Effect of diaspirin cross-linked hemoglobin on normal and postischemic microcirculation of the rat pancreas

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Von Dobschuetz, Ernst, Tomas Hoffmann, Clemens Engelschalk, and Konrad Messmer. Effect of diaspirin cross-linked hemoglobin on normal and postischemic microcirculation of the rat pancreas. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1507–G1514, 1999.—Microcirculatory alterations with reduced nutritive supply to the pancreas could be the cause of hyperamylasemia, which occurs in some patients receiving the vasoactive oxygen carrier diaspirin cross-linked hemoglobin (DCLHb) in clinical studies. Therefore, the effects of DCLHb on rat pancreas microcirculation were evaluated. Anesthetized Sprague-Dawley rats received one of the following treatments during baseline conditions (n = 7 rats/group): 10% hydroxyethyl starch (HAES) (0.4 ml/kg), DCLHb (400 mg/kg), or DCLHb (1,400 mg/kg). After 1 h of complete, reversible pancreatic ischemia, other animals received 10% HAES (0.4 ml/kg) or DCLHb (400 mg/kg) during the onset of reperfusion. The number of red blood cell-perfused capillaries (functional capillary density, FCD) and the level of leukocyte adherence in postcapillary venules in the pancreas were assessed by means of intravital microscopy during 2 h after treatment. In the nonischemic groups, FCD was 15% greater after DCLHb (1,400 mg/kg) than after 10% HAES treatment without any increase in leukocyte adherence. In the ischemia-reperfusion (I/R) 10% HAES group, FCD was significantly (P < 0.05) lowered, leukocyte adherence enhanced, and mean arterial pressure (MAP) reduced by 31% compared with nonischemic animals. DCLHb treatment in the I/R group resulted in a slight increase in FCD, a significant (P < 0.05) reduction of leukocyte adherence, and a complete restoration of MAP compared with the animals of the I/R control group. Thus our data provide no evidence for a detrimental effect on the pancreatic microcirculation or an enhanced risk of postischemic pancreatitis by DCLHb.

DCLHb does not show immunogenicity in humans, has lowered infectious risks compared with blood transfusions, and is immediately available in an emergency situation, since there is no need for cross-matching and blood typing. The beneficial oxygen-carrying properties of DCLHb have been demonstrated in several in vitro and in vivo situations in animals subjected to controlled and uncontrolled hemorrhagic shock (19). Probably due to the nitric oxide (NO)-scavenging mechanism of free hemoglobin, DCLHb also appears to be advantageous in septic shock patients (24) suffering from hyporeactivity to vasoconstrictive drugs and mediators. Endothelium-derived relaxing factor (NO) (28), endothelin, and the potentiation of adrenoreceptors (19) by DCLHb are considered to be involved in the vasoreactive reactions observed after DCLHb injection. DCLHb shows an alteration of blood flow and regional vascular resistance to many organs (28). The pancreas is reported to be a susceptible organ to ischemia-reperfusion (I/R) in patients with hemorrhagic shock. Hypoperfusion of the pancreas is possible during cardiac surgery due to the extracorporal circulation, and I/R reactions would be anticipated following pancreas transplantation and during surgery of the thoracic and thoracoabdominal aorta due to the clamping of the blood supply to the pancreas (7, 8). Experimental acute pancreatitis induced by different means shows microcirculatory perfusion failure with a reduction of the nutritive supply to the pancreatic tissue (13). Vasactive changes that are seen after DCLHb administration could result in an alteration of tissue blood and oxygen supply to the pancreas. The role of enhanced endothelin-1 and lowered NO concentrations, as seen after DCLHb administration, in experimental acute pancreatitis is controversially discussed. Some studies show beneficial and some detrimental effects of these factors in the course of acute pancreatitis (14, 15, 34). To date, the hamster skin muscle is the only organ in which microcirculatory alterations after DCLHb injection have been investigated utilizing intravital microscopy techniques. In muscle, these experiments showed an increase of venular red blood cell velocity under nonischemic conditions after DCLHb treatment, without changes of functional capillary density (FCD) or leukocyte adherence in postcapillary venules (20). After 4 h of pressure ischemia in the dorsal skinfold chamber, there was a reduction of leukocyte adherence in postcapillary venules after DCLHb treatment during reperfusion time (21). In 4 of 14 septic patients who received DCLHb in a phaseI trial, sepsis was caused by acute pancreatitis (24). When considering treatment of such
patients with DCLHb, it is important to exclude possible disturbances of pancreatic microvascular perfusion after induction of acute pancreatitis. In clinical studies, some patients showed hyperamylasemia after DCLHb administration. Microcirculatory alterations of DCLHb could be the trigger mechanism of such enzyme enhancement. Thus the purpose of this study was to investigate microcirculatory reactions and possible side effects after DCLHb administration under normal conditions and after an inflammatory stress induced by 1 h of ischemia-induced acute pancreatitis.

**MATERIALS AND METHODS**

Anesthesia and monitoring. Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 180–260 g were anesthetized by ether and pentobarbital sodium (50 mg/kg body wt ip) after an overnight fast with free access to tap water. After tracheotomy, the rat respiration was volume controlled by a ventilator (frequency: 57–65 breaths/min, tidal volume: 2–2.5 ml, inspired oxygen fraction: 0.25–0.40; rodent ventilator 683, Harvard Apparatus, South Natick, MA). The right carotid artery and the right jugular vein were cannulated by a polyethylene catheter (PE-50, 0.58 mm ID, Portex, Hythe, Kent, UK) for continuous monitoring of mean arterial pressure (MAP), heart rate, and continuous volume replacement (3–5 ml/h iv 0.9% NaCl) by a syringe pump. Adequate anesthesia was maintained by intravenous injection of pentobarbital sodium (12 mg·kg body wt·h−1) and N2O admixture (0.65–0.85). Arterial blood gases were measured intermittently (ABL 300, Radiometer, Copenhagen, Denmark) and were adjusted to the following values for baseline conditions by means of breathing adjustment and 8.4% sodium bicarbonate injection: PO2 = 100–120 mmHg, PCO2 = 30–40 mmHg, pH = 7.39 ± 0.02, and base excess = 0 ± 2. Hematocrit values in arterial blood were measured by Coultercounter T540 (Coulter Electronics, Hi- aleah, FL). The experiments were performed in accordance with German legislation for the protection of animals.

Animal model and experimental protocol. After a transversal incision of the abdomen, the complete ischemia of the pancreas was induced by means of microvascular clamps (closing force of 70 g; Aesculap, Tuttinglen, Germany) on the four arteries supplying blood to the pancreas (left gastric artery, gastroduodenal artery, splenic artery, and caudal pancreaticoduodenal artery). The microsurgical procedure has been described elsewhere (9). Sham-operated animals underwent the same preparation but without induction of ischemia. After a stabilization period of 15 min, animals were randomly assigned to five groups (n = 7 animals/group): 1) sham-operated group without ischemia that received 4 ml/kg body wt of 10% hydroxyethyl starch (HAES) (200/0.5) after operation procedures (sham control), 2) sham-operated group without ischemia that received 400 mg/kg (4 ml/kg) body wt DCLHb (sham DCLHb), 3) sham-operated group without ischemia receiving 1,400 mg (14 ml/kg) body wt DCLHb (sham high-dosage DCLHb), 4) 1-h ischemia group that received 4 ml/kg body wt of 10% HAES (200/0.5) at the onset of reperfusion (ischemia control), 5) 1-h ischemia group that received 400 mg/kg (4 ml/kg) body wt DCLHb during the onset of reperfusion (ischemia DCLHb). "[HAES (200/0.5)]" means the artificial colloidal HAES (macromolecular polymer, which is manufactured from amylopectin and consists of hydroxyethylated glucose molecules linked by α-1,4 bonds) with a molecular weight of 200,000. The degree of hydroxyethylation is 0.5, which means that 5 of 10 glucose molecules are substituted by hydroxethyl groups. HAES was chosen as a control solution for DCLHb to show that possible microcirculatory or macrocirculatory effects of DCLHb are not caused just by its volume-expanding property. An ischemia control group receiving no HAES infusion was not considered necessary, since there was no significant difference in microvascular parameters between these two groups in experiments performed to establish this model (9). The circulatory response of DCLHb is well characterized in rats and pigs and is dose dependent up to a dosage of 400–500 mg/kg body wt. At higher doses of DCLHb, there is no further increase of MAP (3). We assumed that the major microcirculatory effect of DCLHb on the pancreas should occur at the doses chosen. Because other experiments that investigated the circulatory effects of DCLHb by microsphere technique also used a dose of 400 mg/kg body wt, we used the same dose to make our results comparable to those studies (5, 6). The high dose (1,400 mg/kg body wt) injected during baseline conditions allowed us to investigate whether a high, top-load dose of DCLHb would elicit greater microcirculatory effects compared with the 400 mg/kg dose.

Samples of arterial blood (1 ml) were taken before administration of the solutions, 15 min after administration, and at the end of the experiment. The withdrawn blood volume was immediately replaced by 0.9% NaCl solution (1:1). Fifteen minutes after administration of the solution, the pancreas and spleen were exteriorized for intravital microscopy on an adjustable stage and covered by a thin Teflon membrane to prevent drying. MAP was continuously registered on a recorder (XT Kompensograph, Siemens, Munich, Germany). Heart rate was obtained from the phasic blood pressure curves. Heart rate, arterial blood gases, and hematocrit were measured at baseline conditions and 15, 60, and 120 min after administration of the solutions. The experiments were terminated by injection of an overdose of pentobarbital sodium.

Drugs. HemAssist (DCLHb) was provided in 4.5-ml containers by Baxter Healthcare (lot no. 9710AD11-111997, Round Lake, IL). The physichochemical and pharmacokinetic characteristics of HemAssist given by manufacturer were as follows: 10 g/dl hemoglobin cross-fumaril content, methemoglobin concentration <5% oxygen half-saturation value (P50) = 32 mmHg (mean arterial blood P50 = 26 mmHg). Right-shifted dissociation curve compared with that of red blood cells for transfusion, pH adjusted to 7.4 at 37°C, half-life time = 13 h in healthy men and women, and oncotic pressure = 42 mmHg (human plasma = 25 mmHg). Containers were stored in a 70°C freezer and thawed 0.5 h before use. The 10% HAES (200/0.5) was purchased from Fresenius (Bad Homburg, Germany).

Intravital microscopy and quantification of microcirculation. After intravenous injection of 0.15 ml of 0.75% HAES (molecular weight of 200,000) labeled with FITC (FITC-HAES; Laevosan, Linz, Austria) for contrast enhancement of microvessels and 0.1 ml of 0.2% rhodamine 6G (molecular weight of 497; Sigma, St. Louis, MO) for in vivo staining of cytochrome c-containing cells (leukocytes), intravital microscopy of the pancreas was performed using a modified Leitz-Orthoplan microscope (Leitz, Wetzlar, Germany) for in vivo staining of cytochrome c-containing cells (leukocytes). Intravital microscopy of the pancreas was performed with a modified Leitz-Orthoplan microscope (Leitz, Wetzlar, Germany) with a 100 W mercury vapor lamp attached to a Plomo-Pak illuminator with I23 (excitation 450–490 nm, emission >515 nm) and N2 (excitation 530–560 nm, emission >580 nm) filter blocks (Leitz) for epi-illumination. A saltwater immersion objective (×25) was used. Leitz allowed magnification of approximately ×800. The observations were recorded by means of a charge-coupled device video camera (FK 6990, Cohu, Prospect, Measurements, San Diego, CA) and stored on video tape (Panasonic video recorder, 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 number of red blood cell-perfused capillaries (cm) per observation area (cm²). The FCD was determined by analysis of the video tapes according to Schmid-Schönbein et al. (26) by means of superimposing a grid system (squared-type) on the video screen (square side = 50 µm). The number of intersections of red blood cell-perfused capillaries with the grid system were counted, and FCD was calculated as described earlier (9). Ten randomly selected tissue surface areas (400 × 300 µm) of the pancreas were evaluated at each time point. For quantification of leukocyte-endothelial interaction, at least three identical postcapillary venules (diameter of >40 µm and length of <150 µm) 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 30-s observation time. The surface area of the vessel segments was calculated on the basis of diameter measurements, assuming a cylindrical geometry of the vessels. Adherent leukocytes are given as cells per area (mm²).

Measurement of serum values of α-amylase and interleukin-6. Blood samples were withdrawn from the carotid artery and centrifuged at 3,000 g for 10 min at 4°C. Serum samples were stored in a –70°C freezer until assay. Serum α-amylase activity was measured by a random-excess analyzer (COBAS INTEGRA, Roche, Basel, Switzerland). The interleukin-6 (IL-6) serum concentration was measured by means of a rat blood IL-6 ELISA kit (no. 1051502A, Laboserv, Staufenberg, Germany). Interference of DCLHb with the two performed tests was investigated by spiking plasma with increasing concentrations of DCLHb. Because the amylase activity test yielded valid data up to a DCLHb concentration of 200 mg/dl, samples were diluted for measurement. No interference of the IL-6 ELISA test was encountered up to a DCLHb concentration of 1,500 mg/dl. Tests were not performed in the high-dosage group due to technical problems with these two tests.

Light microscopy. Tissue samples from the corpus of the pancreas were taken at the end of the experiment from four animals per group and immediately fixed in 10% neutral buffered Formalin. The samples were dehydrated, embedded in paraffin, cut at ~3 µm (Microtome, Leica, Munich, Germany), and stained with polymorphonuclear esterase staining (Sigma). In 10 regions of interest (diameter of 250 × 250 µm) per animal, granulocytes were counted (cells/mm²) by a blind observer.

Statistics. All data are presented as mean values ± SE. After normal distribution testing was performed, data were subjected to one-way ANOVA and pair-wise Student-Newman-Keuls test between the groups. Non-normally distributed data were tested by one-way ANOVA on ranks followed by a pair-wise Dunn’s method comparison procedure. Within each individual group, one-way repeated-measures ANOVA (normally distributed data) followed by Dunnett’s method, or a Friedman repeated-measures ANOVA on ranks (non-normally distributed data) followed by Dunnett’s method was performed. The amylase values were tested by paired t-test (normally distributed data) or Wilcoxon’s signed rank test (non-normally distributed data) (SigmaStat 2.0, Jandel, San Rafael, CA). P < 0.05 was considered to be statistically significant.

RESULTS

Effects of DCLHb on normal pancreas. To investigate the influence of DCLHb on pancreatic microcirculatory perfusion changes, FCD (Fig. 1A) was assessed by intravital microscopy. DCLHb (400 mg/kg) administered during baseline conditions resulted in no significant difference of FCD compared with 10% HAES treatment. A higher dosage of DCLHb (1,400 mg/kg) increased microcirculatory perfusion by 18% compared with the HAES-treated group at the end of the experiments. In all three sham-operated groups, there was a significant increase of leukocyte adherence during the observation time compared with the 45-min values.
(Fig. 2A). No differences in degree of leukocyte adherence in postcapillary venules, amylase serum activity (Fig. 3), and IL-6 serum concentration (Fig. 4) could be detected between the two sham-operated groups. In groups receiving 400 mg/kg and 1,400 mg/kg DCLHb, respectively (Fig. 5), MAP was elevated by 18% and 21%, respectively, compared with the 10% HAES-treated control group. Hypertension persisted in the 1,400 mg/kg DCLHb group over the whole observation period. In the 400 mg/kg DCLHb group, the MAP returned to baseline values between 15 and 120 min after injection. DCLHb resulted in a significant amelioration of base excess compared with the 10% HAES control group at 60 and 120 min after solution injection (Table 1).

Effects of DCLHb on postischemic pancreas. I/R resulted in a significant ($P < 0.05$) reduction of FCD (Fig. 2). 1-h ischemia of the pancreas resulted in a 3-fold increase in the number of adherent leukocytes compared with nonischemia (sham control). Leukocyte adhesion was significantly ($P < 0.05$) reduced by DCLHb treatment at the end of observation time. Values are means ± SE. # $P < 0.05$ vs. sham-HAES control. + $P < 0.05$ vs. ischemia-HAES control.

Fig. 2. Number of adherent leukocytes in at least 3 postcapillary venules. A: there were no significant differences in nonischemic animals between the groups. Values are means ± SE. + $P < 0.05$ vs. 45 min values. B: 1-h ischemia of the pancreas resulted in a 3-fold increase in the number of adherent leukocytes compared with nonischemia (sham control). Leukocyte adhesion was significantly ($P < 0.05$) reduced by DCLHb treatment at the end of observation time. Values are means ± SE. # $P < 0.05$ vs. sham-HAES control. + $P < 0.05$ vs. ischemia-HAES control.

Fig. 3. Serum amylase concentrations at baseline conditions and at the end of the experiment. Ischemia-reperfusion resulted in a significant ($P < 0.05$) increase in amylase concentration, which was completely absent in the group receiving DCLHb. Values are means ± SE. + $P < 0.05$ vs. baseline.

Fig. 4. Interleukin-6 (IL-6) serum concentration at baseline and after treatment. No changes were detected in the nonischemic animals. Significant elevation of IL-6 was found after 2 h of reperfusion in the 10% HAES-treated ischemia-reperfusion group, which was absent in the DCLHb-treated ischemia-reperfusion group. Values are means ± SE. + $P < 0.05$ vs. baseline and DCLHb-sham. p. inf., Postinfusion.
Effects of DCLHb on normal pancreas. In this study, we have observed a significant enhancement of pancreatic FCD after top-load infusion of a very high dosage of DCLHb into healthy animals. These results are similar to data gained by microsphere and laser-Doppler methods, indicating that, when DCLHb is infused, perfusion of the pancreas is increased in healthy animals (5). As expected from other studies (19), MAP increased significantly after DCLHb injection in a dose-dependent manner. However, no adverse microcirculatory effect of DCLHb, for example a fall of FCD or enhanced leukocyte adherence, could be detected in the normal pancreas; it is therefore unlikely that hyperamylasemia seen after DCLHb injection into patients in clinical studies is due to a disturbance of pancreatic microvascular perfusion. The decrease of FCD and the considerable increase in the number of adherent leukocytes observed after infusion of 10% HAES in the sham group may be explained by an inflammatory reaction due to the exteriorization of the pancreas. These changes appear to be counteracted by DCLHb treatment, as suggested by the lower increase of amylose (Fig. 3) and IL-6 (Fig. 4) levels, as well as stable FCD values (Fig. 1A) in the DCLHb-treated sham groups.

Effects of DCLHb on postischemic pancreas. This is the first study on microvascular perfusion after reversible normothermic ischemia to the pancreas. As we have shown previously, 1 h of complete pancreatic ischemia provokes mild pancreatic injury (9). We assume that both harmful and beneficial effects of DCLHb should be detectable in this preparation. In the postischemic pancreas, DCLHb resulted in a slight improvement of FCD, reduction of leukocyte adherence, and a stable arterial blood pressure; this appears important for the treatment of the inflamed gland, since systemic hypotension is a major complicating factor in the early phase of acute pancreatitis in humans. The concentrations of IL-6 and amylose were slightly reduced in the DCLHb I/R group, a finding in agreement with the microcirculatory data showing that DCLHb does not exacerbate the inflammatory reaction. IL-6 is a valuable prognostic parameter to assess the course and severity of disease during the early onset of acute pancreatitis (27). As in our previous study (9), I/R resulted in a comparable reduction of FCD, enhancement of leukocyte adherence, and elevation of amylose, even though the control group was receiving 10% HAES. This group was included to exclude a major volume effect being responsible for changes observed after infusions of the hyperoncotic HAES solution, since DCLHb has itself plasma-expanding properties (oncotic pressure = 42 mmHg).

I/R in the pancreas. Post-I/R injury is characterized by a microcirculatory perfusion failure, called capillary no-reflow, which causes continuing impediment of oxygen supply to the tissue, thereby exacerbating the primary ischemic damage to tissue. The causes for capillary no-reflow are up to now not completely understood. Among other mechanisms, hemoconcentration in
Table 1. Effect of DCLHb on pH, acid/base balance, heart rate, and hematocrit

<table>
<thead>
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<th>Parameter</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Postinfusion</th>
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<td></td>
<td>pH</td>
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<td>15 min</td>
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<td>7.40 ± 0.01</td>
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<td>4.7 ± 0.9</td>
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<td>Heart rate, beats/min</td>
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<td>426 ± 18</td>
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<td>383 ± 9</td>
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<td>430 ± 10</td>
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<tr>
<td>Ischemia 400 mg/kg DCLHb</td>
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<td></td>
<td>41.2 ± 1.0</td>
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Values are means ± SE. Ischemia-reperfusion resulted in a significantly (P < 0.05) higher metabolic acidosis compared with sham-operated animals. DCLHb, diaspirin cross-linked hemoglobin. *Significant difference (P < 0.05) compared with sham control; †significant difference (P < 0.05) compared with ischemia control.

the capillaries due to the enhanced plasma leakage, disequilibrium of endothelin-1 and NO balance controlling microvascular flow, leukocyte plugging of capillaries, and endothelial cell swelling are discussed as possibilities (18). Further hallmarks of reperfusion injury are the activation of leukocytes and the enhancement of leukocyte-endothelium interactions mediated through upregulation of adhesion molecules on the surface of endothelial cells of postcapillary venules (8, 18). Adherent leukocytes are considered to play a pivotal role in I/R tissue damage due to their release of proteases and generation of superoxide radicals (respiratory burst) (8). These phenomena have been described for striated muscle (18), heart, liver, lung, and many other organs (8). Our group was the first to observe these phenomena in I/R-induced experimental pancreatitis, and we have demonstrated that the high susceptibility of the pancreas to I/R results in histological changes resembling those seen in acute pancreatitis (8, 9). I/R in pancreas causes an inflammatory reaction similar to acute pancreatitis and is considered a major determinant in progression of pancreatitis caused by other factors. For example, ethanol ingestion, next to gallstones, is a major associated factor for acute pancreatitis and causes a significant increase of endothelin-1 levels and a drastic reduction (up to 50%) of pancreas perfusion (33). Indicating that reduced perfusion of the pancreas, as well as consecutive low-flow ischemia, can contribute decisively to pancreas tissue damage. To evaluate if there are potentially harmful microcirculatory effects of DCLHb in patients with acute pancreatitis, we have studied postischemic pancreatitis, particularly since elevated serum amylase and lipase values have in fact been observed after infusion of DCLHb to patients. To date, DCLHb has proven its usefulness in both systemic (20) and organ ischemia in animal in vivo models of kidney (22), heart (17), skin muscle (21), and brain (1). In our study, we only observed a slight improvement of pancreatic FCD failure after I/R by DCLHb. There is a significant reduction of leukocyte adherence in the DCLHb-treated I/R group. These results let us conclude that the beneficial effect of DCLHb on postischemic pancreatitis is, in contrast to other organs, very low. The mechanisms by which DCLHb, injected as a topload infusion, beneficially influences I/R damage could be the following. 1) Treatment of I/R-damaged muscle of the rat and hamster (29) with hyperbaric oxygen enhanced oxygen supply to the tissue and showed a beneficial effect. Thus treatment of I/R damage with the oxygen carrier DCLHb, which obviously enhances the PO2 of postischemic tissue (21), is probably beneficial in I/R injury. In fact, infusion of DCLHb after I/R injury of the kidney did not increase radical generation occurring after ischemia (22). 2) After normothermic I/R of the pancreas, the animals suffered from hypotension. Reduction of the MAP induced by tourniquet ischemia of the rat hindlimb resulted in a reduction of FCD in the pancreas of 40% (13). Similar results were obtained in our model, indicating that reduction of MAP by bradykinin resulted in a tremendous decrease in FCD (11). Because DCLHb stabilizes the macrohemodynamic parameters, this could be another reason for the protection of the microcirculation by DCLHb in our experimental model.
Vasoactive effects of DCLHb and possible influence on pancreas microcirculation and I/R damage. DCLHb induces changes of blood flow and regional resistance to flow in various organs by virtue of its vasoactive properties. Several studies, using the radioactive microsphere technique to investigate the normal perfusion of pancreas and mesentery after DCLHb administration, have shown results similar to those obtained in our experiments. In these studies, DCLHb (400 mg/kg body wt) caused an increase in blood flow velocity to the pancreas of almost 100% from baseline (after 15 min); baseline values were reached again within 60 min (5). These effects of DCLHb on the blood supply of the pancreas were nearly abolished when the endothelin receptor antagonist BQ-123 or the substrate for NO synthase, L-arginine, was present, indicating that endothelin release and scavenging of NO are involved in the regional blood flow changes. There is considerable controversy in the literature concerning NO and endothelin in normal and inflamed tissues. In contrast to the above-mentioned studies, a beneficial effect of DCLHb was observed in a dose-dependent manner without adverse microcirculatory side effects. We did not encounter signs of acute pancreatitis. There is a slight reversal of capillary no-reflow as well as a reduction of leukocyte adherence, serum amylase activity, and IL-6 concentration when DCLHb is administered after induction of postsischemic pancreatitis. DCLHb does not exacerbate the inflammatory and microcirculatory pancreatic damage of postsischemic pancreatitis. DCLHb is able to maintain the MAP during the time of pancreatic reperfusion.

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REFERENCES


