right atrium. The left carotid artery and jugular venous catheters were used to obtain blood samples and administer test article, respectively.

Lithium naphththalocyanine (LiPc) microcrystals (O₂ probe) were deposited at a 2–3 mm depth from the tissue surface by inserting a 25-gauge needle into the kidney or brain. The LiPc microcrystals produced a single sharp electron paramagnetic resonance (EPR) line, the width of which is highly sensitive and specific to the tissue partial pressure of oxygen.²⁸ The oxygen sensitivity of the probe in saline was calibrated at L-band (1.3 GHz) by equilibrating with known partial pressures of oxygen. The calibration measurements demonstrated that the EPR linewidth (ΔB_{pp}) of the LiPc probe showed a linear response in the range of 0–150 mmHg. A slope of 0.89 μ T/mmHg was obtained for oxygen sensitivity. The calibration data were used to convert the experimentally measured EPR line-widths into PO₂ values.²⁸ The area of tissue being measured was approximately 3 mm².

Left ventricular myocardial tPO₂ was determined by advancing a 100 μ diameter fiber optic probe (Oximetric, Abbott, Germany) through an 18 g Teflon over-the-needle catheter following pre-positioning of the needle in a relatively avascular (no visible vessels) area approximately 2–3 mm below the epicardial surface of the left ventricle. The fiber optic probe tip was approximately 1 cm caudal to the left anterior descending (left interventricular) coronary artery and 2 cm ventral to the left circumflex coronary artery. Tissue tPO₂ determinations were representative of local oxygen tension at the probe tip. Proper positioning of the fiber optic oxygen sensing probe was determined by the lack of hemorrhage or discoloration at the site of probe placement, appropriate fluctuations in myocardial blood flow, and stable tPO₂ and temperature values. Only probe insertions that demonstrated no peri-probe hemorrhage and that produced stable tPO₂ values were accepted for data analysis. Local tissue temperature was measured by a fully integrated thermocouple, allowing simultaneous monitoring of tissue PtO₂, temperature and automatic temperature correction.

Measurements

Data were obtained continuously and recorded 5 minutes before each exchange, 20 minutes following each exchange and again at 2 hours after the third (50%) exchange transfusion. One hour elapsed between exchange transfusions. Each data set included arterial and mixed venous hematocrit (Hct; %), total protein (TP; g/dL), red blood cell (RBC) and plasma Hb concentration (g/dL), methemoglobin concentration (%), pH and blood gases (PO₂ and PCO₂; mm Hg; ABL Radiometer, Copenhagen, Denmark), oxygen saturation (%), base excess (mmol/L), lactate (mmol/L), heart rate (beats per minute; bpm), systolic (SAP) diastolic (DAP), mean (MAP) arterial blood pressure (mm Hg), left ventricular end diastolic blood pressure (LVEDP; mm Hg) and body temperature (°C). Tissue oxygen tension (tPO₂; mm Hg) was determined by EPR oximetry in kidney and brain and by fluorescence fiber-optic microprobe in heart.^{8, 15} Initial measurements were performed for tPO₂ immediately following stabilization of the oximetry probes and continuously throughout the experiments. All pigs were euthanized at the end of the experiment by administering sodium pentobarbital, (150 mg/kg, IV) in the auricular vein.

Histopathology

Tissues were harvested from the heart, brain and kidney. The ventricular myocardium, cerebrum and kidney cortex were stained with H&E to detail cellular structures. Trichrome stain was applied to distinguish nuclei (black), collagen (blue) and muscle (red) and von Kossa to determine areas of increased accumulation of calcium. The brain (cortex) was stained with Van Gieson in order to identify collagen. The tissues were prepared using standard techniques and each specimen was microscopically evaluated (× 100–400 magnification) using an objective mounted micrometer.

Data Analysis

Results are expressed as mean ± SD for all groups. With the exception of tPO₂, all hemodynamic, pH and blood gas (PO₂ and PCO₂) parameters from each group (heart, brain, and kidney) were combined to provide a single comparison between HBOC vs. HSA (Table 1). An analysis of variance (ANOVA) for repeated measures with Bonferroni correction for multiple comparisons was used to analyze all data with group and time serving as cofactors. Paired t-tests were used to calculate differences from baseline within groups for all parameters other than tPO₂. One-way ANOVA was used to determine differences among groups across time, followed by t-tests. Statistical significance was assumed at P < 0.05. The statistical program used was Systat version 10.2.

Results

Blood Chemistry

Baseline hematology and blood chemical data were within normal limits for pigs. No differences in baseline values for any variable for any group were identified (data not shown). Hemodynamic and hematologic values did not change throughout the duration of the experiment in Control Group pigs (Table 1, 2).

Hemodynamics

Heart rate increased and arterial blood pressure and LVEDP decreased during blood withdrawal. This pattern was reversed during infusion of either 6% HSA or HBOC-201. The transient hemodynamic changes associated with blood withdrawal and test article infusion are not reflected in Table 1 because hemodynamic data were obtained after the end of each infusion. Infusions of HBOC-201 produced sustained increases in MAP (23% Inf 1; 29% Inf 2; 35% Inf 3) from baseline values (Table 1). MAP did not change from baseline values

following the administration of HSA. Arterial blood pressure was significantly greater in pigs administered HBOC-201 compared to HSA following the second and third exchange transfusions and remained elevated for the duration of the experiment (Table 1).

Hematology

Results of selected hematologic variables before and after transfusion with 6% HSA or HBOC-201 are presented in Table 2. Baseline values for Hct, TP, total blood Hb, RBC Hb, Plasma Hb, and metHb concentrations were not different among groups and did not change from baseline in Control Group pigs. The Hct decreased following the infusion of 6% HSA or HBOC-201. The TP did not change following the administration of 6% HSA and increased significantly to values ranging from 6–8 g/dL in pigs administered HBOC-201 (Table 2). Total Hb values did not change throughout the experiment for pigs administered HBOC-201 but decreased from baseline values for pigs administered 6% HSA. The RBC Hb concentration decreased for all pigs administered 6% HSA or HBOC-201 while plasma Hb concentration did not change for pigs administered 6% HSA and increased after each of the three infusions of HBOC-201. Whole blood metHb did not change in Control pigs and metHb remained below 1% of total Hb in pigs administered 6% HSA and significantly increased to $3.6 \pm 1.1\%$ with individual peak values ranging from 2–6% in pigs administered HBOC-201.

Tissue Oxygenation

Changes in brain and kidney tPO_2 remained relatively stable for the duration of the experiment following exchange transfusion with either 6% HSA or HBOC-201 (Table 3). The average tPO_2 was significantly increased compared to baseline values in heart tissue after the first exchange transfusion with HBOC-201 (Table 3).

Histopathology

No significant histopathologic changes were observed in brain, kidney or heart tissue obtained from Control pigs or in brain and kidney tissue obtained from pigs administered 6% HSA or HBOC-201. One and two small foci of coagulation necrosis, respectively, were observed in two of the six HBOC-201 treated pigs.

Discussion

Exchange transfusion and hemodilution with 6% HSA or HBOC-201 to hematocrit values averaging 16% did not change or decrease tPO_2 in the heart, brain and kidney in a large animal model. Exchange transfusion produced predictable hematologic and hemodynamic effects based upon the physical characteristics and component chemistry of the solution administered. Exchange transfusion with 6% HSA produced minimal or no changes in hemodynamics, pH and blood gas variables other than those induced by hemodilution. The administration of HBOC-201 increased arterial blood pressure and plasma hemoglobin concentration, maintained total blood hemoglobin concentration and reduced blood oxygen saturation. Decreases in the hematocrit and RBC Hb concentration were attributed to the dilutional effects of 6% HSA or HBOC-201. Increases in total protein following HBOC-201 administration were attributed to the concentration of protein infused (13 g/dL Hb). The 2–6% increases in the percent whole blood methemoglobin (ferric heme iron) were expected based upon the known tendency for extracellular hemoglobin to undergo spontaneous autoxidation.^{16, 29} The greater P_{50} and lower cooperativity of HBOC-201 compared to porcine RBC hemoglobin were believed responsible for the observed decreases in the arterial and venous oxygen saturation in pigs administered HBOC-201. None of these changes were of significant magnitude to jeopardize tPO_2 .

The average absolute increase in arterial blood pressure compared to baseline values following the administration of HBOC-201 was approximately 23%, 29% and 35%. These values were greater than that produced by 6% HSA after each exchange transfusion, but are not considered to be abnormally elevated values for anesthetized pigs.^{12, 30} The largest change in arterial blood pressure (23%) occurred after the first exchange transfusion of HBOC-201 while subsequent infusions (30 and 50% of blood volume) did not produce proportional increases in arterial blood pressure. Importantly, we did not observe a significant decrease in any hemodynamic variable compared to control pigs. We adapted an anesthetic protocol known to preserve homeostatic responses in swine and produced concordant results to that study.³⁰ Various anesthetic protocols, especially inhalant anesthetics, may be responsible for the conflicting results published by others since nearly all anesthetic drugs have the potential to produce smooth muscle relaxation, vasodilation and blunt the sympatho-adrenal homeostatic responses to manipulations.³¹

The small changes in macro-hemodynamics observed in this study would not be expected to produce significant changes in tPO_2 unless tissue blood flow, arterial blood oxygen content (CaO_2) or tissue metabolism were markedly altered at the microvascular ($< 30 \mu m$) level. Decreases in tPO_2 below the threshold for anaerobic metabolism ($tPO_2 < 3 \text{ mmHg}$) would be expected to lead to anaerobic metabolism and increases in serum lactate, thus leading to the development of metabolic acidosis.³² Acid-base data and, in particular, the arterial lactate values we recorded suggest that tissue blood flow was adequately maintained by both treatment groups. Transient decreases and increases in tPO_2 values were observed in all three vital organs during blood withdrawal and test article infusion, respectively, suggesting associated changes in tissue perfusion pressure. The tPO_2 , however, never fell below a critical value in spite of significant decreases in Hct following each exchange and was never below baseline values in any organ. The *in vivo* measurements of tPO_2 in the heart, brain and kidney support this inference. Increases in arterial blood pressure would be expected to maintain and potentially increase tPO_2 since tissue perfusion pressure is a prime determinant of tissue blood flow, provided that microvascular resistance to blood flow does not increase significantly.^{24, 33} Our macro-hemodynamic data (HR, ABP, LVEDP) suggest that tissue perfusion pressure and blood flow were maintained while the tPO_2 data confirms the maintenance of tissue oxygenation in the presence of HBOC-201.

Our data conflicts with previous studies which measured tPO_2 in the skin fold of the cheek pouch of hamsters undergoing exchange transfusions using a related polymerized bovine derived HBOC (HBOC-301) solution.^{24, 25} Although the previous and current studies utilized exchange transfusions to produce a similar degree of hemodilution the earlier studies reported that exchange transfusion with HBOC-301 resulted in abnormally low tPO_2 values and suggested that this effect may be attributable to nitric oxide scavenging, vasoconstriction and hypoperfusion and could affect survival.²⁵ There are several critical differences among these experiments, and the current study. We measured tPO_2 in three vital organs: heart, brain and kidney, while the earlier studies measured tPO_2 in a non-vital fatty, subcutaneous tissue or skeletal muscle of the hamster cheek pouch. Significant differences are likely to exist between the regulation of blood flow and tissue oxygenation between subcutaneous tissues and vital organs. It is likely that exchange transfusion induced changes in the microcirculation of the hamster cheek pouch are indicative of shunting of blood away from non-vital organs thereby decreasing tissue oxygenation. Our study indicates that the distribution and maintenance of blood flow and oxygenation to vital organs is maintained. We did not measure cardiac output but based upon the results of a previous study which utilized an anesthetic and experimental protocol almost identical to the current study believe that that HSA (6 g/dL) and HBOC-201 markedly increased or maintained cardiac output, respectively.³⁰ That study attributed increases in cardiac output and vital organ blood flow (heart, brain, kidney), produced by the infusion of HSA, to a

compensatory response to hemodilution and decreased oxygen content in order to maintain oxygen delivery.³⁰ The authors concluded that physiologic coupling of oxygen delivery and consumption is maintained after administration of HBOC-201 and not impaired by local vasoconstriction.³⁰ Furthermore, criticisms have been raised concerning the methodological limitations of the quenching method used in these earlier studies to determine tPO₂.³⁴ Finally, and of greater importance, more recent data has demonstrated that the administration of a dilute solution of bovine derived HBOC-301 (4 g/dl) restores tissue perfusion, functional capillary density and significantly improved tPO₂ and acid base parameters in the hamster cheek pouch window chamber model subjected to moderate hemorrhagic shock (50% bleed).^{13, 26} This observation is consistent with our data in anesthetized swine and experiments which examined the effects of HBOC-201 as salvage therapy for severe neuro- and polytrauma.¹² The administration of HBOC-201 in this latter study reduced pressor and fluid requirements, improved intracranial pressure and increased brain tissue tPO₂.¹²

Our study clearly demonstrated that HBOC-201 is capable of maintaining tissue oxygenation in a clinically relevant large animal model in which the Hct was reduced by approximately 50% due to hemodilution. We chose the pig because of its cardiovascular and physiological similarity to humans. The model was not designed to test the limits of the oxygen carrying ability of HBOC-201 and a more severe model utilizing alternative anesthetic drugs or anesthetic techniques may have modified our results. Our experimental model was developed to obtain a total volume exchange that mimicked previously published articles wherein hemorrhage or isovolemic hemodilution was carried out to remove 50% of the animals total blood volume or attain a cumulative 50% volume exchange.^{4-6, 9-11} These earlier studies suggested that this “stress” would result in the development of a metabolic acidosis (lactic acidosis) presumably from inadequate oxygen delivery or maldistribution of blood flow and low tissue oxygen tension. A recent study, using an experimental design similar to the current study, however, suggested that physiologic coupling of oxygen delivery and consumption after administration of HBOC-201 is not impaired.³⁰ Our study extends this conclusion and suggests that tPO₂ is maintained, at least in the tissues (brain, kidney, heart) that we interrogated. Additional studies will need to be conducted in order to more definitively examine tissue blood flow and oxygenation in animals subjected to a more severe degree of isovolemic hemodilution (Hct = 10%, 5%). There may be a spectrum of critical hematocrits below which various transfusion fluids are unable to maintain tissue oxygenation. The tPO₂ values recorded in the current study are similar to tPO₂ values reported in other EPR studies and those employing polarographic electrodes and fluorescence probes.^{6, 28} The EPR method is highly specific and sensitive but is similar to other technologies in that it is limited to small superficial areas of tissue and is difficult to use in vivo. Interestingly, the heart tPO₂ remained more stable in pigs administered HBOC-201 compared to pigs administered 6% HSA. Potential explanations for this difference include the greater total oxygen carrying capacity and content of blood containing HBOC-201 and differences in microcirculatory blood flow. The PtO₂ values for brain and kidney were lower than that for heart in both the HSA and HBOC groups but similar to values reported for gut and skeletal muscle.³⁻⁶ The reason for these differences cannot be determined from our studies but may be related to differences in the techniques used to measure PtO₂, differences in the factors that regulate individual organ blood flow and blood flow distribution or differences in tissue metabolism. Finally, a review of the effects of HBOC-201 administration on organ (myocardium, lungs, hepatic parenchyma, jejunum, renal cortex/medulla) injury after moderate (40%) and severe (55%) controlled hemorrhage or severe uncontrolled hemorrhage in swine concluded that there is limited evidence for HBOC-201 induced oxidative stress and that observed histological changes are clinically equivocal.³⁵ Our exchange transfusion experiment replaced 50% of the pigs blood volume with HBOC-201 and was not preceded by hemorrhagic shock therefore it is not surprising

that we did not observe consistent histopathologic changes of oxidative stress. One study has demonstrated that intracoronary infusion of preoxygenated HBOC-201 preserved myocardial metabolism, regional oxygen delivery and left ventricular function produced by a three minute coronary artery occlusion.³⁶ Importantly, intracoronary infusion of nonoxygenated HBOC-201, Ringers solution or no treatment resulted in immediate loss of ventricular function in the ischemic region and evidence for anaerobic metabolism.³⁶ These findings have important clinical implications regarding the potential benefits of HBOC-201 as an organ preservation fluid and require further investigation.

Conclusion

Taken together our study supports and extends investigations that have identified that systemic alterations produced by HBOC-201 do not alter regional blood flow, and are unlikely to impair vital organ (heart, brain, and kidney) oxygenation. There was no indirect evidence of altered tissue perfusion (normal serum lactate concentrations), and minimal, if any, evidence for tissue histopathologic changes that could be directly attributed to oxidative stress.

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References

1. Winslow RM. Current status of oxygen carriers ('blood substitutes'): 2006. *Vox Sanguinis*. 2006; 91(2):102–110. [PubMed: 16907870]
2. Awasthi V. Pharmaceutical aspects of hemoglobin-based oxygen carriers. *Current Drug Delivery*. 2005; 2(2):133–142. [PubMed: 16305414]
3. Standl TG, Reeker W, Redmann G, Kochs E, Werner C, Schulte am Esch J. Haemodynamic changes and skeletal muscle oxygen tension during complete blood exchange with ultrapurified polymerized bovine haemoglobin. *Intensive Care Med*. 1997; 23(8):865–872. [PubMed: 9310804]
4. Krieter H, Hagen G, Waschke KF, Köhler A, Wenneis B, Brückner UB, van Ackern K. Isovolemic hemodilution with a bovine hemoglobin-based oxygen carrier: effects on hemodynamics and oxygen transport in comparison with a nonoxygen-carrying volume substitute. *J Cardiothorac Vasc Anesth*. 1997; 11(1):3–9. [PubMed: 9058211]
5. Gottschalk A, Standl TG, Freitag M, Radtke P, Rempf C, Burmeister MA, Horn EP, Strate T, Schulte am Esch J. Effects of isovolaemic haemodilution on oxygenation of liver and skeletal muscle. *Eur J Anaesthesiol*. 2005; 22(3):181–188. [PubMed: 15852990]
6. Freitag M, Standl TG, Gottschalk A, Burmeister MA, Rempf C, Horn EP, Strate T, Schulte Am Esch J. Enhanced central organ oxygenation after application of bovine cell-free hemoglobin HBOC-201. *Can J Anesth*. 2005; 52(9):904–914. [PubMed: 16251554]
7. Manley GT, Hemphill JC, Morabito D, Erickson V, Holcroft JJ, Derugin N, Knudson MM. Small-volume resuscitation with the hemoglobin substitute HBOC-201: effect on brain tissue oxygenation. *Adv Exp Med Biol*. 2003; 530:311–317. [PubMed: 14562727]
8. Stern S, Rice J, Philbin N, McGwin G, Arnaud F, Johnson T, Flournoy WS, Ahlers S, Pearce LB, McCarron R, Freilich D. Resuscitation with the hemoglobin-based oxygen carrier HBOC-201, in a swine model of severe uncontrolled hemorrhage and traumatic brain injury. *Shock*. 2009; 31(1):64–79. [PubMed: 18497706]
9. Fitzpatrick CM, Biggs KL, Atkins BZ, Quance-Fitch FJ, Dixon PS, Savage SA, Jenkins DH, Kerby JD. Prolonged low-volume resuscitation with HBOC-201 in a large-animal survival model of controlled hemorrhage. *J Trauma*. 2005; 59(2):273–283. [PubMed: 16294065]
10. Katz LM, Manning JE, McCurdy S, Pearce LB, Gawryl MS, Wang Y, Brown C. HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. *Resuscitation*. 2002; 54:77–87. [PubMed: 12104112]

11. York GB, Eggers JS, Smith DL, Jenkins DH, McNeil JD, Mueller D, Josephs JD, Kerby JD. Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) leads to a reversal of anaerobic metabolism. *J Trauma*. 2001; 50:1063–1075. [PubMed: 11426122]
12. Dudkiewicz M, Harpaul TA, Proctor KG. Hemoglobin-based oxygen carrying compound-201 as salvage therapy for severe neuro- and polytrauma (Injury Severity Score = 27–41). *Crit Care Med*. 2008; 36(10):2838–2348. [PubMed: 18766094]
13. Cabrales P, Tsai AG, Intaglietta M. Polymerized bovine hemoglobin can improve small-volume resuscitation from hemorrhagic shock in hamsters. *Shock*. 2009; 31(3):300–307. [PubMed: 18636045]
14. Rice J, Philbin N, Handrigan M, Hall C, McGwin G, Ahlers S, Pearce LB, Arnaud F, McCarron R, Freilich D. Vasoactivity of bovine polymerized hemoglobin (HBOC-201) in swine with traumatic hemorrhagic shock with and without brain injury. *J Trauma*. 2006; 61(5):1085–1099. [PubMed: 17099513]
15. Kasper SM, Grüne F, Walter M, Amr N, Erasmi H, Buzellow. The effects of increased doses of bovine hemoglobin on hemodynamics and oxygen transport in patients undergoing preoperative hemodilution for elective abdominal aortic surgery. *Anesth Analg*. 1996; 87(2):284–291. [PubMed: 9706917]
16. Buehler PW, D'Agnillo F, Schaer DJ. Hemoglobin-based oxygen carriers: from mechanisms of toxicity and clearance to rational drug design. *Trends in Molecular Medicine*. 2010; 16(10):447–457. [PubMed: 20708968]
17. Katz LM, Manning JE, McCurdy S, Sproule C, McGwin G, Moon-Massat P, Cairns CB, Freilich D. Nitroglycerin attenuates vasoconstriction of HBOC-201 during hemorrhagic shock resuscitation. *Resuscitation*. 2010; 81:481–487. [PubMed: 20080328]
18. McCarthy MR, Vandegriff KD, Winslow RM. The role of facilitated diffusion in oxygen transport by cell-free hemoglobins: implications for the design of hemoglobin-based oxygen carriers. *Biophys Chem*. 2001; 92(1–2):103–117. [PubMed: 11527583]
19. Tsai AG, Cabrales P. Can the effects of vasoactivity of molecular hemoglobin-based plasma expanders be ignored? *Crit Care Med*. 2006; 34(5):1566–1567. [PubMed: 16633264]
20. Sakai H, Hara H, Yuasa M, Tsai AG, Takeoka S, Tsuchida E, Intaglietta M. Molecular dimensions of Hb-based O₂ carriers determine constriction of resistance arteries and hypertension. *Am J Physiol Heart Circ Physiol*. 2000; 279:H908–H915. [PubMed: 10993749]
21. Cabrales P, Tsai AG, Intaglietta M. Hyperosmotic-hyperoncotic versus hyperosmotic-hyperviscous: small volume resuscitation in hemorrhagic shock. *Shock*. 2004; 22(5):431–437. [PubMed: 15489635]
22. Cortinovis A, Crippa A, Cavalli R, Corti M, Cattaneo L. Capillary blood viscosity in microcirculation. *Clin Hemorheol Microcirc*. 2006; 35(1–2):183–192. [PubMed: 16899925]
23. Martini J, Cabrales P, Tsai AG, Intaglietta M. Mechanotransduction and the homeostatic significance of maintaining blood viscosity in hypotension, hypertension and haemorrhage. *J Intern Med*. 2006; 259(4):364–372. [PubMed: 16594904]
24. Tsai AG. Influence of cell-free Hb on local tissue perfusion and oxygenation in acute anemia after isovolemic hemodilution. *Transfusion*. 2001; 41(10):1290–1298. [PubMed: 11606831]
25. Cabrales P, Tsai AG, Intaglietta M. Isovolemic exchange transfusion with increasing concentrations of low oxygen affinity hemoglobin limits oxygen delivery due to vasoconstriction. *Am J Physiol Heart Circ Physiol*. 2008; 295(5):H2212–H2218. [PubMed: 18835914]
26. Cabrales P. Low oxygen-affinity hemoglobin solution increases oxygenation of partially ischemic tissue during acute anemia. *J Am Coll Surg*. 2010; 210(3):271–279. [PubMed: 20193889]
27. Kramer GC, Kinsky MP, Esenaliev RO. Assessment of tissue oxygenation: ultimate endpoint or simply interesting? *Crit Care Med*. 2006; 34(3):924–926. [PubMed: 16505689]
28. Ilangovan G, Manivannan A, Li H, Yanagi H, Zweier JL, Kuppusamy P. A naphthalocyanine-based EPR probe for localized measurements of tissue oxygenation. *Free Radic Biol Med*. 2002; 32(2):139–147. [PubMed: 11796202]
29. Alayash AI. Hemoglobin-based blood substitutes: oxygen carriers, pressor agents, or oxidants? *Nat Biotechnol*. 1999; 17(6):545–549. [PubMed: 10385317]

30. Mongan PD, Moon-Massat PF, Rentko V, Mihok S, Dragovich A, Sharma P. Regional blood flow after serial normovolemic exchange transfusion with HBOC-201 (Hemopure) in anesthetized swine. *J Trauma*. 2009; 67(1):51–60. [PubMed: 19590308]
31. Ogawa Y, Iwasaki K, Shibata S, Kato J, Ogawa S, Oi Y. Different effects on circulatory control during volatile induction and maintenance of anesthesia and total intravenous anesthesia: autonomic activity and arterial cardiac baroreflex function evaluated by blood pressure and heart rate variability analysis. *J of Clinical Anesthesia*. 2006; 18(2):87–95.
32. Richmond KN, Shonat RD, Lynch RM, Johnson PC. Critical PO₂ of skeletal muscle in vivo. *Am J Physiol*. 1999; 277(6 Pt2):H1831–H1840. [PubMed: 10564137]
33. Cabrales P, Tsai AG, Winslow RM, Intaglietta RM. Effects of extreme hemodilution with hemoglobin-based O₂ carriers on microvascular pressure. *Am J Physiol Heart Circ Physiol*. 2005; 288(6):H2146–H2153. [PubMed: 15637119]
34. Golub AS, Pittman RN. Effect of oxygen consumption by measuring method on PO₂ transients associated with the passage of erythrocytes in capillaries of rat mesentery. *Am J Physiol Heart Circ Physiol*. 2005; 289(4):H1777–H1779. [PubMed: 16162870]
35. Johnson T, Arnaud F, Dong F, Philbin N, Rice J, Asher L, Arrisueno M, Warndorf M, Gurney J, McGwin G, Kaplan L, Flournoy WS, Apple FS, Pearce LB, Ahlers S, McCarron R, Freilich D. Bovine polymerized hemoglobin (hemoglobin-based oxygen carrier-201) resuscitation in three swine models of hemorrhagic shock with military relevant delayed evacuation – Effects on histopathology and organ function. *Crit Care Med*. 2006; 34(5):1464–1474. [PubMed: 16540964]
36. Te Lintel Hekkert M, Dubé GP, Regar E, de Boer M, Vranckx P, van der Giessen WJ, Serruys PW, Duncker DJ. Preoxygenated hemoglobin-based oxygen carrier HBOC-201 annihilates myocardial ischemia during brief coronary artery occlusion in pigs. *Am J Physiol Heart Circ Physiol*. 2010; 298(3):H1103–H1113. [PubMed: 20061545]

fig. 1.

Timeline of three (10%, 30%, 50%) exchange transfusions. Timing of sample and data collected indicated by solid black lines. BL indicates baseline; post, post-exchange transfusion; pre, before subsequent exchange transfusion.

Table 1

Hemodynamic and blood gas summary of all groups combined with statistical significance ($P \leq 0.05$); Average ($\pm$ Standard Deviation)

Time point	Treatment	Baseline	20 Min Post Exchange 1	Pre-Exchange 2	20 Min Post Exchange 2	Pre-Exchange 3	20 Min Post Exchange 3	2 Hr Post Exchange 3
Variable	Control	Minute -5	Minute 41	Minute 76	Minute 143	Minute 178	Minute 245	Minute 345
Heart Rate (bpm)	Control	129 $\pm$ 14	140 $\pm$ 10	136 $\pm$ 10	136 $\pm$ 12	131 $\pm$ 16	117 $\pm$ 17	121 $\pm$ 15
	HBOC-201	152 $\pm$ 33	151 $\pm$ 27	144 $\pm$ 25	144 $\pm$ 25	140 $\pm$ 16	145 $\pm$ 28	147 $\pm$ 35
	6% HSA	145 $\pm$ 31	150 $\pm$ 36	144 $\pm$ 37	152 $\pm$ 38	149 $\pm$ 40	151 $\pm$ 39	140 $\pm$ 42
SAP (mmHg)	Control	115 $\pm$ 16	122 $\pm$ 29	115 $\pm$ 24	113 $\pm$ 14	111 $\pm$ 15	106 $\pm$ 23	114 $\pm$ 18
	HBOC-201	97 $\pm$ 15	115 $\pm$ 16 ^a	112 $\pm$ 19 ^a	123 $\pm$ 17 ^{ab}	126 $\pm$ 19 ^{ab}	128 $\pm$ 22 ^{ab}	118 $\pm$ 19 ^{ab}
	6% HSA	105 $\pm$ 24	103 $\pm$ 19	102 $\pm$ 15	104 $\pm$ 13 ^b	103 $\pm$ 14 ^b	98 $\pm$ 17 ^b	93 $\pm$ 15 ^b
DAP (mmHg)	Control	84 $\pm$ 17	91 $\pm$ 29	85 $\pm$ 24	84 $\pm$ 18	81 $\pm$ 20	76 $\pm$ 26	84 $\pm$ 17
	HBOC-201	72 $\pm$ 14	89 $\pm$ 15 ^a	90 $\pm$ 16 ^{ab}	94 $\pm$ 18 ^{ab}	99 $\pm$ 15 ^{ab}	100 $\pm$ 20 ^{ab}	95 $\pm$ 20 ^{ab}
	6% HSA	77 $\pm$ 19	76 $\pm$ 17	75 $\pm$ 14 ^b	75 $\pm$ 11 ^b	75 $\pm$ 12 ^b	69 $\pm$ 14 ^b	65 $\pm$ 14 ^b
MAP (mmHg)	Control	100 $\pm$ 16	107 $\pm$ 29	100 $\pm$ 22	99 $\pm$ 17	97 $\pm$ 18	91 $\pm$ 25	99 $\pm$ 17
	HBOC-201	84 $\pm$ 13	103 $\pm$ 15 ^a	102 $\pm$ 16 ^a	108 $\pm$ 17 ^{ab}	111 $\pm$ 16 ^{ab}	113 $\pm$ 20 ^{ab}	106 $\pm$ 19 ^{ab}
	6% HSA	93 $\pm$ 21	91 $\pm$ 18	89 $\pm$ 14	91 $\pm$ 12 ^b	91 $\pm$ 13 ^b	85 $\pm$ 16 ^b	80 $\pm$ 14 ^b
LVEDP (mmHg)	Control	2 $\pm$ 3	2 $\pm$ 3	3 $\pm$ 5	3 $\pm$ 4	3 $\pm$ 4	3 $\pm$ 3	3 $\pm$ 4
	HBOC-201	-3 $\pm$ 3	-1 $\pm$ 5	-1 $\pm$ 4	0 $\pm$ 5	0 $\pm$ 5	0 $\pm$ 5	-1 $\pm$ 5
	6% HSA	0 $\pm$ 3	0 $\pm$ 3	0 $\pm$ 3	0 $\pm$ 4	0 $\pm$ 4	0 $\pm$ 3	0 $\pm$ 3
Temperature (°C)	Control	36.6 $\pm$ 1.2	36.9 $\pm$ 1.3	37.0 $\pm$ 1.3	37.0 $\pm$ 1.2	36.9 $\pm$ 1.2	36.6 $\pm$ 1.3	36.7 $\pm$ 1.5
	HBOC-201	36.2 $\pm$ 0.7	36.1 $\pm$ 0.7	36.1 $\pm$ 0.8	36.2 $\pm$ 0.7	36.3 $\pm$ 0.6	36.3 $\pm$ 0.7	36.6 $\pm$ 0.9
	6% HSA	36.5 $\pm$ 1.1	36.4 $\pm$ 1.0	36.3 $\pm$ 1.0	36.3 $\pm$ 1.0	36.3 $\pm$ 1.0	36.2 $\pm$ 1.0	36.5 $\pm$ 1.0
Arterial pH	Control	7.45 $\pm$ 0.09	7.45 $\pm$ 0.05	7.42 $\pm$ 0.13	7.46 $\pm$ 0.06	7.44 $\pm$ 0.08	7.45 $\pm$ 0.07	7.49 $\pm$ 0.05
	HBOC-201	7.43 $\pm$ 0.07	7.44 $\pm$ 0.05	7.47 $\pm$ 0.08 ^a	7.46 $\pm$ 0.08	7.48 $\pm$ 0.09 ^a	7.46 $\pm$ 0.09	7.49 $\pm$ 0.10 ^a
	6% HSA	7.48 $\pm$ 0.07	7.48 $\pm$ 0.07	7.49 $\pm$ 0.07	7.48 $\pm$ 0.06	7.50 $\pm$ 0.05	7.48 $\pm$ 0.05	7.51 $\pm$ 0.05
Arterial PCO ₂ (mmHg)	Control	34 $\pm$ 5	36 $\pm$ 2	34 $\pm$ 3	31 $\pm$ 4	34 $\pm$ 2	32 $\pm$ 3	32 $\pm$ 5
	HBOC-201	39 $\pm$ 7 ^b	38 $\pm$ 5 ^b	37 $\pm$ 6	37 $\pm$ 8	38 $\pm$ 8	39 $\pm$ 8	39 $\pm$ 11

Time point	Treatment	Baseline	20 Min Post Exchange 1	Pre-Exchange 2	20 Min Post Exchange 2	Pre-Exchange 3	20 Min Post Exchange 3	2 Hr Post Exchange 3
Variable	Treatment	Minute -5	Minute 41	Minute 76	Minute 143	Minute 178	Minute 245	Minute 345
Arterial PO ₂ (mmHg)	6% HSA	34 ± 4 ^b	33 ± 5 ^b	34 ± 5	35 ± 5	33 ± 4	34 ± 5	34 ± 4
	Control	88 ± 21	80 ± 5	88 ± 17	91 ± 19	83 ± 15	85 ± 16	80 ± 17
	HBOC-201	85 ± 16	83 ± 23	85 ± 21	87 ± 20	80 ± 23	75 ± 24	80 ± 23
	6% HSA	91 ± 12	89 ± 16	87 ± 15	88 ± 19	90 ± 12	85 ± 19	82 ± 18
Arterial SBEc (mmol/L)	Control	-0.4 ± 3.1	0.8 ± 2.7	-2.0 ± 7.4	-1.2 ± 5.1	-0.4 ± 5.4	-1.4 ± 4.8	1.5 ± 4.7
	HBOC-201	1.2 ± 3.0	1.7 ± 2.3	2.9 ± 2.7 ^a	2.6 ± 2.5	3.5 ± 2.7 ^a	3.4 ± 3.4	5.1 ± 3.1 ^a
	6% HSA	1.5 ± 3.0	1.2 ± 3.1	2.2 ± 2.7	1.6 ± 2.3	2.3 ± 2.2	2.2 ± 1.9	3.9 ± 1.8 ^a
	Control	24 ± 3	25 ± 2	23 ± 6	24 ± 4	24 ± 5	24 ± 4	26 ± 4
Arterial HCO ₃ (mmol/L)	HBOC-201	25 ± 3	26 ± 2	27 ± 3 ^a	27 ± 2	27 ± 2 ^a	27 ± 3	29 ± 3 ^a
	6% HSA	26 ± 3	26 ± 3	27 ± 2	26 ± 2	26 ± 2	26 ± 2	28 ± 2 ^a
	Control	95 ± 5	95 ± 1	96 ± 3	96 ± 4	94 ± 5	95 ± 4	94 ± 6
	HBOC-201	94 ± 6	89 ± 9 ^{a b}	90 ± 6	86 ± 7 ^{a b}	83 ± 8 ^{a b}	75 ± 13 ^{a b c}	78 ± 12 ^{a b c}
Venous Saturated O ₂ (%)	6% HSA	96 ± 4	96 ± 4 ^b	95 ± 7	96 ± 4 ^b	97 ± 2 ^b	96 ± 8 ^b	95 ± 6 ^b
	Control	70 ± 14	66 ± 15	64 ± 16	63 ± 16	63 ± 18	66 ± 19	60 ± 17
	HBOC-201	67 ± 13	57 ± 18 ^a	54 ± 15 ^a	46 ± 19 ^a	43 ± 19 ^a	38 ± 18 ^{a c b}	34 ± 18 ^{a c}
	6% HSA	68 ± 15	66 ± 14	64 ± 14	58 ± 18 ^a	56 ± 18 ^a	56 ± 17 ^{a b}	44 ± 17 ^a
Venous pH	Control	7.42 ± 0.05	7.30 ± 0.19	7.37 ± 0.12	7.39 ± 0.09	7.39 ± 0.09	7.37 ± 0.13	7.44 ± 0.05
	HBOC-201	7.40 ± 0.05	7.41 ± 0.05	7.43 ± 0.06	7.41 ± 0.07	7.42 ± 0.08	7.41 ± 0.09	7.44 ± 0.07 ^a
	6% HSA	7.44 ± 0.06	7.44 ± 0.06 ^c	7.44 ± 0.06	7.43 ± 0.06	7.45 ± 0.06	7.45 ± 0.05	7.47 ± 0.05
	Control	42 ± 4	49 ± 13	42 ± 3	42 ± 3	41 ± 3	42 ± 6	40 ± 6
Venous PCO ₂ (mmHg)	HBOC-201	46 ± 6 ^b	45 ± 6 ^b	44 ± 6	45 ± 7	47 ± 9 ^b	45 ± 7 ^b	44 ± 9
	6% HSA	41 ± 5 ^b	40 ± 5 ^b	42 ± 5	42 ± 5	40 ± 5 ^b	40 ± 5 ^b	40 ± 5
	Control	46 ± 9	45 ± 5	43 ± 8	43 ± 67	42 ± 8	43 ± 9	44 ± 12
	HBOC-201	46 ± 7	40 ± 8	38 ± 6	34 ± 9	33 ± 7	31 ± 10	28 ± 8
Venous PO ₂ (mmHg)	6% HSA	45 ± 9	43 ± 8	42 ± 8	40 ± 8	38 ± 7	37 ± 8	32 ± 6

Time point	Treatment	Baseline	20 Min Post Exchange 1	Pre-Exchange 2	20 Min Post Exchange 2	Pre-Exchange 3	20 Min Post Exchange 3	2 Hr Post Exchange 3
Variable		Minute -5	Minute 41	Minute 76	Minute 143	Minute 178	Minute 245	Minute 345
Venous SBEc (mmol/L)	Control	2.5 ± 1.9	-2.6 ± 8.1	-0.8 ± 7.1	0.7 ± 5.7	0.4 ± 6.0	-0.4 ± 6.8	2.9 ± 4.9
	HBOC-201	2.5 ± 2.9	3.5 ± 2.3 ^c	4.5 ± 2.3 ^{ac}	3.7 ± 2.2	4.7 ± 3.1	3.8 ± 3.7	6.1 ± 2.3 ^a
	6% HSA	3.2 ± 2.9	3.4 ± 2.4 ^c	3.2 ± 2.8	3.0 ± 2.2	3.9 ± 2.5	3.7 ± 1.9	5.4 ± 1.6 ^a
Venous HCO ₃ (mmol/L)	Control	26 ± 2	22 ± 7	23 ± 6	25 ± 5	24 ± 5	24 ± 6	27 ± 4
	HBOC-201	26 ± 4	26 ± 2	27 ± 2 ^c	27 ± 2	27 ± 3	27 ± 4	28 ± 2 ^a
	6% HSA	27 ± 3	27 ± 2 ^c	27 ± 2	27 ± 2	27 ± 2	28 ± 2	29 ± 2
Lactate (mmol/L)	Control	2.4 ± 1.2	2.5 ± 1.0	2.3 ± 0.9	1.8 ± 0.9	1.8 ± 0.8	1.8 ± 0.6	1.3 ± 0.4
	HBOC-201	3.0 ± 1.6	3.1 ± 1.2	2.8 ± 1.1	2.9 ± 1.0	2.6 ± 0.9	2.8 ± 1.5	2.1 ± 1.2
	6% HSA	3.5 ± 1.5	3.6 ± 1.3	3.5 ± 1.1	3.6 ± 1.3	3.3 ± 1.3	3.4 ± 1.5	2.6 ± 1.4

^a Statistically significant difference within group from Baseline

^b Statistically significant difference between treatment groups

^c Statistically significant difference between treatment group and control group

Table 2
Hematologic summary of all groups combined with statistical significance ($P \leq 0.05$); Average ($\pm$ Standard Deviation)

Time point	Treatment	Baseline	20 Min Post Exchange 1	Pre-Exchange 2	20 Min Post Exchange 2	Pre-Exchange 3	20 Min Post Exchange 3	2 Hr Post Exchange 3
Variable		Minute -5	Minute 41	Minute 76	Minute 143	Minute 178	Minute 245	Minute 345
Hct (%)	HBOC-201	31 $\pm$ 6	28 $\pm$ 4 ^{a c}	28 $\pm$ 4 ^{a c}	22 $\pm$ 7 ^{a c}	21 $\pm$ 7 ^{a c}	16 $\pm$ 4 ^{a c}	16 $\pm$ 4 ^{a c}
	6% HSA	31 $\pm$ 4	28 $\pm$ 4 ^a	28 $\pm$ 3 ^{a c}	22 $\pm$ 3 ^{a c}	22 $\pm$ 3 ^{a c}	17 $\pm$ 2 ^{a c}	16 $\pm$ 3 ^{a c}
	Control	32 $\pm$ 3	36 $\pm$ 2	37 $\pm$ 3	33 $\pm$ 5	33 $\pm$ 5	32 $\pm$ 4	33 $\pm$ 3
Total Protein (g/dL)	HBOC-201	4.5 $\pm$ 0.4	5.4 $\pm$ 0.4 ^{a b}	5.4 $\pm$ 0.4 ^{a b c}	6.6 $\pm$ 0.3 ^{a b c}	6.6 $\pm$ 0.3 ^{a b c}	7.4 $\pm$ 0.6 ^{a b c}	7.2 $\pm$ 0.5 ^{a b c}
	6% HSA	4.5 $\pm$ 0.3	4.4 $\pm$ 0.3 ^b	4.3 $\pm$ 0.2 ^{a b}	4.2 $\pm$ 0.3 ^{a b}	4.2 $\pm$ 0.2 ^{a b}	4.0 $\pm$ 0.1 ^{a b c}	4.0 $\pm$ 0.2 ^{a b c}
	Control	4.5 $\pm$ 0.4	4.5 $\pm$ 0.3	4.5 $\pm$ 0.0	4.5 $\pm$ 0.1	4.3 $\pm$ 0.2	4.2 $\pm$ 0.2	4.3 $\pm$ 0.2
Total Blood Hb (g/dL)	HBOC-201	9.7 $\pm$ 0.9	10.4 $\pm$ 1.3 ^b	9.9 $\pm$ 1.1	10.1 $\pm$ 1.9 ^b	9.8 $\pm$ 1.4 ^b	9.9 $\pm$ 1.8 ^b	9.9 $\pm$ 2.3 ^b
	6% HSA	9.7 $\pm$ 0.9	8.7 $\pm$ 1.0 ^{a b}	8.6 $\pm$ 0.6 ^a	6.6 $\pm$ 0.5 ^{a b c}	6.7 $\pm$ 0.7 ^{a b c}	5.2 $\pm$ 0.6 ^{a b c}	5.3 $\pm$ 0.8 ^{a b c}
	Control	9.8 $\pm$ 1.2	9.6 $\pm$ 2.2	9.4 $\pm$ 2.5	9.6 $\pm$ 2.3	9.7 $\pm$ 2.1	9.9 $\pm$ 1.6	10.4 $\pm$ 1.2
RBC Hb (g/dL)	HBOC-201	9.7 $\pm$ 0.9	8.8 $\pm$ 1.3	8.6 $\pm$ 1.1 ^a	6.6 $\pm$ 2.0 ^a	6.4 $\pm$ 1.5 ^{a c}	5.1 $\pm$ 1.7 ^{a c}	5.4 $\pm$ 2.4 ^{a c}
	6% HSA	9.6 $\pm$ 1.0	8.7 $\pm$ 1.0 ^a	8.6 $\pm$ 0.6 ^a	6.6 $\pm$ 0.5 ^{a c}	6.7 $\pm$ 0.7 ^{a c}	5.2 $\pm$ 0.6 ^{a c}	5.3 $\pm$ 0.8 ^{a c}
	Control	9.7 $\pm$ 1.2	9.6 $\pm$ 2.2	9.3 $\pm$ 2.5	9.5 $\pm$ 2.3	9.7 $\pm$ 2.1	9.8 $\pm$ 1.6	10.4 $\pm$ 1.2
Plasma Hb (g/dL)	HBOC-201	0.1 $\pm$ 0.1	1.6 $\pm$ 0.7 ^{a b c}	1.3 $\pm$ 0.2 ^{a b c}	3.5 $\pm$ 0.5 ^{a b c}	3.4 $\pm$ 0.4 ^{a b c}	4.9 $\pm$ 0.5 ^{a b c}	4.5 $\pm$ 0.6 ^{a b c}
	6% HSA	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0 ^b	0.0 $\pm$ 0.0 ^b	0.0 $\pm$ 0.0 ^{b c}	0.0 $\pm$ 0.0 ^b	0.0 $\pm$ 0.0 ^b	0.0 $\pm$ 0.0 ^b
	Control	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.02	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MetHb (%)	HBOC-201	0.6 $\pm$ 0.3	1.0 $\pm$ 0.3 ^{a b c}	1.3 $\pm$ 0.6 ^{a b}	2.1 $\pm$ 0.8 ^{a b c}	2.5 $\pm$ 1.1 ^{a b c}	3.2 $\pm$ 0.8 ^{a b c}	3.6 $\pm$ 1.1 ^{a b c}
	6% HSA	0.6 $\pm$ 0.3	0.6 $\pm$ 0.3 ^b	0.6 $\pm$ 0.3 ^b	0.6 $\pm$ 0.2 ^b	0.6 $\pm$ 0.2 ^b	0.8 $\pm$ 0.6 ^b	0.7 $\pm$ 0.5 ^b
	Control	0.5 $\pm$ 0.2	0.5 $\pm$ 0.3	0.5 $\pm$ 0.2	0.6 $\pm$ 0.2	0.4 $\pm$ 0.2	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1

^a Statistically significant difference within group from Baseline

^b Statistically significant difference between treatment groups

^c Statistically significant difference between treatment group and control group

Table 3

Tissue oxygenation (tPO₂) with statistical significance ($P \leq 0.05$); Average ($\pm$ Standard Deviation).

Time point		Baseline	20 Min Post Exchange 1	Pre-Exchange 2	20 Min Post Exchange 2	Pre-Exchange 3	20 Min Post Exchange 3	2 Hr Post Exchange 3
Variable	Treatment	Minute -5	Minute 41	Minute 76	Minute 143	Minute 178	Minute 245	Minute 345
Heart Group tPO ₂ (mmHg)	HBOC-201	28 $\pm$ 4	37 $\pm$ 10	45 $\pm$ 10 ^a	37 $\pm$ 14	37 $\pm$ 16	28 $\pm$ 8	31 $\pm$ 11
	6% HSA	25 $\pm$ 13	22 $\pm$ 18	31 $\pm$ 13	36 $\pm$ 16	27 $\pm$ 9	24 $\pm$ 8	24 $\pm$ 8
Brain Group tPO ₂ (mmHg)	HBOC-201	9 $\pm$ 2	8 $\pm$ 3	9 $\pm$ 3	9 $\pm$ 3	8 $\pm$ 4	8 $\pm$ 4	8 $\pm$ 4
	6% HSA	8 $\pm$ 4	8 $\pm$ 5	9 $\pm$ 4	10 $\pm$ 4	10 $\pm$ 4	9 $\pm$ 4	9 $\pm$ 4
Kidney Group tPO ₂ (mmHg)	HBOC-201	12 $\pm$ 6	10 $\pm$ 5	11 $\pm$ 5	11 $\pm$ 6	12 $\pm$ 8	11 $\pm$ 7	10 $\pm$ 8
	6% HSA	11 $\pm$ 5	12 $\pm$ 7	12 $\pm$ 6	13 $\pm$ 8	12 $\pm$ 8	11 $\pm$ 8	10 $\pm$ 7

^a Statistically significant difference within group from Baseline