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Glutaraldehyde-polymerized bovine hemoglobin and phosphodiesterase-5 inhibition

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Abstract

Objective—Hemoglobin-based oxygen carriers (HBOC) of several types scavenge nitric oxide from the vasculature resulting in vasoconstriction and hypertension, both systemic and pulmonary. Phosphodiesterase-5 (PDE5) inhibitors promote nitric oxide activity and enhance vasodilation. The purpose of this study was to determine whether combined therapy of glutaraldehyde-polymerized bovine hemoglobin (HBOC) with a PDE5 inhibitor would counter the negative hemodynamic consequences of HBOC therapy alone, resulting in improved hemodynamics and oxygen delivery.

Design—A controlled, experimental study.

Setting—A research laboratory at a university.

Subjects—Conscious male Sprague-Dawley rats.

Interventions—Glutaraldehyde-polymerized bovine hemoglobin (HBOC), sildenafil (PDE5 inhibitor), and lactated Ringer's solution (control).

Measurements and Main Results—Infusion of the HBOC resulted in significant ($p < 0.05$) systemic and pulmonary vasoconstriction, with reduced cardiac output and reduced oxygen delivery to the periphery. Infusion of lactated Ringer's demonstrated no changes in the measured variables. Infusion of sildenafil alone reduced systemic and pulmonary artery blood pressure, while maintaining cardiac output and oxygen delivery. Combined HBOC and sildenafil infusion resulted in stable systemic blood pressure, cardiac output, and oxygen delivery. However, the addition of sildenafil to HBOC did not fully ameliorate the pulmonary vasoconstriction caused by HBOC.

Conclusion—The HBOC used in this study resulted in pulmonary and systemic hypertension, reduced cardiac output, and oxygen delivery. These negative consequences of HBOC treatment can be largely overcome by combining HBOC treatment with a PDE5 inhibitor (sildenafil). Thus, these data support the continued investigation of combined HBOC and PDE5 inhibitor treatment in circumstances in which HBOC therapy is being considered.

Keywords

hemoglobin-based oxygen carrier; hemodynamics; oxygen delivery; phosphodiesterase-5 inhibitors; sildenafil

Addition of cell-free hemoglobin-based oxygen carriers (HBOCs) to blood has the potential to enhance oxygen delivery to tissues during conditions of ischemia and/or hypoxia by increasing blood oxygen carrying capacity (1–3). Consequently, HBOCs have therapeutic potential for a number of clinical conditions, including hemorrhagic shock; and may offer potential supportive therapeutics during chemotherapy and radiation, among others (4–6). However, HBOCs have not reached their full therapeutic potential, in part, due to negative hemodynamic effects (3, 4, 7–9).

Although there are several biochemical versions of HBOCs (3, 8), most polymerized HBOCs have generally been shown to produce systemic and pulmonary vasoconstriction in humans and animals (4, 10–12). Although several potential mechanisms may explain the vasoconstrictive properties of these HBOCs (10, 13, 14), nitric oxide (NO) scavenging by the HBOCs is the most likely (1, 4, 15–17). Recently, this laboratory has confirmed that glutaraldehyde-polymerized bovine HBOC infusion in animals had dire consequences on hemodynamic performance in both normoxic and hypoxic rats (18). In this study, conscious rats responded to HBOC infusion with systemic and pulmonary vasoconstriction, elevated blood pressure, marked decreases in cardiac output, and subsequent reduction in oxygen delivery during both normoxic and hypoxic conditions (18). The results of N (G)-nitro-L-arginine methyl ester (LNAME, a NO blocker) in these animals strongly indicated that these hemodynamic effects were related to NO scavenging by this HBOC. Specifically, LNAME infusion in rats pretreated with HBOC demonstrated no significant further change in vasoconstriction; whereas LNAME infusion in control animals resulted in a marked vasoconstriction. Thus, it is suggested that either enhancing the action of available NO or decreasing NO scavenging by the HBOC should ameliorate the negative hemodynamic consequences of HBOC treatment.

Recently, Yu et al (4) demonstrated that pretreatment of animals with NO inhalation moderated the vasoconstrictive and hemodynamic effects of glutaraldehyde-polymerized bovine HBOC infusion. These results suggest a potential for combined therapy of exogenous NO with HBOC administration. However, inhaled NO requires a significant duration of pretreatment (1 hour) and its effects are transitory (4), unless NO inhalation is continued. Furthermore, this treatment requires a continuous abundance of an exogenous NO source. Thus, a mode of increasing endogenous NO concentration or enhancement of endogenous NO vasoactivity that would produce an effect of clinically significant duration would be of interest.

Phosphodiesterase-5 (PDE5) inhibitors, such as sildenafil, vardenafil, and tadalafil act to inhibit PDE5 and increase the action of available endogenous NO. Inhibition of PDE5 results in accumulation and increased activity of cyclic guanosine monophosphate that enhances the vasodilator consequence of existing endogenous levels of NO (19). Interestingly, PDE5 inhibition has been shown clinically and experimentally to reduce pulmonary hypertension via vasodilation (20–23), which is of importance to this issue. Figure 1 illustrates the pathway by which sildenafil provides enhanced activity of available NO. In the same figure, the conceptual interaction of HBOC and NO is demonstrated. The actual and potential chemical interactions of cell-free hemoglobin and NO are numerous and not the subject of this investigation (7, 8, 24, 25).

Glutaraldehyde-polymerized bovine HBOC is of particular interest because bovine blood offers a ready source of large volumes of blood for preparation of HBOC for clinical use. Furthermore, glutaraldehyde-polymerized bovine HBOC has gained approval for use in both veterinary and human medicine in South Africa; has veterinary approval in the United States; and is in human clinical trials in the United States (3). Thus, it was the purpose of this study to examine the potential benefit of combining sildenafil (PDE5 inhibitor) with glutaraldehyde-polymerized bovine HBOC infusion into conscious rats to determine the potential hemodynamic improvements with combined therapy. It was hypothesized that sildenafil would counter both the systemic and pulmonary vasoconstrictor effects of HBOC infusion, improving cardiac output and oxygen delivery in conscious rats.

METHODS

Animals

Male Sprague-Dawley rats (Charles River, 280–350 g and 10–12 weeks of age) were housed in the University of Colorado Denver Health Sciences Center's Center for Laboratory Animal Care (elevation = 5280 ft). Animals were permitted *ad libitum* access to food and water and kept on a 12-hour day–night cycle. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at University of Colorado Denver Health Sciences Center.

Instrumentation

Rats were acclimated to Denver altitude (5280 ft; 1609 m; $P_B \sim 630$ mm Hg) for a minimum of 7 days before experimental instrumentation. Forty-eight hours before the surgery, animals were provided water supplemented with acetaminophen or codeine (0.5 mg/mL and 0.05 mg/mL, respectively) for postoperative analgesia. The animals were weighed. Rats were anesthetized with a mixture of ketamine:Rompon (xylazine) (75 mg/kg:6 mg/kg intraperitoneal). Under sterile conditions, the left carotid artery was cannulated with a PE-50 (0.58 mm internal diameter [ID], Becton Dickinson, Sparks, MD) catheter. A polyvinyl-1 (0.28 mm ID, Becton Dickinson) catheter with a shallow bend at its tip was inserted into the right ventricle via the right jugular vein and guided into the main pulmonary artery. Pressure recordings confirmed placement in the pulmonary artery. Next, two PE-50 (0.58 mm ID, Becton Dickinson) catheters were placed in the superior vena cava via the right jugular vein for venous blood collection and to determine cardiac output. All catheters were flushed with heparinized saline, tied off, tunneled subcutaneously to the dorsal neck region, and exteriorized at the back of the neck. Animals were allowed at least 48 hours to recover before any treatments. None of the animals demonstrated signs of infection, diarrhea, or distress, so none were excluded from study.

Experimental Design

Animals were placed in one of the four groups: lactated Ringer's solution (Henry Schein, Melville, NY) (control, $n = 10$); glutaraldehyde-polymerized bovine hemoglobin (Oxyglobin, Biopure, Cambridge, MA) (HBOC, $n = 10$); sildenafil (Pfizer, New York, NY) in lactated Ringer's solution (S, $n = 5$); and sildenafil plus polymerized-bovine hemoglobin (S-HBOC, $n = 7$). Control and HBOC groups were completed initially, and then the S and S-HBOC groups were completed. After collecting the baseline measurements, animals were treated with Ringer's solution, bovine-polymerized HBOC, sildenafil, or sildenafil plus bovine-polymerized HBOC. Two hours post-treatment, the final measurements were made.

Hemodynamic Measurements

Rats were placed in a custom-designed small, rectangular, Plexiglas chamber with a portal through which catheters could be passed. Animals were not restrained and had the ability to adjust their position. Catheters were flushed with heparinized saline and then connected to fluid-filled pressure transducers. Then, blood pressures, pulmonary artery blood pressures, heart rates, and cardiac outputs were collected before treatment. All animals then underwent a 30-minute infusion of Ringer's (3 mL/kg), HBOC (3 mL/kg, 13 g/dL, 1.3 g/kg), sildenafil (0.5 mg/mL/hr), or sildenafil plus HBOC (same doses for each as before) through a venous catheter. The volume of 3 mL/kg was chosen to reduce volume overloading although permitting the study of the hemodynamic effects of HBOC, as seen in our previous study (18). Note that sildenafil was infused throughout the 2 hours by adding a volume of 300 μ L. Two hours after infusion, hemodynamic variables were measured along with blood gas variables. The 2-hour postinfusion time point was determined from a previous study in this laboratory that identified HBOC treatment that sustained hemodynamic changes up to 4-hour postinfusion (18). Cardiac output was measured using Cardiogreen (Sigma Aldrich, St. Louis, MO) with the dye-dilution method. During the experimental period, the rats were monitored for any signs of distress, but otherwise left undisturbed. At the conclusion of the experiment, animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg) via a jugular catheter.

Blood Gas and Co-Oximetry Measurements

Blood samples were collected immediately after hemodynamic measurements had been obtained. Arterial (0.2 mL) blood was withdrawn via the carotid catheter into a blood gas syringe and analyzed (ABL5, Radiometer, Copenhagen and co-oximetry via OSM3, Radiometer, Copenhagen) with algorithms specific to rat and bovine hemoglobin. Plasma phase co-oximetry was used for determining hemoglobin in plasma introduced with HBOC treatment. Heparinized microhematocrit capillary tubes (100 μ L; Fisherbrand, Pittsburgh, PA) were immediately filled from each 1-mL blood gas syringe (see above), sealed from room air with a removable cap (Kendall Healthcare, St. Louis, MO), and spun on a capillary centrifuge to separate cell and plasma fractions. The plasma portion of the capillary tube was aspirated into the co-oximeter. Measurements were excluded if any portion of the cell fraction was inadvertently aspirated into the analyzer. Care was taken to avoid exposing blood samples to room air.

Calculation for Oxygen Delivery

Oxygen delivery (DO_2) was calculated for total hemoglobin in whole blood that included HBOC in the plasma phase) was calculated as:

$$\text{DO}_2 = (\text{CaO}_2) \times \text{CI},$$

where $\text{CaO}_2^* = (\text{HbO}_2\% / 100) \times \text{tHb} \times *$ and $\text{HbO}_2^* = \text{HbO}_2\text{SAT}(100 - \text{HbCO} - \text{MetHb})$ fraction, oxygen capacity for rat or bovine hemoglobin. CI is cardiac index and CaO_2 is arterial oxygen content. *OSM3 programmed algorithms.

Statistics

For all groups, means $\pm$ SEM are reported. Statistical comparison among groups was initially analyzed with two factors (treatment and time) with repeated-measures (time) analysis of variance. If this analysis revealed differences among groups for baseline values, an analysis of covariance was performed and adjusted means for the 2-hour point were generated and evaluated. The Tukey-Kramer multiple-comparison test was used to locate

differences between group means. Tables show the actual means and SEM, plus the analysis of covariance adjusted means where appropriate. Percent changes from baseline were calculated for key variables, and a single-factor analysis of variance with a Tukey-Kramer multiple-comparison test was used to determine the statistical differences among groups. Statistical analyses were performed using NCSS 2000 statistical software package (NCSS, Kaysville, UT), with statistical significance set at $p = 0.05$.

RESULTS

Blood pressure rose significantly from baseline with HBOC infusion, decreased with sildenafil, and did not change when sildenafil and HBOC were combined (Table 1). The rise in blood pressure with HBOC was due to the significant increase in peripheral resistance (Table 1). Peripheral resistance was unchanged in the other groups. These effects are shown in Figure 2, in which the percent change from baseline for each group is presented.

Pulmonary artery pressure also rose significantly with HBOC infusion, although the increase was small; and pulmonary pressure declined significantly, but a small amount, with sildenafil (Table 2). Combining sildenafil and HBOC resulted in no change in pulmonary pressure. Similar to the changes in peripheral resistance, HBOC resulted in elevation in pulmonary vascular resistance (Table 2). Sildenafil in combination with HBOC did not fully prevent the rise in pulmonary vascular resistance, as pulmonary vascular resistance did increase with combined treatment. These changes are highlighted in Figure 3, in which the percent changes from baseline are shown.

Cardiac index fell markedly with HBOC infusion (Table 1). This fall was secondary to significant decreases in both heart rate and stroke volume (Table 1). Sildenafil alone had no significant effect on cardiac index, heart rate, or stroke volume. But when infused with HBOC, sildenafil partially prevented the decline in cardiac output seen with HBOC alone. These effects on cardiac index are shown in Figure 4, in which the percent change is plotted.

The delivery of oxygen was compromised by HBOC infusion (Table 3). Sildenafil had no significant effect on oxygen delivery, and it partially offset the decrease seen with HBOC when infused in combination with HBOC. These effects are demonstrated in Figure 4, in which the percent changes are graphed. Hemoglobin concentration was not changed significantly by HBOC, S-HBOC, or S infusion, and was reduced with Ringer's infusion in control. Oxygen content was reduced in control and increased slightly in S-HBOC.

DISCUSSION

The main finding of this study was that the observed increases in pulmonary and systemic vasoconstriction associated with glutaraldehyde-polymerized bovine HBOC infusion may be partially prevented by coadministration of sildenafil, a PDE-5 inhibitor, and therefore reduce the associated decline in cardiac output and oxygen delivery. These data are novel and add support to the concept that glutaraldehyde-polymerized bovine HBOC likely scavenges NO resulting in vasoconstriction; thereby, elevating blood pressure, pulmonary pressure, and reducing cardiac output. Sildenafil, by enhancing the vascular effects of endogenous NO, appears capable of limiting the hemodynamic functional changes associated with glutaraldehyde-polymerized bovine HBOC. Notably, at least at the dose used in this study, sildenafil was less effective in countering the impact of glutaraldehyde-polymerized bovine HBOC on the pulmonary pressure, compared with the systemic pressure. However, sildenafil did reduce the impact of glutaraldehyde-polymerized bovine HBOC on pulmonary vascular resistance.

These results verify the study by Yu et al (4) in which inhaled NO reduced the vasoconstrictive effects of glutaraldehyde-polymerized bovine HBOC infusion, And the current results extend the study by Yu et al (4) to include enhanced NO function by PDE5 inhibition as an effective cotreatment with glutaraldehyde-polymerized bovine HBOC infusion to offset both systemic and pulmonary hemodynamic effects. Thus, the results of this study indicate that enhancing the activity of endogenous NO offers a credible alternative to administering exogenous NO to counter the hemodynamic consequences of glutaraldehyde-polymerized bovine HBOC infusion. This study evaluated only a single dosage of sildenafil, a dose-effect study to determine the magnitude of an optimal sildenafil dose would be of interest.

This study supports those of others that have reported systemic and pulmonary vasoconstriction with polymerized HBOC infusion (12, 17, 26). Although reduction in cardiac output, with polymerized HBOC infusion, has been reported (12), this study indicates that with the glutaraldehyde-polymerized bovine HBOC at the dose used, and in this animal model the reduction in cardiac output can be marked. As a consequence, oxygen delivery is impaired in this circumstance. It should be noted that not all polymerized HBOC versions cause vasoconstriction. Tsai et al (27) have demonstrated that a polyethylene glycol-decorated HBOC did not cause vasoconstriction despite NO binding similar to bovine-polymerized HBOC and -polymerized HBOC, each of which caused vasoconstriction. They speculated that the polyethylene glycol-decorated HBOC had a higher oncotic pressure than the other HBOCs and increased blood volume, thereby, increasing microvascular blood flow. Notably, cardiac index did not fall with polyethylene glycol-decorated HBOC treatment. However, the mechanisms remain undetermined, but of interest.

For the pulmonary circulation, sildenafil did moderate the vasoconstriction caused by HBOC infusion, but pulmonary pressure still rose. It must be noted that the rise in pulmonary pressure induced by HBOC in this study was small and likely not clinically relevant, but must still be considered when using HBOC infusion. The increase in pulmonary pressure, with combined sildenafil and HBOC infusion pressure, is likely the consequence of the moderate increase in pulmonary vascular resistance along with the fact that cardiac output was maintained with combined therapy. In contrast, with only HBOC infusion, the pulmonary pressure increased despite reduced cardiac output, reflecting the marked elevation in pulmonary vascular resistance associated with the HBOC infusion.

It should be noted that the current model was that of adding to vascular volume with HBOC in a euvoletic animal. In contrast, in most other research studies, HBOC has been infused within the setting of low volume and/or low blood pressure (28–31). However, in models of hemorrhagic shock, various versions of HBOC, including polymerized bovine HBOC, infused for resuscitation have also demonstrated vasoconstriction and lowered cardiac output (32). Interestingly, in at least one study using a swine model of hemorrhagic shock, clinical outcome was improved, but the improved outcome was associated with evidence of clinically relevant adverse histologic changes in several organs, including heart, lung, liver, and kidney (32). However, it would be unfair to imply that HBOC infusion is always associated with reduced cardiac output or does not improve survival in a variety of hemorrhagic and other models of trauma, as there are many examples of investigations supporting the use of HBOC infusion in models of trauma (11, 28, 29, 33–35). According to this study, it would be important to know the hemodynamic, clinical outcome, and tissue histologic effects of the combination of PDE5 inhibition with HBOC used for low volume/low pressure resuscitation.

Sildenafil is one example of a PDE5 inhibitor. Vardenafil and tadalafil are other commercial examples. Of particular interest would be the use of tadalafil, because it has a longer-lasting NO effect (24); so perhaps it could be used for less frequent dosing. Sildenafil was effective in this study despite the likely reduction of NO by glutaraldehyde-polymerized bovine HBOC. This would suggest that NO scavenging by the glutaraldehyde-polymerized bovine HBOC, although significant, is incomplete. Thus, significant NO concentrations are still available to permit the enhancement of the NO-vasodilation pathway by PDE5 inhibition.

These results have clinical importance in settings other than hypovolemic shock. In particular, if glutaraldehyde-polymerized bovine HBOC infusion is being considered to enhance oxygen delivery and perfusion of tissue *per se*, then potential vasoconstriction by glutaraldehyde-polymerized bovine HBOC may be a contraindication. For example, the use of HBOC infusion to enhance oxygen delivery has been suggested for a wide variety of potential therapies. These instances include supportive therapy during radiation and chemotherapy (4, 5); management of pedicle flaps in cosmetic or restorative surgery (6); and reducing infarct volume as a result of focal cerebral ischemia (36). In these clinical settings, vasoconstriction by HBOC has the potential of reducing blood flow and oxygen delivery to the target tissue. The combination therapy with sildenafil (or other PDE5 inhibitor) may improve blood flow and permit the enhancement of oxygen delivery to these tissues via the increased oxygen-carrying capacity of added HBOC.

CONCLUSION

In conclusion, this study has provided novel data suggesting that glutaraldehyde-polymerized bovine HBOC administration may be clinically enhanced by combining with a PDE5 inhibitor to counter the NO scavenging impact of glutaraldehyde-polymerized bovine HBOC. In this study, sildenafil was highly effective in restoring hemodynamics and oxygen delivery during glutaraldehyde-polymerized bovine HBOC infusions. Future studies in other animal models, and under other conditions such as low volume/pressure, or hypoxia, would be of interest in determining the extended value of combined PDE5 inhibitor and glutaraldehyde-polymerized bovine HBOC therapy.

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Figure 1.

Conceptual presentation of potential enhancements of nitric oxide (*NO*) vasodilation by sildenafil during scavenging of *NO* by hemoglobin-based oxygen carriers (*HBOC*). *GMP*, guanosine monophosphate; *PDE-5*, phosphodiesterase-5; *cGMP*, cyclic guanosine monophosphate; *GTP*, guanosine triphosphate.

Figure 2.

Percent change in mean arterial blood pressure and total peripheral resistance in experimental groups. *A* and *B*, For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hours). *HBOC*, hemoglobin-based oxygen carriers; *S-HBOC*, sildenafil plus polymerized bovine hemoglobin; *S*, sildenafil in lactated Ringer's solution.

Figure 3. Percent change in mean pulmonary artery pressure and pulmonary vascular resistance in experimental groups. *A* and *B*, For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hours). *HBOC*, hemoglobin-based oxygen carriers; *S-HBOC*, sildenafil plus polymerized bovine hemoglobin; *S*, sildenafil in lactated Ringer's solution.

Figure 4.

Percent change in cardiac index and oxygen delivery in experimental groups. *A* and *B*, For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hours). *HBOC*, hemoglobin-based oxygen carriers; *S-HBOC*, sildenafil plus polymerized bovine hemoglobin; *S*, sildenafil in lactated Ringer's solution.

Table 1

Systemic hemodynamic response to HBOC and sildenafil infusions

	Baseline	2 Hours	Adjusted Means ANCOVA
Mean Arterial Pressure			
Control	12134 ^a	12433 ^a	
HBOC	12134 ^a	14435 ^{b,d}	
S	12332 ^a	11035 ^{c,d}	
S-HBOC	11935 ^a	12136 ^{a,c}	
Total Peripheral Resistance			
Control	139314 ^a	209323	219 ^a
HBOC	147312 ^a	359337	363 ^{b,d}
S	172315 ^{a,b}	180315	169 ^a
S-HBOC	180328 ^b	263340	248 ^{a,b}
Cardiac Index			
Control	214317 ^a	180329	177 ^a
HBOC	224318 ^a	10839	104 ^{b*}
S	171313 ^b	144310	151 ^a
S-HBOC	178333 ^b	119322	124 ^a
Heart Rate			
Control	41039 ^a	368320	368 ^a
HBOC	394318 ^a	314314	313 ^{a,d}
S	454316 ^b	438317	441 ^b
S-HBOC	403321 ^a	346311	345 ^a
Stroke Volume Index			
Control	0.5630.04 ^a	0.5430.06	0.54 ^a
HBOC	0.5530.03 ^a	0.3530.03	0.35 ^{b,d}
S	0.3830.03 ^b	0.3330.03	0.32 ^b
S-HBOC	0.4630.11 ^b	0.3230.06	0.31 ^b

HBOC, hemoglobin-based oxygen carriers; ANCOVA, analysis of covariance; S-HBOC, sildenafil plus polymerized bovine hemoglobin; S, sildenafil in lactated Ringer's solution.

^{a,b,c} For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hr). Units: mean arterial pressure (mm Hg), total peripheral resistance ($[\text{dyne} \cdot \text{sec} \cdot \text{cm}^{-5}] \cdot 10^3$), cardiac index ($\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), heart rate ($\text{beats} \cdot \text{min}^{-1}$), stroke volume index ($\text{ml} \cdot \text{kg}^{-1}$);

^d $p < 0.05$, compared to baseline.

Values are means $\pm$ SEM.

Table 2

Pulmonary hemodynamic response to HBOC and sildenafil infusions

	Baseline	2 Hours	Adjusted Means ANCOVA
Mean Pulmonary Artery Pressure			
Control	2031 ^a	1731	17 ^a
HBOC	2032 ^a	2332	23 ^{b,d}
S	2231 ^b	1731	15 ^{a,d}
S-HBOC	1931 ^a	2133	22 ^b
Pulmonary Vascular Resistance			
Control	22.332.5 ^a	25.832.8	31 ^a
HBOC	29.733.1 ^b	60.537.9	59 ^{b,c,d}
S	30.232.3 ^b	27.432.3	25 ^a
S-HBOC	33.831.3 ^b	51.734.0	46 ^{a,c,d}

HBOC, hemoglobin-based oxygen carriers; ANCOVA, analysis of covariance; S-HBOC, sildenafil plus polymerized bovine hemoglobin; S, sildenafil in lactated Ringer's solution.

^{a,b,c} For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hrs). Units: mean pulmonary artery pressure (mm Hg), pulmonary vascular resistance ([dyne·sec·cm⁵] $\cdot 10^{-3}$);

^d $p < 0.05$, compared to baseline.

Values are means $\pm$ SEM.

Table 3

Oxygen delivery, hemoglobin, and oxygen content with HBOC and sildenafil infusion

	Baseline	2 Hours	Adjusted Means ANCOVA
Oxygen Delivery ($\text{mlO}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)			
Control	3932 ^a	3434 ^a	
HBOC	4034 ^a	2031 ^{b,c}	
S	3032 ^a	2433 ^{a,b}	
S-HBOC	3036 ^a	2034 ^b	
Hemoglobin ($\text{gm} \cdot 100\text{ml}^{-1}$)			
Control	14.531 ^a	12.731	12.2 ^{a,c}
HBOC	13.631 ^b	14.531	14.6 ^b
S	14.131 ^a	12.531	13.5 ^b
S-HBOC	13.031 ^b	14.631	13.8 ^b
Oxygen Content ($\text{mlO}_2 \cdot 100\text{ml}^{-1}$)			
Control	18.431 ^a	15.731	15.1 ^{a,c}
HBOC	17.231 ^a	18.431	18.5 ^b
S	17.631 ^a	16.531	17.1 ^{a,b}
S-HBOC	16.431 ^b	17.431	17.4 ^{a,b,c}

HBOC, hemoglobin-based oxygen carriers; ANCOVA, analysis of covariance; S-HBOC, sildenafil plus polymerized bovine hemoglobin; S, sildenafil in lactated Ringer's solution.

^{a,b} For the group comparisons, same letter designations are not significant; different letter designations represent significant ($p < 0.05$) differences among treatments, within time period (baseline or 2 hrs);

^c $p < 0.05$, compared to baseline.

Values are means $\pm$ SEM.