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A novel hemoglobin-based blood substitute protects against myocardial reperfusion injury

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Caswell, John E., Micah B. Strange, David M. Rimmer III, Michael F. Gibson, Phillip Cole, and David J. Lefer. A novel hemoglobin-based blood substitute protects against myocardial reperfusion injury. *Am J Physiol Heart Circ Physiol* 288: H1794–H1801, 2005; doi:10.1152/ajpheart.00905.2004.—HBOC-201 (Biopure; Cambridge, MA) is a glutaraldehyde-polymerized bovine hemoglobin (Hb) solution that is stroma free, has lower viscosity than blood, and promotes O_2 unloading. We investigated the effects of HBOC-201 in a canine model of myocardial ischemia-reperfusion injury. Dogs were anesthetized and subjected to 90 min of regional myocardial ischemia and 270 min of reperfusion. HBOC-201 or 0.9% saline vehicle equivalent to 10% total blood volume was infused 30 min before myocardial ischemia. Hemodynamic data and peripheral blood samples were taken at baseline, 1 h of myocardial ischemia, and 1, 2, and 4 h of reperfusion. At 270 min of reperfusion, the area at risk (AAR) per left ventricle and the area of infarction (Inf) per AAR were determined. The myocardial AARs in the two study groups were similar. In addition, myocardial blood flow (as measured by radioactive microspheres) in the ischemic zone was similar between the vehicle and HBOC-201 groups. HBOC-201-infused dogs demonstrated a significant ($P < 0.01$) 56% reduction in Inf/AAR. Analysis of blood samples taken at 4 h of reperfusion showed a significant ($P < 0.05$) reduction in creatine kinase MB isoform for the HBOC-201 group. Histological analysis of the myocardium demonstrated significant ($P < 0.01$) reductions in neutrophil infiltration in the HBOC-201 group. These data indicate that treatment with HBOC-201 before myocardial ischemia-reperfusion reduces the extent of myocardial inflammation and ischemia-reperfusion injury in the canine myocardium.

infarction; contractility; neutrophils; ischemia

THERE HAVE BEEN RECENT CONCERNS over the demand and safety of our nation's blood supply. Although the blood supply is safer today than it ever has been, there is concern regarding the risk of viral infection after blood transfusion. Blood has many limitations aside from infection; it has a limited shelf life and must be discarded after 35–42 days, it must be typed and cross-matched before infusion, and it is very expensive to the patient, typically costing $140 to $225 (US dollars; Ref. 3). Bovine hemoglobin (Hb)-based blood substitutes have extended shelf life, do not need to be typed and cross-matched, and do not transmit viral or bacterial infections (4).

HBOC-201 (Biopure; Cambridge, MA) is a glutaraldehyde-polymerized, bovine Hb solution. HBOC-201 has a similar structure to human Hb but has been biochemically altered to enhance O_2 unloading (17). Unlike human Hb, bovine Hb blood substitutes do not require 2,3-diphosphoglycerate to lower the O_2 affinity. The O_2 affinity of HBOC-201 (P_{50}, ~40 mmHg) is regulated by the chloride ion content of human plasma, which is adequate to lower O_2 affinity of the bovine Hb to a satisfactory level for unloading in human tissue. The Bohr effect has also been shown to be more pronounced in bovine Hb, which allows an even better O_2 delivery at lower pH values. It has also been shown that this blood substitute does not change hemodynamics with respect to P_{CO_2} and pH (1, 6, 12, 17, 19, 23). HBOC-201 is modified by cross-linking of the α,β-dimers to form a tetrameric structure that does not dissociate as rapidly as free Hb (14). Similarly, glutaraldehyde polymerization in HBOC-201 results in a prolonged intravascular half-life of polymeric Hb due to reduced clearance of the solution (8).

The Hb in HBOC-201 is stroma free and is not bound to red blood cells; therefore, the Hb may be easily transported in blood plasma to places where red blood cell flow is not capable, which is an exciting characteristic in the possibility of an ischemic event. This characteristic of HBOC-201 along with its heightened ability to unload O_2 into tissue and the easy acquisition and use of the product may allow bovine Hb to be more beneficial in emergency settings than whole blood. It may even prove beneficial in hypoxic settings such as acute coronary artery occlusion, where its unique ability to unload O_2 while traveling in blood plasma may be exploited. The present study was designed to determine whether administration of HBOC-201 before acute coronary artery occlusion could alter the extent of myocardial injury.

MATERIALS AND METHODS

Animal care guidelines. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society of Medical Research and the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center in Shreveport.

HBOC-201. Glutaraldehyde-polymerized bovine Hb in a buffered physiological solution of electrolytes was obtained from Biopure (Cambridge, MA).

Animal preparation. Twenty-two heartworm-free mongrel dogs of either sex that weighed 16.3–27.0 kg (average wt, 20.2 ± 0.6 kg) were fasted overnight. The dogs were randomized and anesthetized with pentobarbital sodium (40 mg/kg iv) and were subsequently endotracheally intubated and ventilated with room air by use of a Harvard

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respirator (model 613; Harvard Apparatus; Boston, MA). Respiratory rate and stroke volume were adjusted to maintain arterial blood gas and pH levels within normal physiological ranges. Anesthesia was maintained by intravenous administration of pentobarbital sodium as needed. A standard lead II ECG was used to continuously monitor heart function. A polyethylene catheter was placed in the right femoral vein for fluid and drug administration. Both femoral arteries were cannulated for collection of hemodynamic data and blood samples. In the right femoral artery, an 8-Fr pigtailed pressure-transducer catheter (MPC-500; Millar; Houston, TX) was advanced to the left ventricle. In the left femoral artery, a 5-Fr pressure-transducer catheter (MPC-350; Millar) was advanced into the abdominal aorta. The chest was opened by a median sternotomy, and the pericardium was incised and tented to cradle the heart. A proximal portion of the left anterior descending artery (LAD) was dissected away from the epicardium to allow a microaneurysm clip to be placed on the vessel to induce complete coronary artery occlusion.

Experimental protocol. Thirty minutes were allowed for stabilization after initial preparation and surgical procedures were complete. Hemodynamic data were obtained at baseline and at 30-min intervals thereafter until the end of the experiment. Blood samples were taken at baseline, 1 h of ischemia, and 1, 2, and 4 h of reperfusion.

On the day of the experiment, the animals were randomly divided into two groups to compare *1*) myocardial ischemia-reperfusion (MI/R) + vehicle and *2*) MI/R + HBOC-201. During the initial surgery, 10% of each animal's total blood volume was removed and replaced with either HBOC-201 or 0.9% saline at a rate of 0.2 $ml \cdot kg^{-1} \cdot min^{-1}$ during the 30-min baseline period. Ten minutes before occlusion of the LAD, a 50-mg bolus of lidocaine (10 ml) was given to minimize ventricular fibrillation; a similar bolus of lidocaine was also given to the animal 10 min before reperfusion. The LAD remained occluded for a period of 90 min and was then reperfused for 4.5 h after removal of the microanuerysm clip.

Hemodynamic data. Hemodynamic data were recorded on a 16-channel MacLab (ADInstruments) system. The hemodynamic and functional data were simultaneously logged onto a Power Macintosh 8500 computer (Apple Computer) using MacLab Chart 3.5 software. The following variables were recorded: heart rate, mean arterial blood pressure, left ventricular (LV) systolic and end-diastolic pressures, and peak positive and negative first-time derivatives of LV pressure (maximum and minimum dP/d*t*, respectively). Lead II of the ECG was used to simultaneously monitor heart rate, arrhythmias, and ST segment alterations.

Regional myocardial blood flow determination. In 10 additional animals, regional myocardial blood flow was measured using sterile 15-μm radiolabeled microspheres (EI du Pont de Nemours) by the reference-withdrawal method. Microspheres were injected into animals at baseline and at 75 min of ischemia. Flow determination was made using one of five available isotopes (^{141}Ce, ^{113}Sn, ^{85}Sr, ^{95}Nb, or ^{46}Sc) in a 0.01% Tween 80 suspension in 10.0% dextran.

Each vial of microspheres was thoroughly mixed by sonication and vortex agitation before injection. Approximately one to two million microspheres were injected via the left atrium (acute MI/R) or directly into the left ventricle via the catheter (extended MI/R) for each flow measurement. The reference arterial samples were obtained from the catheter inserted into the aorta via the femoral artery; withdrawal of samples commenced before the injection of microspheres and continued at a constant rate for 4 min using a peristaltic pump (Rainin Instruments).

Two adjacent transverse slices of the left ventricle that correspond to the central ischemic region were sampled for myocardial blood flow determinations after overlay tracings were completed for quantitation of infarct size. Two transmural sections were obtained from each of the nonischemic and central ischemic regions of each slice. Sections were taken at least 1 cm away from the boundaries between regions demarcated by Evans blue staining. Each transmural wedge of tissue was subdivided into two pieces of approximately equal thickness (endocardial and epicardial); after the location of each piece of tissue was recorded, samples were weighed and placed into counting vials for radioactivity assay in a gamma counter (1282 CompuGamma; LKB Wallac). Corrections for background and overlap were performed using the CompuGamma UltroTerm II system software. Myocardial blood flow was then calculated using the equation $\dot{Q}_m = (C_m \times \dot{Q}_r)/C_r$, where $\dot{Q}_m$ is myocardial blood flow (in ml/min), C_m is the number of counts in the tissue sample (in cpm), $\dot{Q}_r$ is the withdrawal rate of the reference arterial sample (in ml/min), and C_r is the number of counts in the reference arterial sample (in cpm). The amount of flow per gram of tissue was calculated by dividing the flow by the weight of the appropriate tissue sample.

Determination of area at risk and infarct size. At the conclusion of reperfusion, the LAD was reoccluded, and in vivo area-at-risk (AAR) delineation proceeded by injection of 40 ml of 10% Evans blue stain through the 8-Fr pigtailed catheter. A bolus of potassium chloride was injected with the Evans blue to aid in euthanizing the animal. The heart was then excised, the LAD was cannulated, and small polyethylene tubing was inserted. One liter of 2,3,5-triphenyltetrazolium chloride was infused at a constant 100-mmHg pressure through the cannulated LAD for ex vivo determination of necrotic tissue within the ischemic zone (the AAR). The heart was then fixed in 10% formalin for 6 h. After fixation, the hearts were sliced into 7-mm-thick serial cross-sections starting at the apex (14). The myocardial slices were then imaged using a dissecting microscope (model SZ4045; Olympus America) equipped with a charge-couple device color video camera (Iris; Sony Electronics). The LV area, AAR, and area of infarction for each slice were then determined by computer planimetry using NIH Image 1.57 software. The right ventricle was removed from each imaged section, and the LV weight was recorded. The size of the myocardial infarction was determined by the following previously described equation (16): weight of infarct = $(A_1 \times W_1) + (A_2 \times W_2) + (A_3 \times W_3) + \ldots (A_n \times W_n)$, where A is the percent area of infarction by planimetry, subscripted numbers 1–n represent section numbers, and W is the weight of the same numbered sections. Similar calculations were used to determine weights of the noninfarcted AARs and nonischemic zones within the myocardium.

Hematology of peripheral blood. Tests for creatine kinase MB isoform (CK-MB) and troponin (Tn-I) and complete blood counts were performed at the Louisiana State University Health Sciences Center clinical laboratories. Whole blood samples were obtained at baseline, 1 h of ischemia, and 1, 2, and 4 h of reperfusion from the catheterized femoral vein (7, 18).

Myocardial histology. Routine histological staining was performed on multiple midventricular cardiac sections to determine the extent of polymorphonuclear (PMN) infiltration into the ischemic reperfused myocardium. Vehicle- and HBOC-201-treated groups were subjected to 90 min of myocardial ischemia and 4.5 h of reperfusion and were then stained as described. The tissue sections were immediately fixed and stored in a 10% neutral-buffered formalin solution. The tissue slices were paraffin embedded, cut into 1-mm sections, placed on slides, and stained with Gill's no. 3 hematoxylin and eosin. The slides were then viewed microscopically, and the number of neutrophils (PMN cells) per high-power field was determined. For each of the hearts examined, the number of neutrophils was counted in six fields on three separate tissues.

Statistical analysis. All values represent means ± SE of the indicated number of independent experiments. The infarct size, AAR, left ventricle size, leukocyte count, CK-MB, Tn-I, and hemodynamic data were analyzed with ANOVA coupled with post hoc analysis (Scheffé's test of significance). All statistics were calculated using StatVeiw 4.5 (Abacus Concepts). Statistical significance was set at $P < 0.05$.

Table 1. *Hematology data*

	Baseline Value	Ischemia, 1 h	Reperfusion 1 h	Reperfusion 2 h	Reperfusion 4 h
Hematocrit, %					
Vehicle	42±2	44±1	45±1	45±1	43±1
HBOC-201	45±1	39±2*	38±2†	39±2*	39±2
WBCs, no./μl					
Vehicle	5,200±572	5,800±485	7,637±976	9,975±1,411	12,688±1,617
HBOC-201	5,825±338	6,033±573	7,933±847	10,233±977	13,855±1,516
PMNs, no./μl					
Vehicle	2,661±252	3,953±770	5,507±756	7,537±1,060	9,501±1,578
HBOC-201	3,438±293	4,105±502	5,987±830	7,958±998	11,640±1,423
Platelets, 1,000 s/μl					
Vehicle	184±10	192±15	207±9	189±11	179±9
HBOC-201	185±14	165±8	165±9*	170±8	161±2

Values are means ± SE. WBCs, circulating leukocytes; PMNs, circulating polymorphonuclear leukocytes; *$P < 0.05$; †$P < 0.01$.

RESULTS

Exclusion criteria. A total of 22 dogs entered the 90-min ischemia and 270-min reperfusion study. During coronary occlusion, two dogs in the vehicle group and two dogs in the HBOC-201 group developed intractable ventricular fibrillation and were therefore excluded from further analysis. A total of 18 ($n = 8$ for vehicle and $n = 10$ for treatment) canines successfully completed the MI/R experimental protocol and were included for analysis.

Hematology. The circulating levels of leukocytes, neutrophils, and platelets for the vehicle and HBOC-201 groups at all time points are presented in Table 1. Total counts for leukocytes and neutrophils (PMN cells) did not differ significantly between the vehicle and HBOC-201 groups at anytime during the experimental protocol. Platelet counts were significantly lower at 1 h of reperfusion in HBOC-201-treated canines but were not different at other time points. Hematocrit was reduced in HBOC-201-treated compared with vehicle-treated canines; hematocrit was significantly lower at 1 h of ischemia as well as at 1 and 2 h of reperfusion and was nonsignificantly reduced at 4 h of reperfusion.

Hemodynamics. Mean arterial pressure, heart rate, LV systolic pressure, LV diastolic pressure, maximum and minimum dP/d*t*, and LV developed pressure were recorded from each of the animals throughout the MI/R experimental protocol. These data are presented in Table 2. LV developed pressure showed significant increases at 1, 2, and 4 h of reperfusion in HBOC-201-treated over vehicle-treated canines. No other hemodynamic parameters showed significant changes between the HBOC-201- and vehicle-treated groups except end-reperfusion (4-h) LV systolic pressure and maximum dP/d*t*.

Regional myocardial blood flow. Myocardial blood flow values from the epicardium and endocardium during baseline and at 75 min of ischemia are presented in Fig. 1. We observed similar blood flow in myocardia of animals receiving vehicle or

Table 2. *Hemodynamic data*

	Baseline Value	Ischemia, 1 h	Reperfusion 1 h	Reperfusion 2 h	Reperfusion 4 h
MABP, mmHg					
Vehicle	112±10	100±7	89±6	92±6	79±7
HBOC-201	107±5	111±6	100±7	96±6	96±5
HR, beats/min					
Vehicle	145±10	153±8	140±4	137±8	142±15
HBOC-201	146±9	154±5	138±6	139±2	139±7
LVSP, mmHg					
Vehicle	105±15	108±7	94±6	94±6	86±7
HBOC-201	115±5	121±6	111±7	107±6	110±4*
LVEDP, mmHg					
Vehicle	4±2	7.0±2	39±23	39±21	42±20
HBOC-201	6±2	7.0±1	6±1	6±1.0	9±4
LVDP, mmHg					
Vehicle	100±15	101±7	55±17	55±17	45±16
HBOC-201	111±6	112±5	99±9*	100±5*	100±5†
Max dP/d*t*, mmHg/s					
Vehicle	1,932±156	1,725±122	1,681±118	1,676±135	1,279±221
HBOC-201	1,894±113	1,946±81	1,739±118	1,801±104	1,763±75*
Min dP/d*t*, mmHg/s					
Vehicle	−1,916±143	−1,675±141	−1,545±145	−1,639±147	−1,177±235
HBOC-201	−1,856±126	−1,929±106	−1,656±162	−1,718±127	−1,589±129

Values are means ± SE. MABP, mean arterial blood pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure. *$P < 0.05$; †$P < 0.01$.

HBOC-201 at baseline. Baseline myocardial blood flow in the epicardium was 0.86 ± 0.35 ml·min^{-1}·g^{-1} in the vehicle group and 0.92 ± 0.17 ml·min^{-1}·g^{-1} in the HBOC-201 group [P = not significant (NS)]. Similarly, baseline blood flow in the endocardium was 1.1 ± 0.39 ml·min^{-1}·g^{-1} in animals receiving vehicle and 0.80 ± 0.14 ml·min^{-1}·g^{-1} in the HBOC-201 group (P = NS). Figure 1*B* depicts the reductions in myocardial blood flow after ischemia. During myocardial ischemia, epicardial blood flow was decreased by 80 ± 3% in the vehicle group and 74 ± 8% in the HBOC-201 group (P = NS). Blood flow to the endocardium was decreased during ischemia by 87 ± 6% in the vehicle group and 83 ± 7% in dogs receiving HBOC-201 (P = NS).

Markers of cardiac injury. Figures 2 and 3 present plasma CK-MB and serum Tn-I levels, respectively. Levels of CK-MB and Tn-I (in ng/ml) in HBOC-201-treated canines did show decreases from vehicle-treated canines during reperfusion, with CK-MB showing significant decreases ($P < 0.05$) at 1, 2, and 4 h of reperfusion (Fig. 2). Tn-I levels were reduced in HBOC-201-treated animals, but the reductions did not achieve statistical significance until 4 h of reperfusion (Fig. 3).

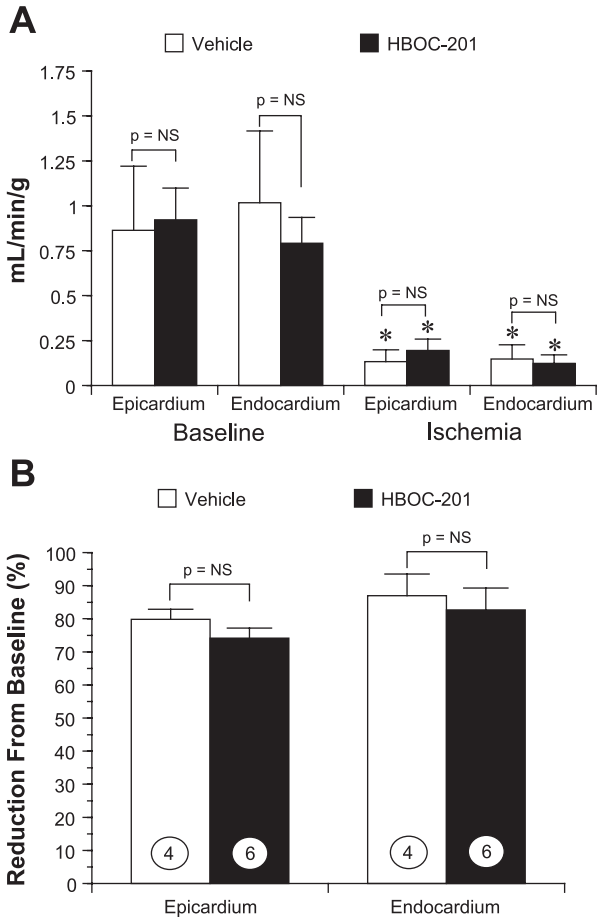

Fig. 1. Regional myocardial blood flow in dogs receiving HBOC-201 or saline vehicle. *A*: myocardial blood flow in the epicardium and endocardium at baseline and at 75 min of ischemia. No significant differences were observed between the HBOC-201 and vehicle groups. Myocardial blood flow was significantly (*$P < 0.001$) reduced in the epicardium and endocardium during ischemia in both study groups. *B*: percent reduction in ischemic zone blood flow in the epicardium and endocardium for HBOC-201 and vehicle groups. No significant group differences were observed; *n*, no. of dogs, in circles. NS, not significant.

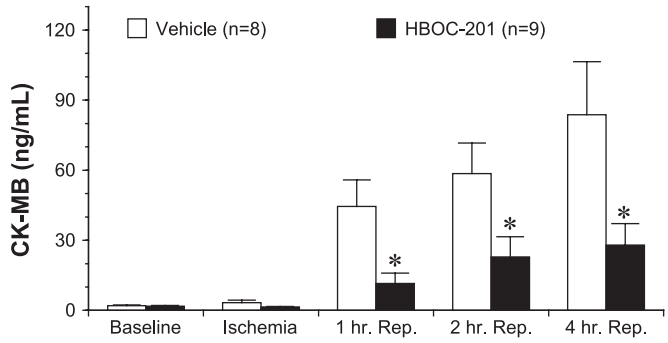

Fig. 2. Plasma creatine kinase MB isoform (CK-MB) levels were measured at specified time points during the experimental protocol including baseline, 90 min of ischemia, and 1, 2, and 4 h of reperfusion (Rep) in animals receiving saline vehicle or HBOC-201. *$P < 0.05$ vs. vehicle.

Myocardial AAR and infarct size. Representative photographs of AARs and infarct staining for vehicle and treatment groups after 90-min ischemia and 270-min reperfusion are presented in Fig. 4. Despite similar-sized AARs, the vehicle group suffered a significantly larger area of infarction after MI/R compared with the HBOC-201 group. Summary data for AAR and infarct size are shown in Fig. 5. Both experimental groups experienced similar-sized AARs per left ventricle after coronary occlusion; values were 44.2 ± 4.1 and 44.5 ± 3.0% for vehicle and HBOC-201 groups, respectively (P = NS). Infarct size per AAR was significantly lower ($P < 0.01$) in the HBOC-201 group at 11 ± 3% compared with 26 ± 4% in the vehicle group. In addition, infarct size expressed as a percentage of the left ventricle was 11 ± 1% in the vehicle group compared with 5 ± 1% in the HBOC-201 group ($P < 0.01$).

Myocardial neutrophil accumulation. Graphic representation of neutrophil (PMN cell) counts within the ischemic reperfused myocardium after 90 min of myocardial ischemia and 270 min of reperfusion is presented in Fig. 6. Neutrophil accumulation was determined histologically by an observer blinded to the treatment groups. Cell accumulation values in the ischemic-reperfused myocardium were 75 ± 8 PMN cells/field in the vehicle and 19 ± 2 PMN cells/field in the HBOC-201 group ($P < 0.01$ between the groups).

DISCUSSION

The present study demonstrates a significant reduction of myocardial infarct size after pretreatment with HBOC-201 in a

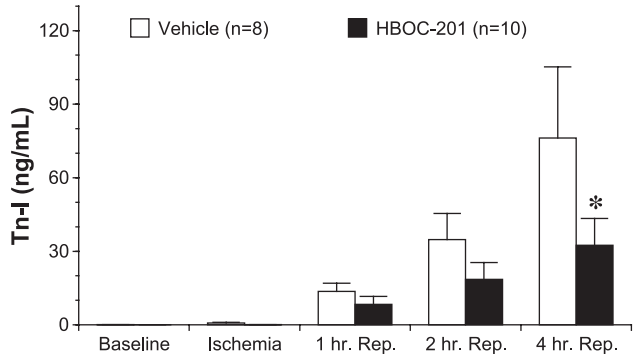

Fig. 3. Myocardial troponin-I (Tn-I) release was measured from plasma samples at specified time points during the experimental protocol including baseline, 90 min of ischemia, and 1, 2, and 4 h of reperfusion in animals receiving saline vehicle or HBOC-201. *$P < 0.05$.

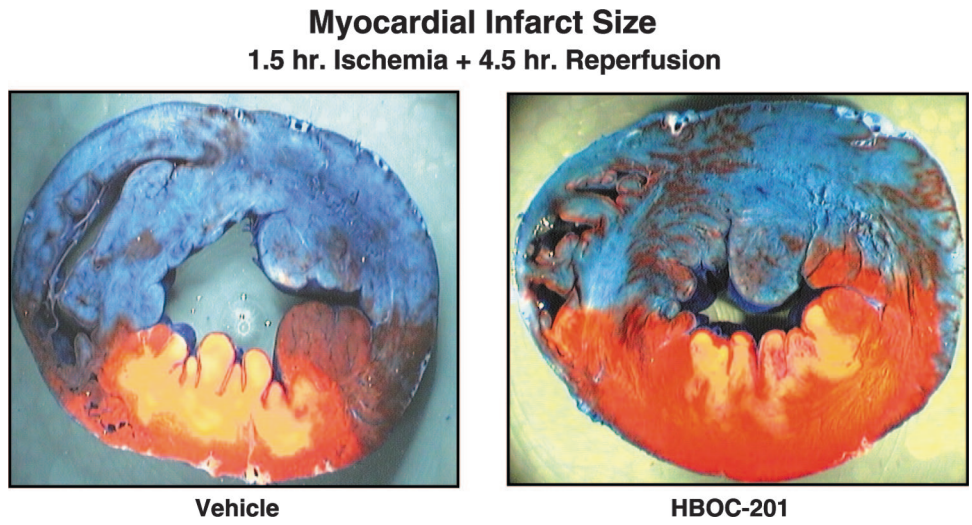

Fig. 4. Representative photomicrographs of heart sections from dogs receiving saline vehicle or HBOC-201. Area at risk appears red in color, and areas of infarction appear pale. Nonischemic myocardium is stained blue.

canine model of acute coronary artery occlusion and reperfusion. Myocardial necrosis within the AAR was reduced by ~56% in hearts treated with HBOC-201. Furthermore, treatment with HBOC-201 also resulted in significant reductions in myocardial neutrophil infiltration and myocardial release of CK-MB and Tn-I. This protective effect cannot be attributed to changes in circulating neutrophils, because there was no significant difference between the vehicle and HBOC-201 groups, although HBOC-201 did inhibit neutrophil infiltration into the ischemic reperfused myocardium. It has been previously shown (7) that neutrophil accumulation is a mechanism of tissue injury capable of extending myocardial infarction. Finally, the cardioprotection observed with HBOC-201 treatment is not attributable to changes in hemodynamic status or myocardial blood flow during myocardial ischemia.

Our data strongly suggest that pretreatment with this glutaraldehyde-polymerized bovine Hb solution (i.e., HBOC-201) attenuates myocardial reperfusion injury. Reperfusion injury is the conversion of normal or injured cells to more severely injured cells during the restoration of blood flow; this resultant damage is mediated by molecular O_2 (23). It has been shown that ischemia is an initiator of inflammatory mediators and O_2 radicals that lead to irreversible tissue injury (15). Introduction of an O_2-rich product such as bovine Hb before ischemia may reduce the severity of the ischemia by preloading the myocardium with O_2 or by aiding in O_2 delivery to hypoxic tissue. In either case, a reduction in the severity of ischemia would result in a reduction in ischemia-induced inflammation and a subsequent reduction in reperfusion injury.

Previous studies of cerebral, skeletal muscle, and gut ischemia (5, 11, 22) have demonstrated a reduction in tissue injury. Horn et al. (11) and Standl et al. (21) reported that intravenous administration of HBOC-201 restored the decreased skeletal muscle tissue O_2 tensions after artificial arterial stenosis. Their results demonstrate that bovine Hb solution improves poststenotic tissue oxygenation. It has also been shown (22) that a Hb solution administered to pigs in severe hemorrhagic shock was able to restore systemic blood pressure and also improve microvascular oxygenation in the gut. Cole et al. (5) showed that infusion of a Hb solution was able to significantly reduce cerebral ischemia with a prolonged reperfusion of 72 h. All of these studies as well as ours indicate that infusion of Hb solution decreases the severity of ischemic end-organ tissue injury.

Nitric oxide (NO) is involved the regulation of vascular tone. The use of Hb-containing agents such as HBOC-201 are thought to cause vasoconstriction by scavenging of NO in the vascular lumen (10). This reduction in local NO concentration leads to vascular constriction (9, 13). One mechanism that

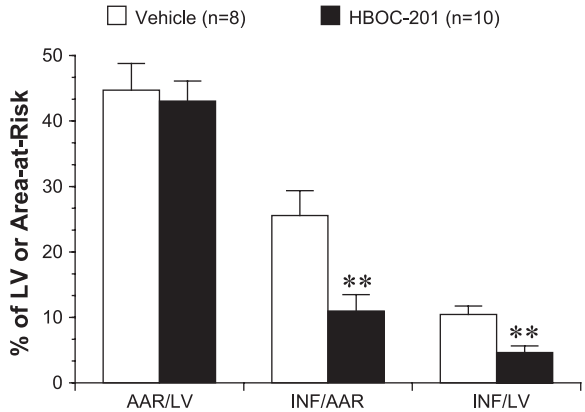

Fig. 5. Data for area at risk (AAR) per left ventricle (LV), infarct (INF) per area at risk, and infarct per left ventricle. **$P < 0.01$ vs. vehicle.

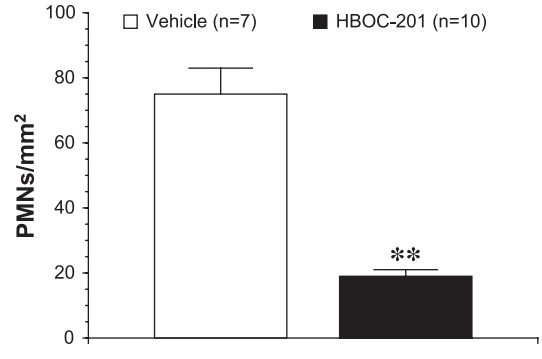

Fig. 6. Myocardial polymorphonuclear leukocyte (PMNs) infiltration after 1.5 h of ischemia and 4.5 h of reperfusion in animals receiving saline vehicle or HBOC-201. **$P < 0.01$ vs. vehicle.

appears to induce vasoconstriction is the high-affinity binding of NO by acellular Hb (8). In the present study, we did observe an increase in blood pressure after HBOC-201 infusion in our model of MI/R. However, the increase in mean arterial blood pressure did not achieve statistical significance and did not adversely affect myocardial injury after ischemia and reperfusion.

The present study demonstrates that HBOC-201 has a number of positive attributes in the setting of MI/R injury. One limitation in the present study is that HBOC-201 was administered as a pretreatment, and this does not closely mimic what occurs during the evolution of acute myocardial infarction in humans. Another limitation is the short reperfusion time of 4.5 h. Additional studies to investigate 48–72 h of reperfusion will provide a better real-life analysis of the potential protective effects of HBOC-201.

In summary, the present study clearly demonstrates that pretreatment with a Hb-based blood substitute significantly ameliorates MI/R injury. In addition, the extent of myocardial inflammation was also attenuated with this Hb-based blood-substitute therapy. Additional studies are indicated to define the precise cellular and molecular mechanisms involved in this cardioprotective effect.

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DISCLOSURES

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