

Recombinant Human Hemoglobin with Reduced Nitric Oxide-scavenging Capacity Restores Effectively Pancreatic Microcirculatory Disorders in Hemorrhagic Shock

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Background: Scavenging of nitric oxide by hemoglobin-based oxygen carriers could aggravate microcirculatory failure in splanchnic organs after hemorrhagic shock as a consequence of vasoconstrictive side effects. The aim of this study was to compare the effects of two recombinant human hemoglobin solutions, a second-generation product bearing reduced nitric oxide-scavenging properties (rHb2.0) due to site directed mutagenesis of the heme pocket and a first-generation recombinant hemoglobin (rHb1.1) with scavenging capacity similar to native hemoglobin, on the pancreatic microcirculation after hemorrhagic shock.

Methods: Twenty-eight pentobarbital-anesthetized rats were bled to a mean arterial pressure of 40 mmHg and maintained at this level for 1 h. Using an intravital microscope, the length of erythrocyte-perfused pancreatic capillaries per observation area (functional capillary density) were measured in animals resuscitated by volumes of hydroxyethyl starch, rHb1.1, or rHb2.0 equivalent to the shed blood volume. Animals without shock induction served as control.

Results: As compared with control ($438 \pm 10 \text{ cm}^{-1}$), animals treated with hydroxyethyl starch ($315 \pm 44 \text{ cm}^{-1}$) and rHb1.1 ($288 \pm 67 \text{ cm}^{-1}$) showed a significant reduction of functional capillary density after 2 h of resuscitation. rHb2.0 was able to restore functional capillary density ($410 \pm 42 \text{ cm}^{-1}$) and mean arterial pressure to baseline values.

Conclusion: rHb2.0 was effectively able to restore pancreatic microcirculation after hemorrhagic shock. This may be related to the compound's effective lack of nitric oxide-scavenging properties. This hemoglobin solution or ones similar to it might be uniquely valuable for resuscitation from hemorrhagic shock.

SEVERAL forms of first-generation modified hemoglobins,¹ and perfluorochemical emulsions² are currently undergoing clinical testing as blood substitutes. A major problem during the first therapeutic use of hemoglobin-based oxygen carriers (HBOCs) in a hemorrhagic shock patient 50 yr ago by Amberson *et al.*³ was renal toxicity due to the presence of stromal elements and fast dissociation of the tetrameric molecule. Using special cross-linking and polymerization techniques, modern hemoglobin-based blood substitutes lack these renal side

effects, show higher oxygen affinity, and have prolonged systemic circulation time. Still, some of these solutions result in moderate hypertension and microvascular vasoconstriction. The mechanisms of these undesirable side effects are not completely understood. Nitric oxide (NO) acts as a chemical messenger by activating soluble guanylyl cyclase, resulting in dilation of vascular smooth muscle cells. It is well known that NO reacts rapidly with oxyhemoglobin to form methemoglobin or it binds to deoxy hemoglobin A, thus scavenging NO from the blood and resulting in vasoconstriction. By alteration of the heme pocket in *Escherichia coli*-expressed recombinant hemoglobin, a genetically modified second-generation blood substitute (rHb2.0 for injection) was constructed that has been shown to have a reduced NO-scavenging rate *in vitro*⁴ and no hypertensive side effects *in vivo*⁵. Recently, rHb2.0 was shown to be highly effective for resuscitation of swine after perioperative blood loss.⁶

Pancreatic hypoxia, as a consequence of shock-induced microcirculatory failure, is considered to be a causative factor in the initiation and progression of pancreatic tissue injury⁷ in patients with hemorrhagic shock, contributing to leukocyte activation⁸ and multiple organ failure by the release of activated pancreatic enzymes into systemic circulation.⁹ Therefore, the objective of this study was to investigate the effect of the recombinant hemoglobin-based oxygen carrier with 20- to 30-fold lower rate of NO-scavenging (rHb2.0) in comparison with a human recombinant hemoglobin solution with wild-type scavenging rate (rHb1.1) and the non-oxygen-carrying 6% hydroxyethyl starch (HES) solution on the microhemodynamics and leukocyte adherence to postcapillary venules of the pancreas after hemorrhagic shock.

Materials and Methods

Anesthesia and Monitoring

The experimental protocol of this study was approved by the ethical governmental committee of Bavaria and conforms to the Guiding principles in the Care and Use of Animals as approved by the Council of the American Physiologic Society. Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 180-260 g were anesthetized with ether and pentobarbital (50 mg/kg body weight intraperitoneally) after an overnight fast with free access to tap water. After tracheotomy, respiration was volume controlled (frequency, 57-65 breaths/min; tidal

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volume, 2–2.5 ml; fraction of inspired oxygen [F_{IO_2}], 0.25–0.40; Harvard Rodent Ventilator 683; Harvard Apparatus, Inc., Holliston, MA). The right carotid artery and the right jugular vein were cannulated with a polyethylene catheter (PE-50, 0.58 mm ID; Portex, Hythe, Kent, United Kingdom) for continuous monitoring of mean arterial pressure (MAP) and heart rate. Rectal temperature was kept between 36.5° and 37.5°C by means of a heating pad (Fa. Effenberger, Pfaffing, Germany). Adequate anesthesia was maintained by intravenous infusion of pentobarbital (12 mg · kg body weight⁻¹ · h⁻¹) and nitrous oxide admixture (0.65–0.75) to the inspired gas. Arterial blood gases, acid–base status, and blood lactate concentration were measured intermittently by a blood gas analyser (Ciba Corning 860; Chiron Diagnostics GmbH, Fernwald, Germany) and were adjusted to the baseline values by means of ventilator adjustment and intravenous injection of 8.4% Na-bicarbonate: partial pressure of oxygen (P_{O_2}), 100–120 mmHg; partial pressure of carbon dioxide (P_{CO_2}), 30–40 mmHg; pH, 7.39 ± 0.02; base excess, 0 ± 2. During the experiment, arterial P_{O_2} was kept above 90 mmHg by alteration of F_{IO_2} (shown as $P_{aO_2}:F_{IO_2}$ ratio) and P_{CO_2} below 45 mmHg by alteration of the respiratory rate. Hematocrit of arterial blood was measured by a Coultercounter T540 (Coulter Electronics, Hiialeah, FL).

Animal Model and Experimental Protocol

The hemorrhagic shock model used in the current study is a well-established model of fixed pressure hemorrhage.^{10,11} After recording of baseline value, animals were assigned to a sham-operated control group or blood was withdrawn *via* the arterial catheter to decrease the MAP to 40 mmHg within 10 min. MAP was controlled at 40 mmHg for 60 min by further withdrawal of blood if necessary. At the end of this shock interval, animals were randomly assigned to one of the following resuscitation groups: (1) 6% HES solution (n = 7), (2) recombinant hemoglobin with a wild-type NO-scavenging rate (rHb1.1; n = 7), or (3) recombinant hemoglobin with a reduced NO-scavenging rate (rHb2.0; n = 7). Sham-operated animals without induction of hemorrhagic shock served as a control group (n = 7). Shock animals were resuscitated by means of intravenous infusion given over 7 min of the aforementioned solutions in volumes equivalent to the shed blood volume. The volume (100% of shed volume) has proven its efficacy in several hemorrhagic shock experiments in rats.^{12,13} After resuscitation and a 15-min stabilization period, animals underwent a transverse laparotomy for exteriorization of the pancreas and spleen. The organs were placed on an adjustable microscope stage; both organs were covered by a thin Teflon[®] membrane to prevent drying. To prevent cooling of the organs, the whole animal and the stage were covered by swabs during the time between the microscopy measurements. MAP was contin-

uously recorded on a recorder (Siemens XT Kompenso-graph; Siemens, Munich Germany). Blood gases, lactate concentration, and hematocrit of arterial blood were measured at baseline conditions, at the end of shock, and at 15, 60, and 120 min after administration of the solutions. The experiments were terminated by intravenous injection of an overdose of pentobarbital.

Drugs

Hydroxyethyl starch, 6%, 200,000/0.5 (oncotic pressure: 36 mmHg, isotonic in 0.9% NaCl solution), was purchased from Fresenius AG (Bad Homburg, Germany).

rHb2.0 for injection was supplied as a deoxy solution. The material was supplied in 5 ml polystyrene tubes packaged within oxygen-impermeable pouches (Baxter Hemoglobin Therapeutics, Boulder, CO). rHb2.0 is a second-generation recombinant human hemoglobin in which the reaction rate with NO has been reduced by 20- to 30-fold. It is expressed in *E. coli* and extensively purified; the α chains are also fused to prevent dissociation. Amino acid substitutions were made in the distal heme pocket by site-directed mutagenesis to reduce the rate of NO scavenging. rHb2.0 is polyethylene glycol polymerized and derivatized and is formulated at a concentration of approximately 100 g/l in a gluconated electrolyte solution. At this hemoglobin concentration, rHb2.0 has a viscosity of approximately 2.3 cP and a colloid osmotic pressure of approximately 62 mmHg. The oxygen half-saturation pressure of rHb2.0 is 34 mmHg. Containers were stored at 4°C.

The rHb1.1 was supplied frozen (–70° to –80°C) in the oxyhemoglobin form (Baxter Hemoglobin Therapeutics). It is a first-generation recombinant human hemoglobin expressed in *E. coli*.^{14,15} The hemoglobin is a stabilized pseudotetramer with a molecular weight of 64 and a oxygen half-saturation pressure of 32 mmHg. rHb1.1 is formulated at a concentration of 100 g/l in phosphate-buffered saline with a viscosity of approximately 1.9 cP and a colloid osmotic pressure of approximately 40 mmHg. rHb1.1 was used within 2 h after thawing.

Intravital Microscopy and Quantification of Microvascular Parameters

Intravital microscopy of the pancreas was performed using a modified Leitz-Orthoplan microscope (Leitz, Wetzlar, Germany) with a mercury lamp (100 W, oxygenated hemoglobin) attached to a Ploemo-Pak illuminator (Leitz) with $I_{2/3}$ (excitation 450–490 nm, emission greater than 515 nm, used for leukocyte adherence) and N_2 (excitation 530–560 nm, emission greater than 580 nm, used for functional capillary density [FCD]) filter blocks (Leitz) for epi-illumination. Bovine serum albumin, 0.15 ml (0.75%), was labeled with the fluorochrome fluorescein isothiocyanate (Sigma, St. Louis; MO) before the first microcirculatory measurement time

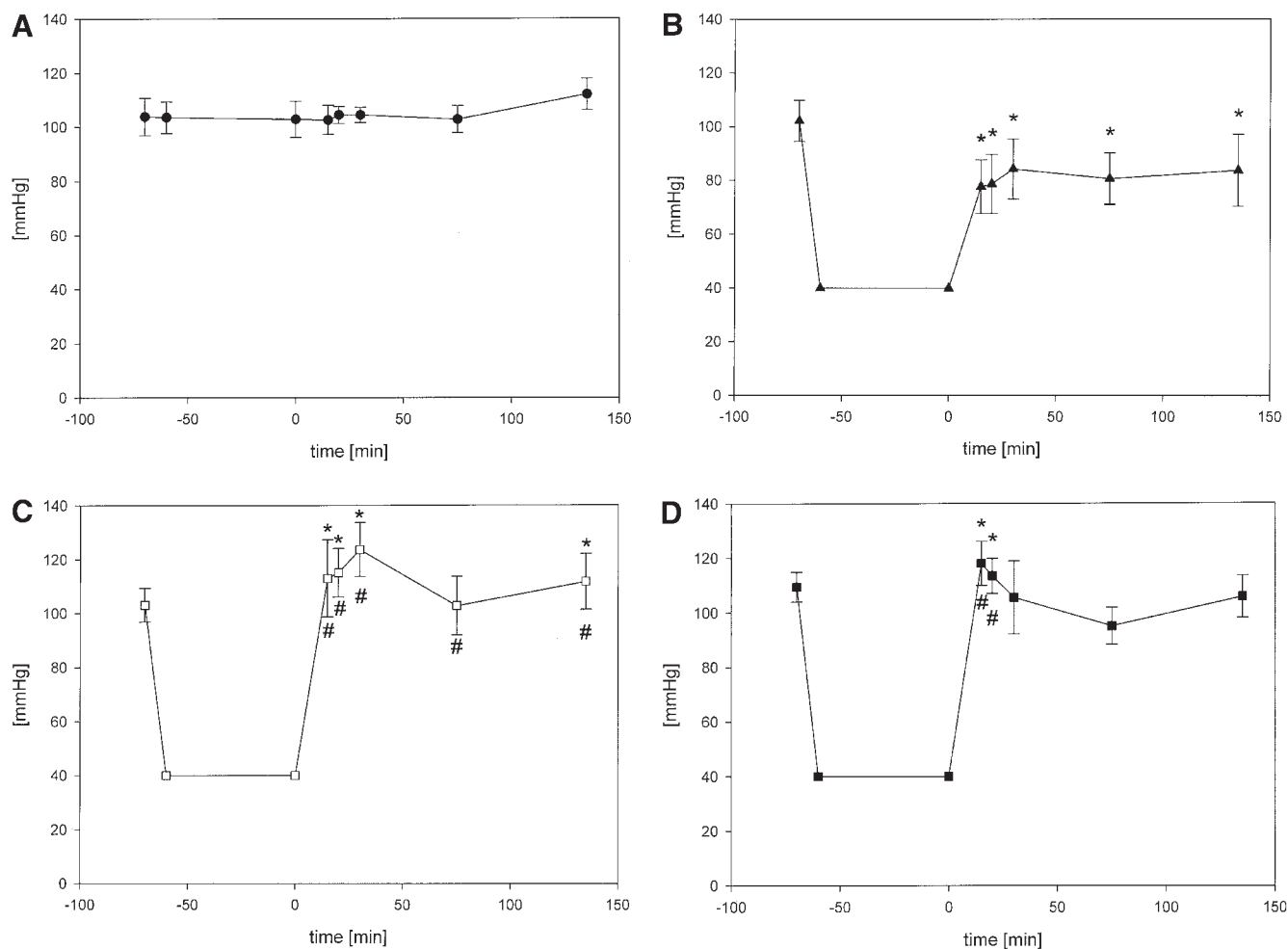


Fig. 1. Mean arterial pressure during the experiment in sham-operated animals (A) and in hydroxyethyl starch (B), rHb1.1 (C), and rHb2.0 (D) treated animals. Values are presented as mean \pm SD. * $P < 0.05$ is significant versus baseline; # $P < 0.05$ is significant versus HES.

point. A saltwater immersion objective (SW $\times 25/0.6$; Leitz) allowed magnification of approximately $800\times$. The observations were recorded by means of a charge-coupled device video camera (FK 6990; Cohu, Prospective Measurements, San Diego, CA) and stored on videotape (video recorder; AG-Panasonic, Munich, Germany) for off-line evaluation. FCD was measured at 60 and 120 min after injection of the solutions. FCD is defined as the length of erythrocyte-perfused capillaries (cm) per observation area (cm^2).¹⁶ The FCD was determined by analysis of the videotapes using CAMAS image-analyzing system (Dr. Zeintl Inc., Biomedical Engineering, Heidelberg, Germany). Ten randomly selected regions of interest ($400 \times 300 \mu\text{m}$) of the pancreas were evaluated at each time point.

Statistical Analysis

For analysis between groups, the Kruskal-Wallis test followed by a pairwise Dunn comparison procedure were used. For analysis within the groups, the Fried-

mann and Wilcoxon tests were used. Differences were considered statistically significant at $P < 0.05$. All data are presented as mean \pm SD.

Results

Macrobiodynamics, Acid-Base Status, and Blood Lactate Concentration

The volumes of blood withdrawn during the shock period were 7.6 ± 0.4 , 7.8 ± 0.9 , and 7.9 ± 0.9 ml in the HES, rHb1.1, and rHb2.0 groups, respectively, with no statistically significant differences. In the sham-operated group, MAP (fig. 1) stayed above 100 mmHg during the whole experiment. Resuscitation with HES could not restore the macrocirculatory parameters to baseline values. After resuscitation with HES, all values were significantly lower as compared to baseline. rHb2.0 restored MAP to similar values as in sham-operated animals. rHb1.1 resulted in a higher blood pressure compared with HES and rHb2.0 during the first 15 min after injec-

Table 1. Heart Rate, Pao₂/Fio₂ Ratio, and Systemic Hematocrit

Parameter	Baseline	Shock	Time Postresuscitation		
			15 Min	60 Min	120 Min
Heart rate, beats/min					
Sham control	348 ± 20	381 ± 15	378 ± 31	368 ± 36	364 ± 42
HES	381 ± 27	365 ± 38	390 ± 38	392 ± 33	381 ± 49
rHb1.1	390 ± 38	356 ± 26	368 ± 34	375 ± 35	381 ± 30
rHb2.0	397 ± 27	357 ± 36	365 ± 25	394 ± 31	410 ± 46
Pao ₂ /Fio ₂ ratio					
Sham control	334 ± 70	314 ± 35	338 ± 92	314 ± 44	299 ± 35
HES	301 ± 41	425 ± 59*†	265 ± 48	296 ± 42	330 ± 63
rHb1.1	326 ± 66	420 ± 37*	264 ± 42	316 ± 51	314 ± 41
rHb2.0	316 ± 30	416 ± 69*	337 ± 303	354 ± 94	321 ± 32
Hematocrit, %					
Sham control	41 ± 2.6	41 ± 2.2	41 ± 2.8	42 ± 2.1	42 ± 1.6
HES	41 ± 2.1	31 ± 2.8*	16 ± 2.6*†	19 ± 3.0*†	20 ± 3.2*†
rHb1.1	43 ± 2.6	31 ± 3.2*	19 ± 2.1†	20 ± 2.0†	20 ± 2.5*†
rHb2.0	42 ± 1.8	31 ± 2.6*	16 ± 1.7*	17 ± 1.2*†	18 ± 1.1*†

* P < 0.05 is significant vs. sham control. † P < 0.05 is significant vs. baseline.

Fio₂ = fraction of inspired oxygen; HES = hydroxyethyl starch; Min = minutes; Pao₂ = arterial oxygen tension; rHb1.1 = first-generation recombinant hemoglobin; rHb2.0 = second-generation recombinant hemoglobin.

tion, and the difference was significant as compared with the HES-resuscitated group. There were no significant differences in heart rate (table 1) between the individual groups. The Pao₂:Fio₂ ratio was significantly lower during shock as compared with the sham-operated control group. Systemic hematocrit significantly decreased during hemorrhagic shock and furthermore after resuscitation without significant differences between the three resuscitation regimes. There was a significant reduction of negative base excess in all shock groups with a slower return of negative base excess 15 min after HES resuscitation. rHb1.1 and rHb2.0 immediately reversed acidosis (table 2). Arterial lactate concentration increased to the same degree during shock in all groups but significantly more in the HES group. It was restored to baseline values in all resuscitation groups.

Functional Capillary Density

Functional capillary density (fig. 2) was not restored by HES resuscitation and was significantly lower (315 ±

44 cm⁻¹) as compared with sham-operated animals (438 ± 10 cm⁻¹) after 2 h of resuscitation. rHb1.1, the hemoglobin solution with a wild-type NO-scavenging rate, showed an even less efficient restoration of FCD (288 ± 67 cm⁻¹). In contrast to these two resuscitation regimes, rHb2.0 restored FCD (410 ± 42 cm⁻¹) to baseline values.

Discussion

In the current experimental study, we showed that recombinant human hemoglobin solution with a reduced NO-scavenging rate (rHb2.0) is superior to an oxygen carrier with scavenging rates similar to wild-type hemoglobin (rHb1.1) in restoring pancreatic capillary perfusion after hemorrhagic shock. In contrast to a non-oxygen-carrying colloid (6% HES, 200,000/0.5), both hemoglobin products restored macrohemodynamics and systemic shock parameters, such as negative base excess and lactate, immediately after resuscitation.

Table 2. Base Excess and Lactate Values

Parameter	Baseline	Shock	Postresuscitation		
			15 Min	60 Min	120 Min
Base excess (mm)					
Sham control	-0.26 ± 1.7	-1.36 ± 2.8	-0.11 ± 3.6	-1.21 ± 2.7	-2.13 ± 3.2
HES	-1.09 ± 2.1	-9.17 ± 2.5*†	-4.9 ± 3.2*	-2.83 ± 2.6	-3.04 ± 3.1
rHb1.1	0.5 ± 1.5	-8.2 ± 2.3*†	-0.96 ± 6.1	0.99 ± 4.6	0.6 ± 3.1
rHb2.0	-0.5 ± 1.9	-7.7 ± 2.6*†	-1.74 ± 2.7	-0.37 ± 2.4	-0.16 ± 1.7
Lactate (mm)					
Sham control	1.92 ± 0.7	1.38 ± 0.6	1.54 ± 0.7	1.59 ± 1.1	1.03 ± 0.4*
HES	1.41 ± 0.8	4.8 ± 1.35*†	2.49 ± 1.9	1.56 ± 0.8	1.38 ± 0.8
rHb1.1	1.8 ± 0.8	4.7 ± 1.0	1.13 ± 0.9	0.89 ± 0.5	0.97 ± 0.3
rHb2.0	1.54 ± 0.8	4.0 ± 0.9	1.18 ± 0.5	0.88 ± 0.3	0.82 ± 0.6

* P < 0.05 is significant vs. baseline. † P < 0.05 is significant vs. sham control.

Fio₂ = fraction of inspired oxygen; HES = hydroxyethyl starch; Min = minutes; Pao₂ = arterial oxygen tension; rHb1.1 = first-generation recombinant hemoglobin; rHb2.0 = second-generation recombinant hemoglobin.

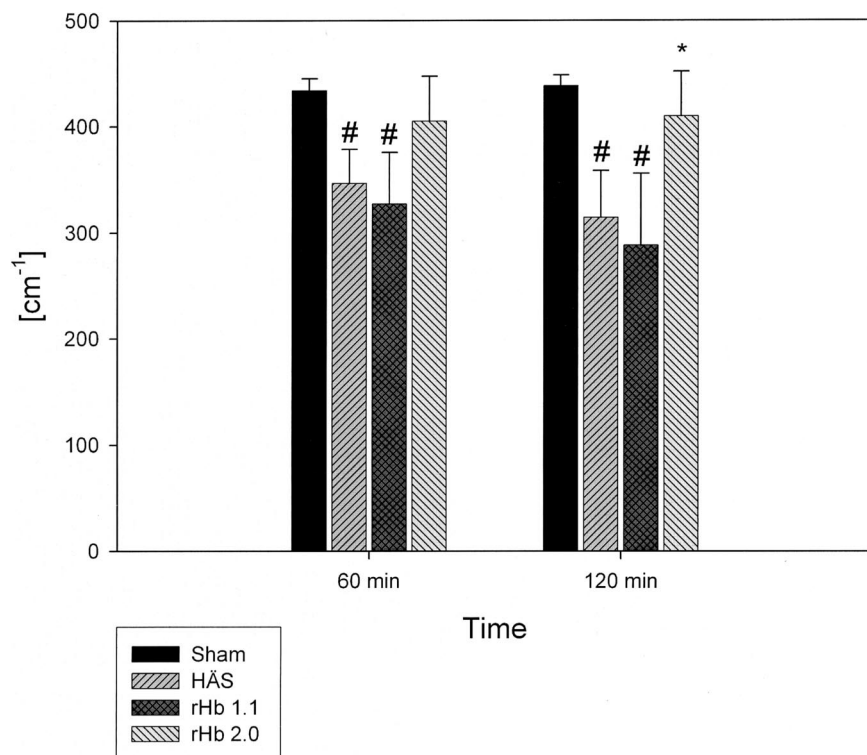


Fig. 2. Functional capillary density of the exocrine pancreas. * Significant versus rHb1.1, $P < 0.05$; # significant versus sham, $P < 0.05$.

Next to rapid surgical intervention, blood substitution is the treatment of choice in patients with hemorrhagic shock and blood loss. Although the initial cell injury induced by the reduced oxygen supply to vital organs during hemorrhage can probably not be reverted completely by oxygen carriers,¹⁷ restoration of the microcirculation is the main target of sufficient resuscitation after primary restoration of macrohemodynamics to prevent further damage. Both experimental studies and clinical trials have demonstrated systemic and vasoconstrictive side effects of the different first-generation stroma-free hemoglobin solutions.¹ Several mechanisms such as scavenging of NO,¹⁸ increased endothelin synthesis apparent in clinical studies by vasoconstriction,¹⁹ sensitization of α -adrenergic receptors,²⁰ and disturbance of precapillary autoregulation are discussed.²¹ There is speculation that the higher endothelin concentration on infusion of HBOC is due to concomitant decreasing of NO because there is a close balance between the endothelium-derived relaxation by NO and the constriction due to endothelin 1.²² Therefore, although the main mechanisms of pressor actions of hemoglobin-based oxygen carriers are not completely clear, the NO-scavenging property of extravascular tetrameric hemoglobin seems to be predominantly involved. Up to now, the vasoconstrictive side effects of stroma-free hemoglobins could only be reduced by polymerization of the tetramer, presumably resulting in reduced extravasation of the molecules with less vasoconstriction and a therapeutic benefit in trauma patients.²³

Because of progress in biotechnology, Doherty *et al.*⁵

synthesized different recombinant hemoglobin solutions with genetic alteration of the distal heme pocket, which exhibited reduced scavenging of NO. The systemic hypertensive response in conscious rats was directly related to the rate of *in vitro* NO oxidation by hemoglobin. In the current study, both hemoglobin solutions tested restored macrohemodynamics immediately after infusion. However, in contrast to rHb2.0, the hemoglobin solution with scavenging property similar to wild type (rHb1.1) showed a hypertensive effect during the first 15 min after injection.

Nitric oxide itself is a well-known mediator of regulation of splanchnic microcirculation. Influences on pancreatic microcirculation and inflammatory damage are described. The administration of NO donors resulted in an amelioration of experimental acute pancreatitis, whereas the administration of NO inhibitors caused an aggravation of experimental acute pancreatitis.²⁴ In an intravital microscopy study, administration of an NO synthase inhibitor was found to be associated with an increase in leukocyte adherence in postcapillary venules of rats with cerulein induced acute pancreatitis.²⁵ Another animal study showed a significant increase of NO production after ischemia-reperfusion of the pancreas.²⁶ Contradictory to the above studies, a beneficial impact of inhibiting NO synthase in severe experimental pancreatitis with a 100% mortality in rats due to hypotension in the untreated control group has been described.²⁷ Therefore, the potential relevance of effects of hemoglobin-based oxygen carriers on pancreatic micro-

circulation by changing the NO-scavenging property is obvious.

The role of the pancreas in the pathophysiology of hemorrhagic shock has been under examination for many years.²⁸ By blocking pancreatic enzymes in the ischemic intestine during shock, systemic activation of leukocytes could be prevented.⁸ Therefore, the pancreatic tissue damage in hemorrhagic shock seems mainly to be caused by the ischemia and the ongoing microcirculatory failure during reperfusion probably contributing to the systemic inflammatory response syndrome and multiple organ failure.

During the study of the human hemoglobin-based oxygen carrier DCLHb²⁹ and the currently used rHb1.1,³⁰ clinical cases of hyperamylasemia and acute pancreatitis occurred. Although hyperamylasemia is a common phenomenon in critical care patients, the correlation with infusion of hemoglobin solutions could never be excluded. First, the NO-scavenging properties of hemoglobin-based oxygen carriers were suspected to cause microcirculatory failure and spasmus of the sphincter of Oddi with consecutive induction of inflammation in the pancreas. These hypotheses have been investigated in experiments with the opossum, suggesting that HBOCs affect duodenal and transsphincteric flow by an NO-scavenging effect, which was weaker after DCLHb administration³¹ as compared with rHb1.1.³² In our previous studies, we could not find a detrimental effect of DCLHb on pancreatic microcirculation after top-load infusion either on pancreatic ischemia-reperfusion damage³³ or on hemorrhaged microvascular perfusion¹⁰ of rats. In contrast to these former results, we have shown a detrimental effect of rHb1.1 on pancreatic microcirculation in the current study. This finding is consistent with the results of the study by Loeb *et al.*,³⁴ who demonstrated a reduction of gastrointestinal perfusion after top-load infusion of rHb1.1. FCD is an index of the quality of capillary perfusion and has proven to be a suitable indicator of successful resuscitation after severe hemorrhagic shock.³⁵ It should be noted that only resuscitation with rHb2.0 resulted in a complete restoration of pancreatic capillary perfusion.

Next to the above discussed reasons for improvement of FCD by reduction of NO-scavenging capacity of hemoglobin, other investigators have shown that different hemoglobins with the same NO kinetics have different vasoactivities as evident from the increase of MAP. They have attributed this property to differences in oxygen affinities and rheologic properties of the hemoglobin solutions such as molecular diffusion across the endothelial barrier, which is a function of molecular size, and viscosity.³⁶ Furthermore, it is known from studies with extreme hemodilution in hamsters that colloidal fluids with a higher viscosity can preserve FCD and herewith organ viability after a reduction of more than 60% of systemic hematocrit.³⁷ In contrast, capillary perfusion

cannot be provided by low-viscosity fluids in states with very low blood viscosity.³⁸ It could also be shown that resuscitation with an HBOC with a left-shifted oxygen diffusion curve, a low hemoglobin oxygen half-saturation pressure of 5.5 mmHg, a colloid osmotic pressure of 49 mmHg, and solution viscosity of 2.4 cP could more effectively restore microvascular perfusion after hemorrhagic shock as compared with HES or autologous blood, an effect which was thought to be due to the increased viscosity of the HBOC.³⁹ From our results, it cannot completely be excluded that capillary perfusion is influenced by such a specific property of the infused fluids because none of these solutions can ever have fully identical properties. Regarding the pulmonary vascular permeability, it was shown that both hemoglobins used have similar effects.⁴ All three solutions studied have similar viscosities (2.0, 1.9, and 2.1 cP for HES, rHb1.1, and rHb2.0, respectively). The transfusion viscosity trigger during extreme hemodilution at which viscosity properties of the infused colloid, which affected functional capillary perfusion, is reached at much lower hematocrit values than measured in our study.^{37,38} A difference of plasma retention time and the higher colloid osmotic pressure of rHb2.0 may contribute to an increased plasma expansion by the solution as compared with the other two fluids. Systemic hematocrit does not show significant differences between the three solutions during resuscitation time (table 1). Therefore, viscosity differences and different plasma expansion characteristics may not be the main factors responsible for the improved functional capillary perfusion after rHb2.0 injection.

Using the same shock model and resuscitation with a cross-linked human hemoglobin solution, a much higher FiO_2 was needed to maintain a normal arterial PaO_2 immediately after injection.¹⁰ This need was apparent from the significant decrease of $\text{PaO}_2:\text{FiO}_2$ ratio 15 min on infusion in contrast to the other groups. We interpret this finding as an NO-dependent increase of pulmonary pressure after infusion, as shown with other HBOCs. In a recent study in isolated rat lungs, pulmonary hypertensive responses were greatly reduced when rHb2.0 was compared to rHb1.1.⁴ In the current study, we could not detect such pulmonary side effects after resuscitation with either rHb2.0 or rHb1.1, as evidenced by a stable $\text{PaO}_2:\text{FiO}_2$ ratio during the whole experiment, indicating its safety in respect to pulmonary circulation.

In conclusion, rHb2.0 is an effective resuscitation fluid enabling adequate restoration of the pancreatic microcirculation after hemorrhagic shock. The positive effects of rHb2.0 are probably due to the reduction of NO scavenging because the vasoconstrictive side effects and inflammation-enhancing properties seen with rHb1.1 could not be detected with rHb2.0. Therefore, this novel recombinant hemoglobin product, rHb2.0, or ones sim-

ilar to it might be uniquely valuable for resuscitation from hemorrhagic shock.

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