Supplementary Data

Supplementary Methods

ANIMALS were supplied by the Experimental Animal Center of West China Hospital, Sichuan University in Chengdu, China. All animal experimental procedures were performed in accordance with the policies of the Animal Care and Use Committee of Sichuan University, and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The ACE inhibitor in this study was captopril (Sigma-Aldrich). Its dosage (100 μM) was determined by the preliminary experiment (Supplementary Fig.S2A, B) and with reference to recent publications (8, 9).

Reagents

Polyclonal or monoclonal antibodies against the NAD(P)H oxidase subunits ($p47^{phox}$, $p67^{phox}$, $gp91^{phox}$, Nox1, and Nox4), CD68, von Willebrand factor (vWF), E-selectin (CD62E), intercellular adhesion molecule-1 (ICAM-1), 3-nitrotyrosine (3-NT), Bcl-2, Bax, caspase-3, eNOS, phosphorylated eNOS at Ser1177 (peNOS), iNOS, and β-actin were obtained from Abcam, AbD Serotec, or Santa Cruz Biotechnology. Secondary antibodies were from Invitrogen, DAKO, or GeneTex. ACE ELISA kits were from R&D systems. Other chemicals, if not indicated, were purchased from Sigma-Aldrich.

Measurement of methemoglobin formation

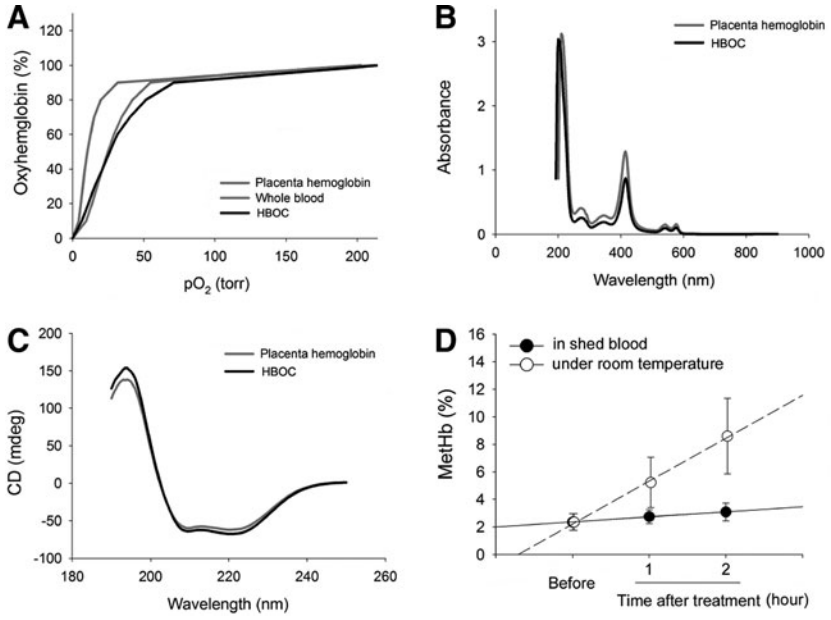
To measure the methemoglobin (MetHb) formation under physiological condition, five adult male SD rats (245 ± 9 g) were used. After animal preparation, fifteen percent of total blood volume was withdrawn from femoral artery, and then, an equivalent volume of hemoglobin-based oxygen carriers (HBOC) was infused *via* the femoral vein (~2–3 ml). The blood volume was calculated as 7.5% of the body weight. Two hours later, the blood sample (5 ml) was collected from the femoral vein, and the HBOC was separated as a supernatant by centrifugation (1200 rpm × 10 min). In another group, the HBOC was bubbled with 95%O_2 + 5%CO_2 for 15 min and exposed to room temperature for 2 h. The percentage of MetHb was measured as previously reported (6).

Preparation of MetHb form of HBOC

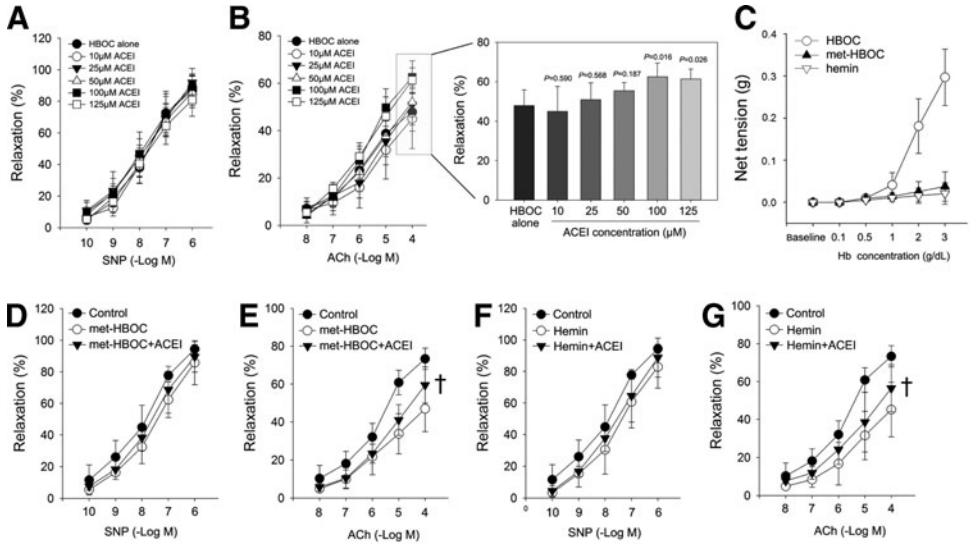
To prepare 100% MetHb form of HBOC (met-HBOC), the HBOC solution was directly exposed to pure NO gas for 15 min through a membrane gas exchanger (1500 ml/min; Kewei Medical Ltd.). All prepared met-HBOC solutions were dialyzed against 0.9% saline overnight at 4°C before being used.

Dog cardiopulmonary bypass model

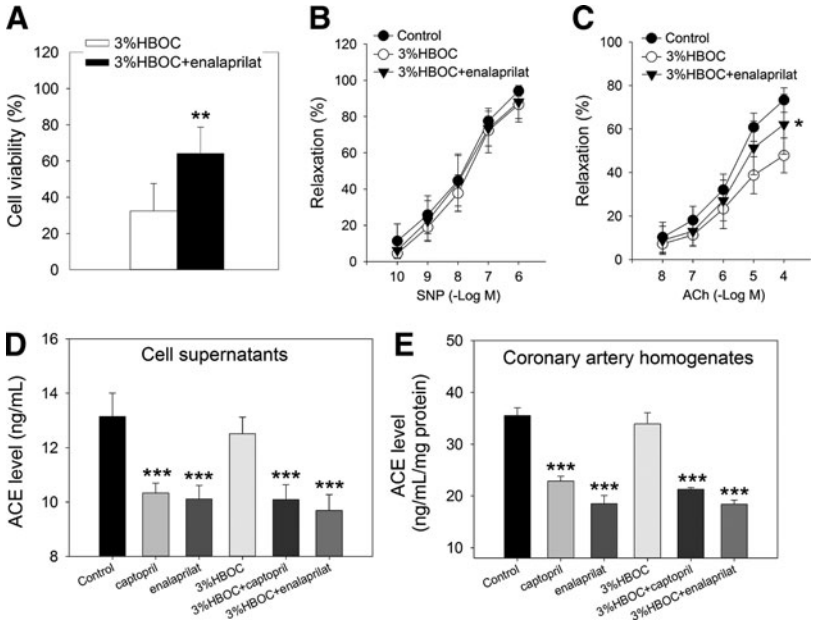
A beagle dog cardiopulmonary bypass (CPB) model was previously established as described in the literature (5). In brief, adult male beagle dogs, weighing 8–10 kg, were used. After induction (4 mg/kg propofol, 0.1 mg/kg midazolam, and 5 μg/kg fentanyl) and muscle relaxation (1 mg/kg scoline), all the dogs were intubated with an Fr. 7.5 endotracheal tube and mechanically ventilated using an air/O_2 mixture (1:4) with a tidal volume of 10 ml/kg (Datex-Ohmeda Excel 210; Soma Technology). Each group received a continuous



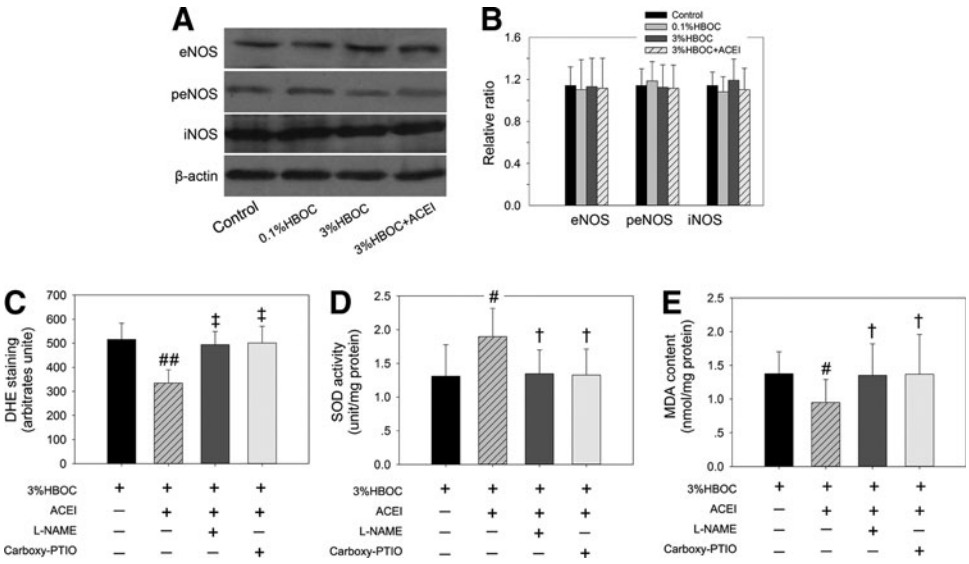
SUPPLEMENTARY FIG. S1. The characteristics and auto-oxidation rate of hemoglobin-based oxygen carriers (HBOC). (A) Respective oxygen equilibrium curves of placenta hemoglobin, whole blood, and HBOC. **(B)** Representative absorbance spectra of placenta hemoglobin and HBOC. **(C)** Respective circular dichroism (CD) spectra of placenta hemoglobin and HBOC. **(D)** The percentage of methemoglobin (MetHb) formation of the HBOC in the rat blood or under room temperature. Values are presented as mean ± SD ($n = 5$ per group).



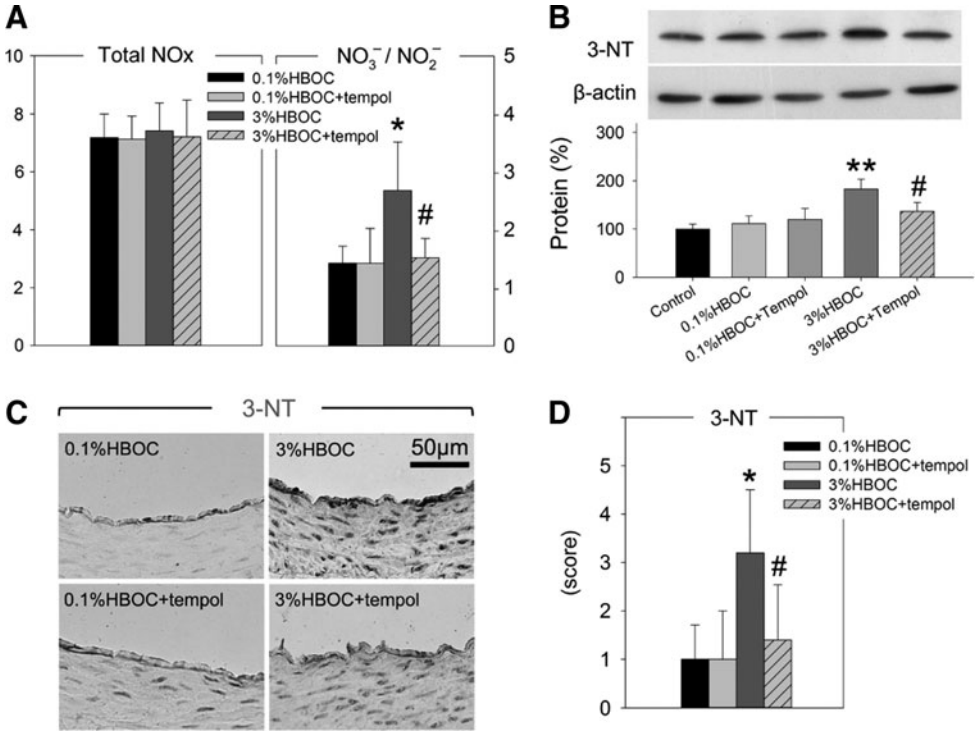
SUPPLEMENTARY FIG. S2. The optimal dosage of captopril and its effect on hemin and met-HBOC induced endothelial damage. (A, B) The preliminary study indicated that 100 μM is the optimal dosage under our experimental condition ($n = 3$ to five per group). Hemin and met-HBOC equally impair endothelial function, which can be attenuated by captopril. **(C)** The net tension of coronary artery rings after incubation with HBOC, met-HBOC, or hemin. It should be noted that the concentrations of hemin were determined with reference to the calculated heme levels of HBOC. For example, the calculated heme level of 3%HBOC is about 1.875 mM, so 1.875 mM hemin was used corresponding to 3%HBOC. **(D–G)** SNP-induced endothelium-independent relaxation and ACh-induced endothelium-dependent relaxation in coronary arteries after indicated treatment. Control vessels were treated with KH solution alone ($n = 5$ to 6 per group). Values are presented as mean ± SD. †$p < 0.05$ *versus* the met-HBOC group or the hemin group. ACEI in the figure indicates ACE inhibitor captopril.



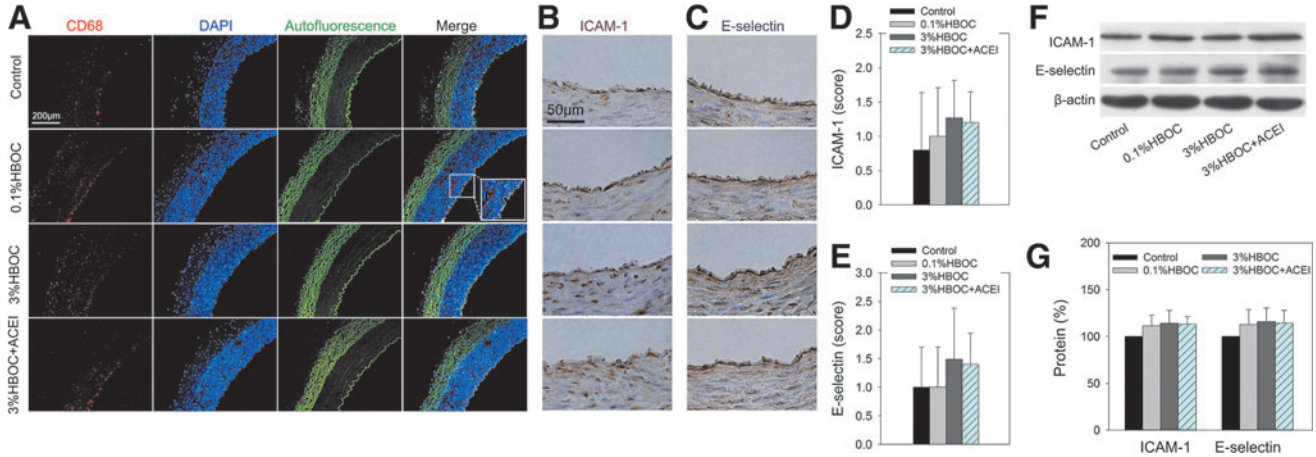
SUPPLEMENTARY FIG. S3. Enalaprilat reduces HBOC-induced endothelial damage by down-regulation of ACE level. (A) Cell viability of human umbilical vein endothelial cells (HUVECs) measured by MTT test ($n = 5$). **(B, C)** SNP-induced endothelium-independent relaxation and ACh-induced endothelium-dependent relaxation in coronary arteries after incubation with 3%HBOC or 3%HBOC + 100 μM enalaprilat. Control vessels were treated with KH solution alone ($n = 5$ to 6 per group). **(D)** The angiotensin-converting enzyme (ACE) levels in cell-free supernatants of HUVECs. Control group was treated with culture medium alone ($n = 6$). **(E)** The ACE levels in coronary artery homogenates. Control vessels were treated with KH solution alone ($n = 6$). Values are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ *versus* the 3%HBOC group.



SUPPLEMENTARY FIG. S4. Captopril does not influence major sources of NO and preserves bioavailable NO. (A, B) Western blot analysis of total eNOS, phosphorylated eNOS at Ser1177 (peNOS), and iNOS expressions in coronary arteries after treatment with KH solution (control), 0.1%HBOC, 3%HBOC, or 3%HBOC + 100 μM captopril. The blot is a representative of blots obtained from five independent experiments. **(C–E)** The reactive oxygen species (ROS) production, superoxide dismutase (SOD) activity, and malonaldehyde (MDA) content in HUVECs after indicated treatment ($n = 5$ per group). Values are presented as mean ± SD. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *versus* the 3%HBOC group; †$p < 0.05$ and ‡ $p < 0.01$ *versus* the 3%HBOC + ACEI group.



SUPPLEMENTARY FIG. S5. ROS overproduction is responsible for the reduction of bioavailable NO. (A) Total NOx and NO_3^-/NO_2^- ratio measured in coronary arteries after incubation with 10 μM tempol and HBOC. **(B)** The levels of 3-NT measured by Western blotting. **(C, D)** Representative of immunohistochemical staining and quantification analysis for 3-NT-positive protein in coronary arteries. Scale bar: 50 μm. Values are presented as mean ± SD ($n = 5$ to 6 per group). $^{*}p < 0.05$ and $^{**}p < 0.01$ *versus* the 0.1%HBOC group; $^{\#}p < 0.05$ *versus* the 3%HBOC group.



SUPPLEMENTARY FIG. S6. HBOC-induced endothelial dysfunction is not attributable to macrophage infiltration and inflammation. Representatives of immunohistochemical staining for CD68 **(A)** ICAM-1 **(B)** and E-selectin-positive proteins **(C)** in coronary arteries. Scale bar: 200 or 50 μm. **(D, E)** Quantifications for ICAM-1 and E-selectin-positive proteins. **(F, G)** Western blot analysis of expressions of ICAM-1 and E-selectin. Values are presented as mean ± SD ($n = 5$ to six per group).

infusion of fentanyl at 0.3 μg/kg/min and vecuronium bromide at 0.2 mg/kg/h during surgery. Anesthesia was maintained with 150 μg/kg/min propofol. After heart exposure through a mid-sternal incision and heparinization (3 mg/kg), the ascending aorta and the right atrial appendage were cannulated. The CPB circuit was composed of a rolling pump (StÖckert II), a membrane oxygenator (1500 ml/min; Kewei Medical Ltd.), and an arterial filter (Kewei Medical Ltd.). The CPB was primed with Lactate Ringer's solution containing 5% sodium bicarbonate (10 ml/L), 20% mannitol (2.5 ml/L), furosemide (0.5–1.0 mg/L), dexamethasone (5 mg/L), heparin (10 mg/L), and 10% potassium chloride (5 ml/L). In addition, a 10% calcium gluconate (2–4 ml) was added every 30 min for four times.

Effect of HBOC on dog heart during CPB

Thirty adult male beagle dogs were randomly divided into five groups ($n = 6$). After the establishment of CPB and equilibration, cardiac arrest was induced by infusion of 40 mL/kg St.Thomas' solution (STS) alone (I/R group), STS with 0.1%HBOC (0.1%HBOC group), or STS with 3%HBOC (3%HBOC group). For 3%HBOC + ACEI group, the dogs were pretreated with an intravenous injection of captopril (3 mg/kg) for 10 min; then, cardiac arrest was achieved *via* intra-aortic infusion of 40 ml/kg STS with 3%HBOC. After 2 h of cardiac arrest, the hearts were reperfused by aortic declamping. After 30 min of reperfusion, the dogs were weaned from CPB and observed for an additional 90 min. The group received sham operation but without CPB, and cardiac arrest was used as sham control. After the experiment, all the dogs were sacrificed with an intravenous bolus injection of sodium pentobarbital (120 mg/kg).

SUPPLEMENTARY TABLE S1. PARAMETERS OF THE HEMOGLOBIN-BASED OXYGEN CARRIER SOLUTION

Parameters	*Results or range*
Hemoglobin concentration (gHb/L, in 0.9% saline)	8–10
Percentage of methemoglobin (%)	< 3%
P_{50} (mmHg)	21–24
Hill coefficient	1.9–2.3
Colloid osmotic pressure (mmHg)	20–25
Osmolality (mosm/kg)	290–310
Molecular weight (kDa)	64–600
Tetrameric hemoglobin (%)	< 1%
Antioxidant enzyme	None

P_{50}, the PO_2 level at that hemoglobin is half saturated with O_2.

To measure cardiac function, a water-filled latex balloon attached to a pressure sensor (model SP844; MEMSCAP, Inc.) was inserted into the left ventricle (LV) *via* the mitral valve. Then, the cardiac functional parameters, including heart rate (HR), LV systolic pressure, and LV end-diastolic pressure, were collected by a PowerLab data-acquisition system (ADInstruments Pty). In addition, a Swan–Ganz Float Catheter (No.7; Edwards Laboratories) was inserted *via* the femoral vein and advanced to the pulmonary artery to measure cardiac output (CO), pulmonary artery wedge pressure, pulmonary arterial pressure, and central venous pressure. Mean arterial pressure was monitored by a polyethylene catheter that was placed in the left femoral artery. To assess the level of cardiac oxygen (O_2) utilization, blood samples from the artery and coronary vein sinus were collected. Cardiac O_2 consumption (VO_2) and O_2 extraction index (O_2EI) were calculated from the values of CO, hemoglobin concentration (Hb), arterial O_2 partial pressure (PaO_2), venous O_2 partial pressure (PvO_2), arterial O_2 saturation (SaO_2), and venous O_2 saturation (SvO_2) (ABL800 FLEX blood gas analyzer, Radiometer Medical A/S) by using the following formula:

$$VO_2(\text{ml/min}) = CO \times [1.38 \times Hb \times (SaO_2 - SvO2) + 0.0031 \times (PaO_2 - PvO_2)] \times 10$$

$$O_2EI(\%) = 1 - [(1.38 \times Hb \times SvO_2 + 0.0031 \times PvO_2)/(1.38 \times Hb \times SaO_2 + 0.0031 \times PaO_2)] \times 100\%$$

Determination of myocardial necrosis

Myocardial necrosis estimated by the releases of creatine kinase-MB (CK-MB) and cardiac troponin-I (cTnI) were measured as previously described (7). In addition, hematoxylin and eosin (H&E) staining was employed to assess the histopathological changes in the myocardium.

Coronary angiography

Male adult beagle dogs, weighing 8–10 kg, were used. After anticoagulation and anesthesia, an Fr.4 (1.40 mm) angiographic catheter (Radifocus, Terumo) was introduced through the femoral artery and advanced to either the left or right coronary artery. The inner lumen of the catheter was used for intracoronary infusion of drugs. After the infusion of nonionic contrast agent Omnipaque (Amersham Health) to acquire basal coronary angiogram (AXIOM Artis dTA system; Siemens Medical Solutions), 10 ml of HBOC with a concentration of 0.1%, 0.5%, 1%, 2%, or 3% was randomly infused at a rate of 10 ml/min ($n = 5$ for each concentration). For an additional group, five dogs were pretreated with captopril (3 mg/kg) for 10 min and followed by an intracoronary infusion of 3%HBOC. At the end of each HBOC infusion, a coronary angiogram was acquired and washed out with 10 ml normal saline. The procedure described earlier was repeated once to confirm the vascular reaction with an interval of 10 min. The obtained coronary angiograms were analyzed by an independent, blinded investigator using quantitative coronary analysis software (QuantCor QCA; Siemens Medical Systems). The percentage of change in coronary artery luminal diameter and the rate of vasoconstriction after HBOC infusion were calculated. Negative values for coronary artery changes indicate vasoconstriction.

Echocardiography

Immediately after each coronary angiography, echocardiography was performed using a commercially available ultrasound system (Vivid 7; GE Healthcare) with a 3-MHz transducer (M3S). Standardized protocol for image acquisition was previously reported (3). Image acquisition was completed within 2 min by an experienced sonographer, with image interpretation performed later. The echocardiographic parameters used to evaluate LV function included LV ejection fraction measured by the two-dimensional echocardiography (2-DE) biplane Simpson method and LV longitudinal strain measured by the speckle tracking method.

Measurement of vascular reactivity on isolated vessel rings

Arterial rings (3–4 mm in length) from beagle dog femoral and coronary artery, free of fat and connective tissue, were mounted between two stainless steel hooks in organ bath chambers (PanLab Systems; Harvard apparatus). Each chamber contained 10 ml of Krebs–Henseleit (KH) solution (118 m*M* NaCl, 4.7 m*M* KCl, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 1.77 m*M* $CaCl_2$, 25 m*M* $NaHCO_3$, and 11.4 m*M* glucose; pH 7.4, 37°C) and was aerated continuously with 95% O_2 and 5% CO_2. Special attention was paid during the preparation to avoid damaging endothelium. During 60 min of equilibration period, resting tension of 3.5 g was periodically adjusted, and the KH solution was changed every 30 min. The arterial viability was checked by stable and reproducible constriction to the addition of potassium chloride (KCl, 60 m*M*). Contracted arteries were then washed and subjected to 30 min of equilibration. After that, these arteries were incubated with HBOC, met-HBOC or an equivalent amount of hemin for 10 min, and isometric tension of each vessel was recorded. To measure the effect of HBOC on the endothelium, isolated coronary arterial rings were incubated with increasing concentrations of HBOC at 37°C for 2 h. In additional groups, the arterial rings were treated by 3%HBOC with captopril (100 μM), enalaprilat (100 μM), tempol (10 μM), apocynin (100 μM), Nω-Nitro-L-arginine methyl ester (L-NAME, 100 μM), or carboxy-PTIO (200 μM) at 37°C for 2 h. After being washed and equilibrated for 60 min under a resting tension of 3.5 g, these arteries were evoked using phenylephrine (10^{-7} *M*) to elicit reproducible contractile responses. Acetylcholine (ACh; 1×10^{-8} to 1×10^{-4} *M*) or sodium nitroprusside (SNP; 1×10^{-10} to 1×10^{-6} *M*) was then progressively added to induce endothelium-dependent or -independent relaxation, respectively.

TUNEL staining

Levels of endothelial cell apoptosis in dog coronary artery were detected with terminal dUTP nick-labeling (TUNEL) assay according to the manufacturer's instructions (Roche Diagnostics). More than 10 fields selected at random were analyzed in a blinded manner. Images were captured using an Olympus BX51 microscope and a DP70 digital camera (Olympus corp.). The percentage of TUNEL-positive endothelial cells among the total number of endothelial cells was calculated using Image-Pro Plus 5.0 software (Media Cybernetics, Inc.).

Transmission electron microscopy

The LV tissues harvested after CPB study and the coronary artery rings after treatment were immediately fixed for 24 h in 3% glutaraldehyde in 0.1 *M* phosphate buffer saline (pH 7.4), and then fixed with 1% osmium tetroxide for 1 h. After the samples were dehydrated in a series of ethanol and were embedded in Epon 812, ultrathin sections were prepared and counterstained with uranyl acetate and lead citrate. The stained sections were examined under a Hitachi H-600IV electron microscope (Hitachi Ltd.) at 75 kV. The levels of change were assessed in a blinded fashion by two pathologists.

Immunohistochemistry

Paraffin sections (5 μm) or cryosections (8 μm) of dog coronary arteries were prepared and stained for CD68, vWF, E-selectin, ICAM-1, and 3-NT by using standard and widely accepted immunostaining techniques. CD68, also known as macrosialin, specifically stains for macrophages. E-selectin and ICAM-1 were markers of endothelial inflammation. vWF was used to indicate the endothelium. In addition, paraffin sections of dog LV tissue were stained with H&E and assessed in a blinded fashion by a pathologist for the following histological examination: acute myocardial necrosis, interstitial edema, perinuclear halo, and fatty changes. Semiquantitative analysis of histopathological changes was performed using an arbitrary grading system from score 0 to 5 (score 0: $< 10\%$ positive cells; score 1: 10%–20% positive

cells; score 2: 21%–30% positive cells; score 3: 31%–40% positive cells; score 4: 41%–50% positive cells; score 5: >50% positive cells).

Cell culture

The human umbilical vein endothelial cells (HUVECs) obtained from West China Hospital were grown in DMEM containing 2 m*M* L-glutamine, 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (37°C, 5% CO_2). Cells were plated onto 96-well plates at 5000 cells per well in 100 μl of culture medium. Twenty-four hours after plating, 100 μl of medium containing 0.1%, 0.5%, 1%, 2%HBOC, 3%HBOC, or 3%HBOC + captopril (100 μM) was added to the wells (in triplicate) and incubated for 2 h at 37°C and 5% CO_2. After being washed with PBS, the cells were observed under a microscope. Cell viability was evaluated using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) test, and absorbance was read at 562 nm in a microplate reader (MR-96A; Mindray). Results are expressed as percent of viability compared with the control (without HBOC treatment).

Measurement of ACE levels in HUVECs and coronary artery

After treatment, the levels of ACE in either cell-free supernatants of HUVECs or tissue homogenates of coronary artery were measured by using commercially available ELISA kits (R&D systems).

Western blot analysis

Cell lysates or tissue homogenates were subjected to Western blot analysis. The protein concentration of its supernatant was determined by the BCA method (Pierce). As previously described (4), 20 μg of protein was separated by SDS–PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with primary antibody, followed by horseradish peroxidase-conjugated secondary antibody. Protein bands were developed with a Supersignal chemiluminescence detection kit (Pierce) and visualized with a Kodak X-ray Processor 102 (Eastman Kodak). The intensity (area × density) of the individual bands on blots was measured by Quantity One software (Bio-Rad Laboratories). The background was subtracted from the calculated area. We used the control group (without HBOC treatment) as 100%.

DHE-derived fluorescence assay of ROS production and NAD(P)H oxidase activity

Isolated dog coronary artery after treatment was embedded in aluminum cups of about 1 ml of OCT resin (Tissue Tek, Sakura) and frozen in liquid nitrogen. To assess ROS production, cryosections (8 μm) were stained with the superoxide-sensitive dye dihydroethidine (DHE, 10 μM in PBS) and incubated for 30 min at 37°C. Red DHE fluorescence was detected with an Olympus BX51 microscope and a DP70 digital camera (Olympus corp.) at room temperature. In addition, HUVECs after treatment were incubated with DHE (10 μM) for 30 min; then, ROS production was quantified by fluorescent measurement under Em/Ex = 480/580 nm (LS55 fluorescence spectrometer, Perkin-Elmer corp.). The NAD(P)H oxidase activity of HUVECs was measured as previously described (1). Briefly, 20 μg of protein was incubated with DHE (10 μM) and DNA (1.25 μg/ml) in PBS with the addition of NAD(P)H (50 μM), in a final volume of 120 μl, for 30 min at 37°C in the dark. Fluorescence intensity was recorded in a microplate reader under Em/Ex = 480/580 nm (LS55 fluorescence spectrometer).

Superoxide dismutase activity and malondialdehyde formation assays

As markers of oxidative stress, the superoxide dismutase (SOD) activity and malondialdehyde (MDA) formation in HUVECs were measured by using commercially available kits (Nanjing Jiancheng corp.).

Determination of NOx

The levels of nitrite (NO_2^-) and nitrate (NO_3^-) in the coronary artery were measured by using a modified Griess reaction as previously reported (4). For total NOx, NO_3^- was first converted to NO_2^- by adding nitrate reductase.

Electrophysiology recording

Vascular smooth muscle cells (VSMCs) were isolated from dog coronary arteries using an enzyme procedure with modifications (2). The VSMCs with bright and smooth appearance were selected for recording. Whole-cell large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) currents were recorded at room temperature (22°C–24°C) with the conventional voltage clamp configuration. In brief, holding potential was set at −80 mV. Current densities were obtained by normalizing currents to the cell membrane capacitance. The extracellular (bath) solution contained 135 m*M* NaCl, 5.2 m*M* KCl, 1.0 m*M* $MgCl_2$, 10 m*M* HEPES, 0.5 m*M* $CaCl_2$, 10 m*M* glucose, and 5.0 m*M* 4-aminopyridine (4-AP); pH was adjusted to 7.2 by NaOH. 4-AP in the bath solution was used to exclude the interference from voltage-dependent K^+ (K_V) channel currents. The internal (pipette) solution contained 70 m*M* KCl, 60 m*M* K^+-Asp, 5 m*M* K_2ATP, 2.5 m*M* EGTA, 1 m*M* $MgCl_2$, 1.8 m*M* $CaCl_2$, and 10 m*M* HEPES; pH was adjusted to 7.4 by KOH. Patch electrodes were made from the fire-polished borosilicate glass capillaries (Sutter Instrument Co.) using a micropipette puller (model P-97, FLAMING/BROWN micropipette puller; Sutter Instrument Co.). Electrode resistance was 4 to 5 MΩ. Currents were sampled at 10 kHz using an Axon 200B amplifier, digitized *via* a Digidata 1440A interface, and analyzed using pClamp 10.0 (Axon/Molecular Devices). HBOC solution was diluted with extracellular solution to required concentrations, and then applied locally to attached VSMCs at a speed of 150 μl/min through a perfusion pipette (diameter, 0.2 mm) that was positioned 30 to 50 μm away from the patched cells. BK_{Ca} channel currents of VSMCs were recorded before and after exposure to indicated treatment.

Statistical analysis

All values are presented as mean ± SD. An unpaired Student *t* test was used to detect significant differences when two groups were compared. One-way or two-way ANOVA was used to compare the differences among three or more groups followed by Bonferroni's multiple-comparison tests as applicable (SPSS 16.0 software). p values < 0.05 were considered statistically significant.

Supplementary References

1. Fernandes DC, Wosniak J, Pescatore LA, Bertoline MA, Liberman M, Laurindo FR, and Santos CX. Analysis of DHE-derived oxidation products by HPLC in the assessment of superoxide production and NADPH oxidase activity in vascular systems. *Am J Physiol Cell Physiol* 292: C413–C422, 2007.
2. Jaggar JH, Li A, Parfenova H, Liu J, Umstot ES, Dopico AM, and Leffler CW. Heme is a carbon monoxide receptor for large-conductance Ca^{2+}-activated K^+ channels. *Circ Res* 97: 805–812, 2005.
3. Li C, Li T, Zhang J, Wu W, Zhu D, Dian K, and Rao L. Performance of echocardiographic parameters in sequential monitoring of left ventricular function in an animal model of acute heart failure. *Echocardiography* 27: 1274–1281, 2010.
4. Li T, Li J, Liu J, Zhang P, Wu W, Zhou R, Li G, Zhang W, Yi M, and Huang H. Polymerized placenta hemoglobin attenuates ischemia/reperfusion injury and restores the nitroso-redox balance in isolated rat heart. *Free Radic Biol Med* 46: 397–405, 2009.
5. Li T, Wu W, You Z, Zhou R, Li Q, Zhu D, Li H, Xiang X, Irwin MG, and Xia Z. Alternative use of isoflurane and propofol confers superior cardioprotection than using one of them alone in a dog model of cardiopulmonary bypass. *Eur J Pharmacol* 677: 138–146, 2012.
6. Li T, Yu R, Zhang HH, Wang H, Liang WG, Yang XM, and Yang CM. A method for purification and viral inactivation of human placenta hemoglobin. *Artif Cells Blood Substit Immobil Biotechnol* 34: 175–188, 2006.
7. Li T, Zhang P, Liu J, Zhou R, Li Q, You Z, and Dian K. Protective effects of hemoglobin-based oxygen carrier given to isolated heart during ischemia via attenuation of mitochondrial oxidative damage. *Free Radic Biol Med* 48: 1079–1089, 2010.
8. Rafatian N, Milne RW, Leenen FH, and Whitman SC. Role of renin-angiotensin system in activation of macrophages by modified lipoproteins. *Am J Physiol Heart Circ Physiol* 305: H1309–H1320, 2013.
9. Velez JCQ, Ierardi JL, Bland AM, Morinelli TA, Arthur JM, Raymond JR, and Janech MG. Enzymatic processing of angiotensin peptides by human glomerular endothelial cells. *Am J Physiol Renal Physiol 3*02: F1583–F1594, 2012.