

Diaspirin Cross-linked Hemoglobin Effectively Restores Pancreatic Microcirculatory Failure in Hemorrhagic Shock

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Background: Microvascular reperfusion failure of splanchnic organs is a crucial hallmark in organ damage induced by hemorrhagic shock, which should be prevented by a resuscitation solution. Because the vasoactive properties of the hemoglobin-based oxygen carrier diaspirin cross-linked hemoglobin (DCLHb) could adversely influence restoration of pancreatic capillary perfusion during resuscitation, the authors investigated its effects on the microcirculation of the rat pancreas in comparison with whole blood and 6% hydroxyethylstarch resuscitation from severe hemorrhagic shock.

Methods: Twenty-eight pentobarbital-anesthetized rats were bled to a mean arterial pressure (MAP) of 40 mmHg and maintained at this level for 1 h. Using an intravital microscope, mean arterial pressure, the length of erythrocyte-perfused pancreatic capillaries per observation area (functional capillary density), the adherence of leukocytes in postcapillary venules, and pancreatic lipid peroxidation, measured as thiobarbituric acid-reactive material in pancreatic tissue, were determined in animals resuscitated by volumes of hydroxyethylstarch, DCLHb, and whole blood (WB) equivalent to the shed blood volume or in

control animals without shock induction for a period of 2 h after resuscitation.

Results: Compared with control animals ($366 \pm 28 \text{ cm}^{-1}$), animals resuscitated with DCLHb ($294 \pm 45 \text{ cm}^{-1}$), WB ($306 \pm 11 \text{ cm}^{-1}$), and hydroxyethylstarch ($241 \pm 34 \text{ cm}^{-1}$) showed a significant reduction of functional capillary density after 2 h of resuscitation. DCLHb was as effective as WB and superior to hydroxyethylstarch in restoring functional capillary density and mean arterial pressure. Leukocyte adherence in postcapillary venules was not enhanced by DCLHb ($369 \pm 148/\text{mm}^2$) infusion when compared with hydroxyethylstarch ($615 \pm 283/\text{mm}^2$) and WB-treated ($510 \pm 415/\text{mm}^2$) animals. Lipid peroxidation of pancreatic tissue was significantly elevated after treatment with both oxygen-carrying solutions compared with hydroxyethylstarch.

Conclusion: DCLHb is as effective as WB for preservation of the pancreatic microcirculation. (Key Words: Acute pancreatitis; blood substitute; hemoglobin solution; leukocyte-endothelium interaction; transfusion.)

SEVERE hemorrhagic shock is characterized by inadequate microcirculatory perfusion and subsequent reperfusion, altered cell membrane permeability, subsequent organ failure caused by cellular dysfunction, and reaction of the immune system.¹ The recovery of macrohemodynamics and the fast restoration of microvascular perfusion and oxygen supply to vital organs are the major aims of primary shock therapy. The oxygen-carrying blood substitute diaspirin cross-linked hemoglobin (DCLHb) is free from immunogenicity in humans² and has lower risks of infections compared with homologous blood.³ Hypoperfusion of the pancreas and consecutive ischemia-reperfusion damage of the organ are considered to be the main causes of acute pancreatitis that occur in patients after hemorrhagic shock.^{4,5} Another hallmark of this postischemic organ injury is the generation of oxygen free radicals produced by activated leukocytes⁶ and xanthine oxidase⁷ during the process of reperfusion. These radicals are implicated in several toxic pathways, including lipid peroxidation and damage of DNA.⁸ Cell-free hemoglobin could also be the source of deleterious oxygen free radicals when admin-

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istered as a resuscitation fluid after hemorrhagic shock.⁹ In animals and humans,^{10,11} DCLHb produces an elevation of mean arterial pressure (MAP), which is probably caused by its nitric oxide (NO)-scavenging¹² and endothelin-1-releasing¹³ properties. Endothelin is known to reduce pancreatic perfusion.¹⁴ The vasoactive effects of DCLHb could influence microcirculatory shock-induced damage to the pancreas. No studies relevant to shock-related pathology have investigated alterations of capillary perfusion and leukocyte adherence by DCLHb treatment in splanchnic organs after hemorrhage. Therefore, the objective of this study was to investigate the effects of DCLHb on the microhemodynamics, leukocyte adherence, and degree of lipid peroxidation of the rat pancreas in comparison with resuscitation with whole blood (WB) and a non-oxygen-carrying colloid solution after hemorrhagic shock.

Materials and Methods

Anesthesia and Monitoring

The experimental protocol of this study was approved by the ethical governmental committee of Oberbayern 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 volume: 2–2.5 ml; fraction of inspired oxygen [$F_{I_{O_2}}$]: 0.25–0.40, Harvard Rodent Ventilator 683; Harvard Apparatus, South Natick, 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, UK) for continuous monitoring of MAP and heart rate. Rectal temperature was kept between 36.5 and 37.5°C by means of a heating pad (Firma 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) in the inspired gas. Arterial blood gases, acid-base status, and blood lactate concentration were measured intermittently by a blood gas analyzer (Ciba Corning 860; Chiron diagnostics GmbH, Fernwald, Germany) and were adjusted to the following values for baseline conditions by means of ventilator adjustment and intravenous injection of 8.4% sodium 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 ; and base excess: 0 ± 2 mm. During the experiment, arterial P_{O_2} was kept above 90 mmHg by alteration of $F_{I_{O_2}}$ (shown as arterial oxygen tension ($P_{a_{O_2}}$)/ $F_{I_{O_2}}$ ratio) and P_{CO_2} was kept below 45 mmHg by alteration of the breathing frequency. Hematocrit in arterial blood was measured using a Coultercounter T540 (Coulter Electronics, Hialeah, FL).

Animal Model and Experimental Protocol

The hemorrhagic shock model used in the current study is a well-established model of fixed-pressure hemorrhage.¹⁵ After recording of baseline values, blood was withdrawn *via* the arterial catheter to decrease the MAP to 40 mmHg within 10 min. Blood pressure was controlled at 40 mmHg for 60 min by further withdrawal of blood if necessary. After this shock interval, animals were randomly assigned to one of the following resuscitation groups: (1) 6% hydroxyethylstarch solution (n = 7); (2) 10% DCLHb solution (n = 7); (3) WB (n = 7). (4) Sham-operated animals without induction of hemorrhagic shock served as the control group (n = 7). Shock animals were resuscitated by means of intravenous infusion of the aforementioned solutions in volumes equivalent to the shed blood volume administered over 7 min. The dose of DCLHb (100% of shed volume) has proven its effectiveness in several hemorrhagic shock experiments in rats.^{16,17} After resuscitation and a 15-min stabilization period, animals underwent 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 transparent plastic membrane to prevent drying. To prevent cooling of the organs, the whole animal and the stage were covered by swabs between the microscopy measurements. MAP was continuously registered on a recorder (Siemens XT Kompensograph, Siemens, Munich, Germany). Heart rate was obtained from the phasic blood pressure curves. Heart rate, arterial blood gases, lactate concentration, and hematocrit 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 discontinued by intravenous injection of an overdose of pentobarbital.

Drugs and WB Preparation

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

DCLHb was provided by Baxter Healthcare Corp. (Round Lake, IL; lot number 97J10AD11-111997). According to the manufacturer, 250 ml DCLHb contains 25 g hemoglobin, 1.425 g NaCl, 0.950 g sodium DL-lactate, 0.075 g KCl, 0.0325 g calcium chloride, 0.010 g magnesium chloride, water for injections to a volume of 250 ml. methemoglobin concentration was less than 5%; P_{50} was 32 mmHg; pH was adjusted to 7.4 at 37°C; oncotic pressure was 42 mmHg. Containers were stored in a -70°C freezer and thawed 0.5 h before use. The animals in the WB group received their autologous shed blood, which was drawn into a syringe containing CPDA-1 in the same mixture relation as in donation packs (63 ml CPDA-1/450 ml blood). CPDA-1 was withdrawn from original packs provided for blood donations (Baxter S.A., Maurepas, France).

Intravital Microscopy and Quantification of Microvascular Parameters

Hydroxyethylstarch, 0.75%, 0.15 ml, (molecular weight 200,000 d), labeled with the fluorochrome fluorescein isothiocyanate (Laevosan, Linz, Austria) for contrast enhancement of microvessels and 0.1 ml Rhodamin 6G, 0.2%, (molecular weight 497; Sigma, St Louis, MO) for *in vivo* staining of cytochrome C-containing cells (leukocytes) were injected intravenously in the right jugular vein before the first microcirculatory measurement time point. Intravital microscopy of the pancreas was performed using a modified Leitz-Orthoplan microscope (Leitz, Wetzlar, Germany) with a mercury lamp (100 W, HBO) 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 epiillumination. A saltwater immersion objective (SWX25/0.6; Leitz) allowed magnification of approximately 800×. The observations were recorded by means of a charge-coupled device (CCD) video camera (FK 6990; Cohu, Prospective Measurements, San Diego, CA) and stored on video tape (video recorder; AG-Panasonic, Munich, Germany) for off-line evaluation. Quantitative assessment of the microcirculation included determination of the FCD and the number of adherent leukocytes in the postcapillary venules. These parameters were measured at three time points: 45, 90, and 120 min after injection of the solutions. FCD is defined as the length

of erythrocyte-perfused capillaries (cm) per observation area (cm²).¹⁸ The FCD, as determined by analysis of the video tapes according to Schmid-Schönbein¹⁹ by means of superimposing a grid (squared-type) on the video screen (square side, 50 μm). The number of intersections of erythrocyte-perfused capillaries with the grid system were counted, and FCD was calculated using the following two formulas:²⁰ $FCD = Lc_0 = \pi/2 \times N_c/L$; $L = 2 \times P \times d$; where Lc is the length of perfused capillaries, N_c is the number of intersections, P is the number of squares of the grid, d is the length of the edge of the grid. Ten randomly selected regions of interest (400 × 300 μm) of the pancreas were evaluated at each time point. For quantification of leukocyte-endothelial interaction, at least three postcapillary venules (diameter < 40 μm and < 150 μm length) per animal were recorded for 30 s at each time point. Adherent leukocytes were defined as cells remaining stationary on the surface of the endothelium for the whole observation time of 30 s. The surface area of the vessel segments was calculated based on diameter measurement assuming a cylindrical geometry of the vessels. Adherent leukocytes are given as cells/endothelial surface (cells/mm²).

Measurement of Lipid Peroxidation in Pancreatic Tissue

Lipid peroxidation of pancreatic tissue was quantified by an assay that measured the thiobarbituric acid-reactive materials (TBARs).²¹ Pancreatic tissue was harvested and shock-frozen immediately after the end of the experiments. Samples were stored in a -70° freezer until analysis. Before analysis, samples were cut by a shaving knife into small pieces. 10 ml KCl solution, 1.1%, was mixed with 1 g pancreatic tissue. The suspension was homogenized at 1,200 rpm for 5 min (homogenisator; Braun, Melsungen, Germany). The homogenate was immediately analyzed photometrically.

Light microscopy

Tissue samples from the corpus of the pancreas were taken at the end of the experiment and immediately fixed in 10% neutral-buffered formalin. The samples were dehydrated, embedded in paraffin, cut at approximately 3 μm (Microtome; Leica, Munich, Germany), and stained with hematoxylin and eosin. One tissue section/animal was evaluated by a blinded observer for edema, inflammation (infiltration of granulocytes), parenchymal necrosis, and hemorrhage.

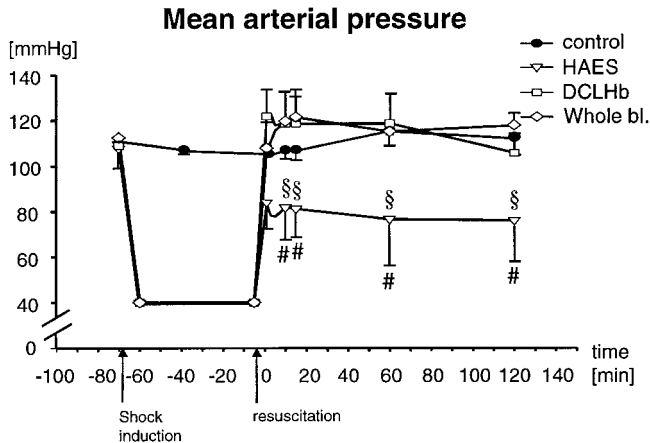


Fig. 1. Changes of mean arterial pressure (MAP) during 1 h hemorrhagic hypotension and resuscitation time. Values are expressed as the mean \pm SD. # $P < 0.05$ is significant versus control, DCLHb, and WB; § $P < 0.05$ is significant versus baseline.

Statistical Analysis

All data are presented as the mean values \pm SD. Data analysis was performed using a statistical software package (STATISTICA; StatSoft GmbH, Hamburg, Germany). Statistical comparison was performed by a two-way analysis of variance (one-way in TBARMs) followed by a Student-Newman-Keuls test. $P < 0.05$ was considered to be statistically significant.

Results

Macrobiodynamics and Hematocrit and Hemoglobin Concentrations

The volume of withdrawn blood during shock was 6.6 ± 0.8 ml, 8.1 ± 0.8 ml, and 8.4 ± 1.0 ml in the hydroxyethylstarch, DCLHb, and WB groups, respectively. DCLHb and WB restored MAP to the values of the control group immediately after injection (fig. 1). Hydroxyethylstarch resulted in a significantly lower MAP after resuscitation compared with control, DCLHb, and WB animals. There were no significant differences in heart rate among the three experimental groups (table 1). The hematocrit values of the DCLHb and hydroxyethylstarch groups were significantly lower than the values in the control and WB groups during the resuscitation time. During shock, the hematocrit value in the hydroxyethylstarch group was significantly higher compared with the DCLHb and WB groups. However, hydroxyethylstarch resuscitation 15 min after injection resulted in a significantly lower hematocrit value than did

DCLHb. DCLHb resuscitation showed significantly higher hemoglobin concentrations during resuscitation compared with hydroxyethylstarch.

Functional Capillary Density

Functional capillary density was significantly reduced in all resuscitation groups compared with the control group during the whole observation time (fig. 2). Two hours after injection, FCD was restored to 66 ($P < 0.0002$ vs. control), 80 ($P < 0.003$) and 84% ($P < 0.012$) of control values (366 ± 28 cm^{-1}) by hydroxyethylstarch, DCLHb, and WB resuscitation, respectively. At the end of the experiment, FCD was significantly higher in the DCLHb ($P < 0.04$) and WB ($P < 0.01$) animals compared with the hydroxyethylstarch-resuscitated animals. There was no significant difference of FCD between the DCLHb and WB groups during the experimentation.

Leukocyte-Endothelium Interaction

Hydroxyethylstarch-treated animals showed a significant ($P < 0.01$) 4.1-fold increase of leukocyte adherence in postcapillary pancreatic venules compared with the control group (149 ± 111 cells/ mm^2) at the end of the experiment (fig. 3). Although there is an elevation of leukocyte adherence in all other resuscitation groups, the values were not significantly different at any time point among the groups or within each group during the three measurements.

Acid-Base Balance, $P_{\text{O}_2}/F_{\text{I}\text{O}_2}$ Ratio, Blood Lactate

The bolus injection of DCLHb resulted in a rapid need to increase $F_{\text{I}\text{O}_2}$ by at least 20% to maintain normal arterial P_{O_2} blood gas values during the first 15 min after infusion. This resulted in a significant ($P < 0.0003$) reduction of the $P_{\text{O}_2}/F_{\text{I}\text{O}_2}$ ratio, compared with the other groups (table 2). Shock increased negative base excess and blood lactate concentration significantly ($P < 0.05$) in all groups, compared with the control group. DCLHb improved recovery from acidosis more effectively than hydroxyethylstarch resuscitation at the end of the observation time. There were no significant differences of blood lactate concentration at any time after resuscitation in the three experimental groups.

Thiobarbituric Acid-reactive Materials Concentration in Pancreatic Tissue

Thiobarbituric acid-reactive materials tissue concentration, as a parameter of peroxidative tissue injury, was measured 2 h after resuscitation. TBARMs could not be

Table 1. The Effect of DCLHb on Heart Rate, Hematocrit, and Hemoglobin

Parameter	Baseline	Shock	15 min Postresuscitation	60 min Postresuscitation	120 min Postresuscitation
Heart rate (beats/min)					
Sham control	370 ± 10	370 ± 8	367 ± 15	361 ± 8	364 ± 21
1 h shock + HES	355 ± 21	352 ± 43	327 ± 32	321 ± 25	324 ± 26
1 h shock + DCLHb	383 ± 26	330 ± 30*	352 ± 21	346 ± 40	324 ± 26*
1 h shock + whole blood	358 ± 30	334 ± 35	346 ± 26	330 ± 21	355 ± 27
Hematocrit (%)					
Sham control	39 ± 2	39 ± 2	40 ± 1	41 ± 2	39 ± 2
1 h shock + HES	41 ± 2	31 ± 5*†§	15 ± 3*†§	18 ± 3*†§	19 ± 3*†§
1 h shock + DCLHb	40 ± 3	25 ± 2*†‡	18 ± 6*†‡§	19 ± 4*†‡	18 ± 4*†‡
1 h shock + whole blood	42 ± 2	26 ± 5*†	38 ± 3	40 ± 2	37 ± 4
Hemoglobin (g/dl)					
Sham control	12.5 ± 0.4	12.8 ± 0.4	12.9 ± 0.8	12.9 ± 0.9	12.7 ± 1.0
1 h shock + HES	13.0 ± 0.6	9.7 ± 0.9*†	5.4 ± 0.8*†§	6.1 ± 1.0*†§	6.0 ± 0.8*†§
1 h shock + DCLHb	12.7 ± 1.0	8.8 ± 0.5*†	9.0 ± 1.4*†‡	9.8 ± 2.0*†‡	8.0 ± 1.3*†‡
1 h shock + whole blood	13.7 ± 0.7	8.6 ± 1.6*†	12.6 ± 0.8	12.7 ± 0.9	12.1 ± 0.8

Values are mean ± SD.

* $P < 0.05$ significant versus baseline.

† $P < 0.05$ significant versus control.

‡ $P < 0.05$ significant versus HES.

§ $P < 0.05$ significant versus whole blood, Newman-Keuls test.

DCLHb = diaspirin cross-linked hemoglobin; HES = hydroxyethylstarch.

detected in the control group, but were detected at lower levels in the hydroxyethylstarch-treated group (fig. 4). Resuscitation with both oxygen-carrying solutions, DCLHb and WB, resulted in a significant ($P < 0.05$) increase of TBARs concentration (4.8 ± 0.7 and 3.7 ± 0.7 nmol/g, respectively) compared with the control and hydroxyethylstarch-treated group, without sig-

nificant differences between these two groups. Histopathologic changes (edema, necrosis, cell infiltration, and hemorrhage) were absent in all three shock groups and the control group.

Discussion

The results of this study indicate that DCLHb as an oxygen-carrying resuscitation fluid restores systemic MAP and pancreatic capillary perfusion as effectively as

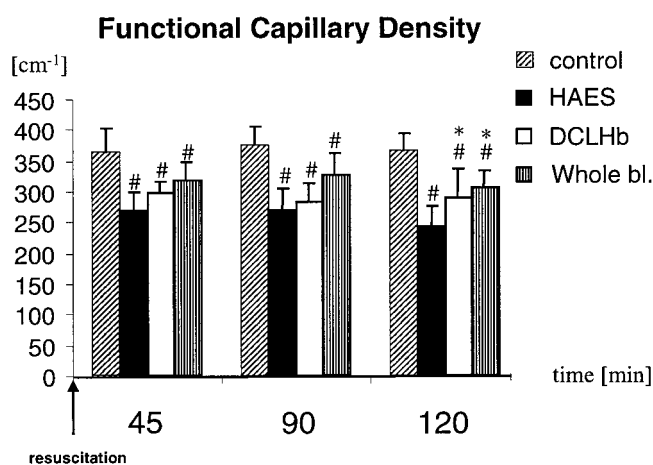


Fig. 2. Functional capillary density (length of erythrocyte-perfused capillaries per area) of pancreatic acini after resuscitation. Values are expressed as the mean ± SD. # $P < 0.05$ is significant versus control, * $P < 0.05$ is significant versus hydroxyethylstarch; † $P < 0.05$ is significant versus WB; no significant changes within each group.

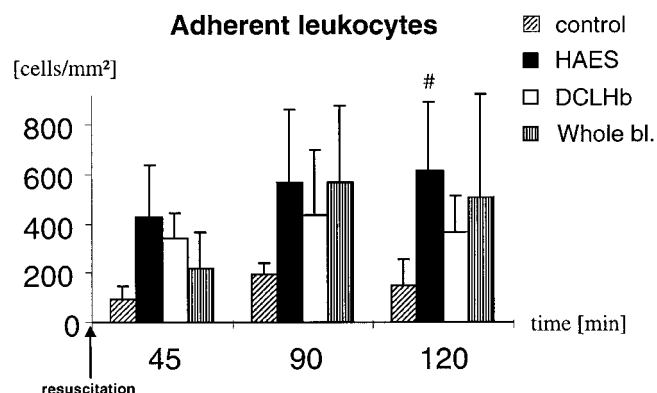


Fig. 3. Number of adherent leukocytes ("stickers") in postcapillary venules. Values are expressed as the mean ± SD. # $P < 0.05$ versus control, no other significances between the groups at each time point or within each group were detected.

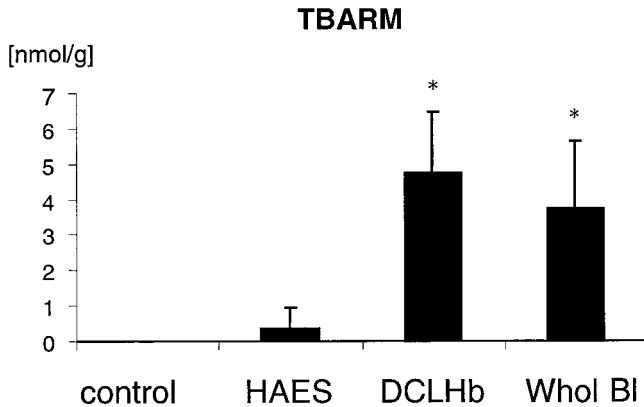


Fig. 4. Pancreatic tissue concentration of thiobarbituric acid-reactive materials (TBARMs) as a parameter of lipid peroxidation damage. No significant difference was detected between the DCLHb and the WB group. Values are expressed as the mean \pm SD. * $P < 0.05$ is significant versus control and hydroxyethylstarch.

does resuscitation with WB. Although there was a higher amount of lipid peroxidation damage of the pancreatic tissue after resuscitation with the oxygen carriers DCLHb and WB, compared with hydroxyethylstarch, probably because of the initiation of reperfusion damage, both oxygen carriers were found to restore capillary perfusion better than did hydroxyethylstarch resuscitation. DCLHb did not enhance the degree of leukocyte adherence in postcapillary venules or the degree of pancreatic lipid peroxidation, compared with WB resuscitation.

The oxygen-carrying properties of DCLHb have been shown in many experimental studies.^{22,23} However, the phase III trial in trauma patients recently was discontinued prematurely because of a higher mortality in the treatment group.²⁴ In clinical phase I and II studies, DCLHb increased MAP after infusion in a dose-independent manner, indicating that the human pressor response to DCLHb is similar to the response observed in preclinical studies.^{10,11} This pressor effect was also observed in other hemoglobin-based oxygen-carrying solutions.²⁵ Although the main mechanisms of pressor actions of DCLHb are not completely clear, the NO-scavenging property of cell-free hemoglobin appears to be directly involved. Clinical investigations showed a threefold elevation of the potent vasoconstrictor endothelin-1 concentration after DCLHb infusion in stroke patients without affecting other vasoactive mediators.²⁶ There is speculation that the higher endothelin concentration at infusion of DCLHb is caused by the DCLHb-induced lowering of nitric oxide because there is a close

balance between the endothelium-derived relaxation by NO and the endothelium-derived constriction factor endothelin-1.²⁷

Effect of Resuscitation on the Microcirculation

The pancreatic tissue damage in hemorrhagic shock is considered to be mainly caused by the ischemia that occurs during hemorrhagic shock and the ongoing microcirculatory failure during reperfusion, although the macrocirculation was restored sufficiently by resuscitation.^{4,5,28} Elevation of endothelin and NO-scavenging involved in treatment with DCLHb could have multiple effects on the microcirculation of the pancreas. Endothelin-1, when infused into anesthetized dogs, has proven to reduce pancreatic blood flow.¹⁴ In hamster skin muscle, DCLHb resulted in a 25% reduction of FCD after hemorrhagic shock, compared with control values.²⁵ After 1 h of normothermic pancreatic ischemia, DCLHb did not alter microvascular perfusion failure.²⁹ So far, there exist only data of DCLHb effects on the splanchnic blood flow, as investigated by the microsphere technique after hemorrhagic shock.³⁰ Because there could be arteriovenous shunting in the pancreas,³¹ using this technique to assess blood flow to splanchnic organs does not show information about nutritive supply to the pancreas that occurs on the capillary level of microcirculation, which is gained by using an intravital microscopy technique. FCD is an index of the quality of capillary perfusion and has proven to be a predictor of survival after severe hemorrhagic shock.³² The number of erythrocyte-perfused capillaries is critical for the vitality of an organ after shock and ischemia-reperfusion damage regarding nutritive and metabolite extracting properties.³³ Infusion of DCLHb showed an enhancement of blood flow to the gastrointestinal system during normal conditions³⁰ and after hemorrhagic shock.¹⁷ Our study proved that DCLHb as a resuscitation fluid restores capillary perfusion of the pancreas as effectively as WB. Although hydroxyethylstarch resuscitation resulted in comparable hematocrit values, as obtained after infusion of DCLHb (indicating similar volume expanding properties), the restoration of capillary perfusion was more effective with the oxygen-carrying hemoglobin solution in contrast with hydroxyethylstarch resuscitation.

Effect of Resuscitation on Leukocyte-Endothelium Interaction

Activation of leukocytes is a crucial hallmark of the immunologic response after hemorrhagic shock. In the pancreas microcirculation, depletion of granulo-

Table 2. The Effect of Resuscitation on Pa_{O₂}/F_{I_{O₂} Ratio, Acid–Base Balance, and Blood Lactate Concentration}

Parameter	Baseline	Shock	15 min Postresuscitation	60 min Postresuscitation	120 min Postresuscitation
Pa_{O₂}/F_{I_{O₂} ratio}					
Sham control	332 ± 45	299 ± 38	306 ± 53	292 ± 28	290 ± 28
1 h shock + HES	279 ± 46	359 ± 29	256 ± 48	281 ± 33	309 ± 51
1 h shock + DCLHb	278 ± 35	393 ± 49†	151 ± 46*†‡§	251 ± 84	331 ± 86
1 h shock + whole blood	335 ± 59	369 ± 36	282 ± 27	306 ± 33	304 ± 31
pH					
Sham control	7.38 ± 0.02	7.38 ± 0.02	7.38 ± 0.01	7.37 ± 0.03	7.35 ± 0.02
1 h shock + HES	7.41 ± 0.03	7.35 ± 0.05	7.27 ± 0.9*†§	7.33 ± 0.05*	7.33 ± 0.04*
1 h shock + DCLHb	7.40 ± 0.02	7.37 ± 0.06	7.34 ± 0.05‡	7.39 ± 0.04‡	7.43 ± 0.03†‡
1 h shock + whole blood	7.42 ± 0.02	7.36 ± 0.04	7.33 ± 0.05*	7.37 ± 0.03	7.36 ± 0.02
Base excess (mm)					
Sham control	-1.1 ± 1.6	-1.1 ± 1.6	-1.3 ± 2.1	-1.7 ± 1.9	-2.2 ± 1
1 h shock + HES	-1.3 ± 1.0	-7.0 ± 3.4*†	-6.2 ± 4.0*†	-3.9 ± 3.8	-4.0 ± 4.2
1 h shock + DCLHb	-0.6 ± 1.5	-5.9 ± 1.2*†	-1.5 ± 1.6‡	0.4 ± 3.4	0.5 ± 2.6
1 h shock + whole blood	-1.6 ± 0.9	-6.5 ± 3.5*†	-4.8 ± 3.6	-3.5 ± 2.3	-3.0 ± 1.0
Lactate (mm)					
Sham control	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.3	1.0 ± 0.2	0.7 ± 0.2
1 h shock + HES	0.7 ± 0.3	3.6 ± 1.6*†	1.9 ± 1.3	1.0 ± 2.0	1.1 ± 0.6
1 h shock + DCLHb	0.8 ± 0.6	3.3 ± 2.3*†	1.6 ± 0.4	0.8 ± 0.9	0.9 ± 0.1
1 h shock + whole blood	0.6 ± 0.1	2.4 ± 0.6*	0.9 ± 0.1	0.7 ± 0.2	0.6 ± 0.1

Values as mean ± SD.

* $P < 0.05$ significant versus baseline.

† $P < 0.05$ significant versus control.

‡ $P < 0.05$ significant versus HES.

§ $P < 0.05$ significant versus whole blood, Newman–Keuls test.

DCLHb = diaspirin cross-linked hemoglobin; HES = hydroxyethylstarch.

cytes resulted in a significant reduction of capillary no-reflow in an experimental study of hemorrhagic shock.⁶ Application of monoclonal antibodies against the neutrophil adhesion molecule CD18 in a primate model of hemorrhagic shock reduced the resuscitation volume requirement and improved survival.^{3,4} These findings highlight the crucial effect of adherence and activation of leukocytes in the pathophysiology of hemorrhagic shock. Leukocyte adherence was significantly elevated compared with that in the control group only 120 min after hydroxyethylstarch infusion. DCLHb showed an elevation of leukocyte adherence of resuscitation that was not more pronounced than in the other resuscitation groups, indicating that DCLHb does not enhance the inflammatory reaction, compared with WB resuscitation.

Effect of Resuscitation on Breathing Adjustment

After resuscitation with DCLHb a much higher F_{I_{O₂} was needed for maintenance of normal Pa_{O₂}. This need was indicated by a significant decrease of the Pa_{O₂}/F_{I_{O₂} ratio 15 min after DCLHb infusion, compared with all other groups. We interpret this finding as an NO-dependent increase of pulmonary pressure after DCLHb infusion.³⁵}}

Effect of Resuscitation on Lipid Peroxidation

One of the main events of ischemia-reperfusion damage that occurs in hemorrhagic shock is the generation of oxygen radicals. They are able to provide up-regulation of leukocyte adhesion molecules and damage nucleic acids and can deactivate important enzymes and peroxidase in cell membrane lipids.⁸ Antioxidant treatment with vitamin E and superoxide dismutase has proven to be beneficial after hemorrhagic shock³⁶ and ischemia-reperfusion.³⁷ In the inflammatory process of hemorrhagic shock, several sources generate oxygen radicals, such as the xanthine oxidase, the oxidative burst of activated granulocytes, and the reaction of nitric oxide with superoxide anion to form peroxynitrite. Additionally, in our study free hemoglobin could act as a Fenton reagent with the potential to catalyze hydroxyl radical generation by autooxidation of the hemoglobin heme iron atom to methemoglobin.⁹ All these radicals can initiate peroxidation of polyunsaturated fatty acids of cell membranes. Lipid peroxidation products such as malondialdehyde represent highly active aldehydes with their own spectrum of toxicity. Measurement of lipid peroxidation is used as an indirect quantitative measure-

ment of reactive oxygen radical generation.³⁸ Pancreatic levels of malondialdehyde, one of the main degradation products of oxidatively peroxidized lipids, can be measured as TBARs. It is unclear whether TBARs are very specific markers of lipid peroxidation because other substances, such as bilirubin, can react with thiobarbituric acid.³⁹ The test we used showed a negligible interference with bilirubin.²¹ In our study, no enhanced production of oxygen radicals by DCLHb, compared with the WB resuscitation, could be detected. Hemodilution with DCLHb after induction of kidney ischemia-reperfusion did not enhance radical generation.⁴⁰ Furthermore, DCLHb has proven its usefulness in the treatment of systemic⁴¹ and organ ischemia in animal *in vivo* models of the heart,⁴² skin muscle,⁴³ and the brain,^{44,45} suggesting an absence of further generation of oxygen radicals. Although lipid peroxidation is significantly higher after resuscitation with oxygen carriers than after colloid resuscitation, hydroxyethylstarch did not reverse capillary perfusion deficit, indicating a lower potential to restore microvascular perfusion with the oxygen-carrying solutions.

In conclusion, our study showed a similar restoration of macrocirculatory parameters by a hemoglobin-based oxygen carrier, compared with WB resuscitation, after hemorrhagic shock. DCLHb did not exacerbate the adherence of leukocytes in postcapillary pancreatic venules compared with the other solutions. Most importantly, the capillary perfusion of the pancreas was restored to values seen after WB resuscitation. There was an increased need of oxygen to maintain oxygen saturation after DCLHb infusion. DCLHb did not cause enhanced lipid peroxidation of pancreatic tissue compared with WB resuscitation. Thus, DCLHb was as effective as WB resuscitation fluid in our experimental model.

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