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Intravenous Contrast Medium Aggravates the Impairment of Pancreatic Microcirculation in Necrotizing Pancreatitis in the Rat

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Background

Previous reports demonstrated that radiographic contrast medium, as used in contrast-enhanced computed tomography, increases acinar necrosis and mortality in experimental pancreatitis. The authors studied the possibility that these changes may be related to an additional impairment of pancreatic microcirculation.

Methods

Fifty Wistar rats had acute pancreatitis induced by intraductal glycodeoxycholic acid (10 mmol/L for 10 min) and intravenous cerulein (5 μg/kg/hr for 6 hrs). After rehydration (16 mL/kg), pancreatic capillary perfusion was quantified by means of intravital microscopy at baseline before intravenous infusion of contrast medium ($n = 25$) or saline ($n = 25$), and 30 and 60 minutes thereafter. In addition to total capillary flow, capillaries were categorized as high- or low-flow (> or <1.6 nL/min).

Results

Pancreatic capillary flow did not change in either high- or low-flow capillaries after saline infusion. However, contrast medium infusion induced a significant decrease of total capillary flow ($p < 0.001$). Analysis according to the relative flow rate revealed that this was primarily because of a significant additional reduction of perfusion in low-flow capillaries ($p < 0.0001$). Furthermore, complete capillary stasis was observed in 15.9 ± 3.4% after contrast medium as compared with 3.2 ± 1.2% after saline infusion ($p < 0.006$).

Conclusion

Radiographic contrast medium aggravates the impairment of pancreatic microcirculation in experimental necrotizing pancreatitis.

Contrast-enhanced computed tomography currently is a standard diagnostic procedure to differentiate between mild edematous and severe necrotizing pancreatitis in the clinical setting.[1-5] Contrast-enhanced computed tomography (CT) allows for early grading of severity of pancreatitis and for later identification of necrosis[3] or abscesses before surgical intervention.[6,7] Although clinically useful, this procedure requires that the seriously injured pancreas be perfused repeatedly with large volumes of radiographic contrast medium. Furthermore, with the development of new and faster scanners, the administered volume per time is likely to increase. Previous reports demonstrated that intravenous contrast medium can convert ischemic renal tissue to irreversible tubular necrosis.[8-10] Until very recently, the possibility that intravenous contrast medium could similarly aggravate tissue damage in the early phase of evolving tissue necrosis in acute pancreatitis never was investigated. In a recent study, we demonstrated that administration of radiographic contrast medium is followed by an increase in acinar necrosis and mortality in experimental necrotizing pancreatitis.[11]

Because the evolution of acute necrotizing pancreatitis is paralleled by a concomitant reduction of pancreatic blood flow,[12,13] we speculated that contrast medium-induced pancreatic injury may aggravate the impairment of pancreatic microcirculation in the early critical phase of evolving necrotizing pancreatitis. This concept is supported by a recent study that demonstrates decreased tissue oxygenation in the pancreas after intravenous contrast medium administration.[14] If the hypothesis of an additional iatrogenic impairment of already reduced pancreatic perfusion was true, the common practice of an early contrast-enhanced CT before areas of necrosis have become established would deserve serious reconsideration.

MATERIALS AND METHODS

Seventy-three male Wistar rats (350–450 g) were used and housed individually in rooms maintained at 21 C ± 1 C using a 12-hour dark cycle. The animals were given a standard rat chow and fasted overnight before the experiment with water allowed *ad libitum*. Care was provided in accordance with the German law for the care and use of laboratory animals. The study was approved by the Regierungspräsidium Karlsruhe, committee on animal care.

Surgical anesthesia was induced with vaporized ether followed by intraperitoneal pentobarbital (20 mg/kg, Nembutal, Pharmazeutische Handelsgesellschaft, Garbsen, FRG) and ketamine (40 mg/kg, Ketanest, Parke, Davis & Company, Berlin, FRG). The right internal jugular vein was cannulated (Luer Lock, I.D. 0.5 mm, B. Braun AG, Melsungen, FRG). Another catheter of the same type was placed in the left carotid artery for blood sampling and blood pressure measurements. Both catheters were tunneled subcutaneously to the suprascapular area and brought out through a tether, which permitted monitoring of the unrestrained animal. Animals were kept under analgesic medication by continuous ketamine administration (2.5 mg/kg/hr). At 5 hours, the animals were fully anesthetized again with intravenous ketamine and pentobarbital. Complete anesthesia was maintained by intravenous administration of sodium pentobarbital (10 mg/kg/hr). The animals were placed on a specially constructed heating plate to maintain body temperature at 37 C ± 1 C. Subsequently, the abdominal incision was re-opened, and the pancreas with the duodenum was gently exteriorized and placed in an immersion chamber containing Ringer's lactate maintained at 37 C ± 1 C by means of a feed-back controlled heating system.

Animals were excluded from the study before randomization to one of the study groups, when one of the following criteria was present: surgical trauma to the preparation or cardiorespiratory derangement as indicated by MAP < 80 mm Hg; $PCO_2 > 50$ mm Hg; $P_{O2} < 80$ mm Hg; or pH < 7.3 or > 7.5 at at any point before administration of the test solution. Nine animals died of pancreatitis during the experiments, and 14 were excluded according to the described criteria. The remaining 50 animals were entered into statistical analysis (n = 25/group).

Induction of Pancreatitis and Volume Administration

Induction of acute necrotizing pancreatitis was achieved using the technique described in detail previously.[15,16] In all animals, glycodeoxycholic acid (Glycodeoxycholic acid, Sigma Chemical, No. G-3258, Deisenhofen, FRG) in glycylglycine-NaOH buffered solution (pH 8.0, room temperature) at a concentration of 10 mmol/L was infused intraductally for 10 minutes at a pressure of 30 mm Hg controlled via mercury manometer with the volume controlled by means of an interposed scaled glass tube. At the end of the intraductal infusion, the catheter was removed, and the duodenal wall was

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closed. Intravenous cerulein (Takus, 5 μg/kg/hr, Farmitalia, Freiburg, FRG) was then infused continuously for 6 hours.

Simultaneously, a continuous infusion of Ringer's lactate at 8 mL/kg/hr for the first 5 hours was given. To achieve adequate rehydration and normalization of the hematocrit in all animals before contrast medium infusion, fluid resuscitation was increased to 16 mL/kg/hr until 6 hours and continued with 8 mL/kg/hr thereafter.

Quantitation of Pancreatic Capillary Blood Flow by Intravital Microscopy

Quantitation of pancreatic capillary blood flow by intravital microscopy was performed by the methods described by Butcher[17] and Sarelius[18] and modified by Mithöfer et al.[19]

Erythrocytes were labeled using fluorescein isothyocyanate (FITC, Isomer I, Sigma Chemical, No. F-7250, Deisenhofen, FRG). Blood was collected from Wistar donor rats and anticoagulated with heparin. Plasma and buffy coat were removed, and erythrocytes were washed three times in glucose-saline buffer (pH 8.2) by repeated suspension and centrifugation. Cells were resuspended in bicine-saline buffer (pH 6.8) containing FITC (9 mg/mL). The mixture was incubated in a gently shaking water bath at 25 C for 3 hours. This procedure results in a covalent binding of the fluorochrome to cytoplasmic elements and allows for complete staining of all erythrocytes.

Labeled cells were then separated from the solution and washed several times until the supernatant was free of fluorescent dye. Finally, fluorescent cells were resuspended in 0.9% NaCl containing citrate-phosphate dextrose (Sigma Chemicals, Deisenhofen, FRG, No. C-7165) and stored at 1 to 6 C in the dark.

All animals received an intravenous injection of 0.5 mL/kg FITC-labeled erythrocytes (hematocrit 50%) and were allowed to stabilize for at least 30 minutes. The animal was placed under a fluorescence microscope (Leitz, Wetzlar, FRG). Epi-illumination was achieved with a short arc xenon lamp (XBO 100W/2, Osram, Berlin, FRG) in the presence of a heat-protecting and an excitation (450–490 nm) filter. The microscopic image was transferred to a monitor by video camera (Cohu CCD-4810, San Diego, CA) and recorded on U-matic videotape (KCA-60-BRS, Sony Deutschland, Cologne, FRG) for subsequent analysis.

Off-line analysis of coded video recordings was performed by a blinded observer counting the number of labeled erythrocytes passing through each observed capillary site in 30 to 60 capillaries from three different regions of the pancreas during 1 minute.

The concentration of fluorescent erythrocytes per unit of arterial blood (n_{FITC}) was measured in all animals at each time point by counting their number in 50 different fields of a Neubauer chamber. The fraction of labeled erythrocytes per total unit of erythrocytes (N_{FITC}) was derived by dividing the labeled fraction per blood volume (n_{FITC}) by systemic hematocrit (Hct_{sys}).

$$N_{FITC} = n_{FITC}/Hct_{sys}.$$

Volumetric red cell flux (Q_{Rbc}) was computed by determining the frequency of fluorescent cells in each observed vascular segment per unit of time (f_{FITC}) and analyzing the whole blood withdrawn from the same animal for the number of fluorescent cells per unit erythrocyte volume (N_{FITC}) at each time point by dividing f_{FITC} by N_{FITC}.[20] This equation is based on the assumption that the ratio of labeled to native cells in capillary and arterial blood is identical, as demonstrated previously.[18]

$$Q_{Rbc} = n_{FITC}/N_{FITC}.$$

Volumetric blood flow (V) was calculated from capillary red cell flux (Q_{Rbc}) by using a previously described equation.[21]

$$V = Q_{Rbc}/Hct_{micro}.$$

Capillary hematocrit (Hct_{micro}) is defined as the volume fraction of red cells in a specified volume of capillary and represents the number of cells available for oxygen exchange.[22] In contrast, discharge hematocrit (Hct_{disch}) is defined as the portion of red cells flowing out of a capillary, a measure of cell flux through the capillary.[22] Axial accumulation of red cells passing through a capillary causes mean velocity of red cells to exceed plasma velocity with a concomitant fall in hematocrit, a phenomenon known as the Fahraeus effect.[23] Previous studies on narrow capillaries described a ratio of Hct_{micro}/Hct_{disch} of 0.76[21] for the capillaries of the size found in the rat pancreas.[24] Because there is consistent agreement of Hct_{disch} and Hct_{sys} under various conditions, volumetric blood flow can be calculated as follows:

$$V = Q_{Rbc}/Hct_{sys} \times 0.76.$$

High- and Low-Flow Capillaries

Among the characteristic microcirculatory features in necrotizing pancreatitis is the loss of vasomotion and the existence of high-flow areas with predominantly edematous changes and low-flow regions that are associated geographically with areas of evolving necrosis. Therefore, in addition to total capillary flow, pancreatic microcirculation was determined in high- and low-flow capillaries, with the cut-off level empirically determined at 1.6 nL/min/capillary.

Administration of the Test Solution

Six hours after pancreatitis induction, baseline measurements of pancreatic microcirculation were taken. Subsequently, either physiologic saline (n = 25) or the nonionic contrast medium iopamidol (Solutrast, Byk Gulden, Konstanz, FRG, n = 25) was injected at 2 mL/kg for 1 minute. This dose corresponds to the dose given to humans in the clinical setting. Measurement of capillary flow was repeated 30 and 60 minutes after infusion of the test solution.

Systemic Hemodynamics and Laboratory Parameters

Mean arterial pressure and heart rate were recorded on a Gould-Brush MK 2400 recorder (Gould-Brush, Cleveland, OH) just before and 30 and 60 minutes after infusion of the test solution. Hematocrit and arterial blood gases were measured at 0, 6, and 7 hours after the induction of pancreatitis (ABL 3, Radiometer A/S Copenhagen, Denmark).

Quantitation of Pathological Trypsinogen Activation

Generation of trypsinogen activation peptides (TAPs) occurs when inactive trypsinogen is cleaved and converted to active trypsin.[25-27] In healthy subjects, TAPs are produced in the intestine by the action of enterokinase on trypsinogen. Trypsinogen activation peptides then are degraded rapidly by enteric bacteria so that no measurable TAP levels occur. In pancreatitis, however, TAPs are generated already in the pancreas and can be measured in body fluids. Levels of TAPs have been shown to correlate with acinar necrosis and predict mortality in our model.[28,29] Thus, they were used as a biochemical index of severity of pancreatitis. All TAP samples were collected in edetic acid (0.20 Mol/L; plasma 0.05 mL/sample, ascites 0.1 mL/sample). Then they were stored frozen at −20 C until assayed. Quantitation of TAPs in plasma (5 hrs) and ascites (5 hrs) was performed using a newly developed enzyme-linked immunosorbent assay employing calcium-independent rabbit anti-TAP antibodies, as previously described.[26,27]

Statistical Analysis

Data are presented as individual data points or means ± SEM. Continuous variables were tested for group differences using the Student's t test between groups and the paired t test within groups. A probability of type I experimental error 5% ($p \leq 0.05$) was accepted for statistical significance.

RESULTS

Group Comparability with Regard to the Severity of Pancreatitis Before Administration of the Test Solution

Hemodynamic and Laboratory Parameters

Mean arterial pressure and arterial blood gases are depicted in Table 1. There were no relevant differences with regard to these parameters between control subjects receiving saline and animals receiving radiographic contrast medium. Initial hemoconcentration occurred in all animals, but hematocrit returned to baseline values after volume resuscitation in both groups before administration of the test solution (Fig. 1).

Trypsinogen Activation Peptides

Trypsinogen activation peptides in plasma and ascites were measured in all animals just before infusion of the test solution at 5 hours. There was no significant difference for TAPs in ascites (NaCl: 23.99 ± 2.65, contrast medium: 27.54 ± 2.77 nMol/L) and in plasma (NaCl: 19.69 ± 1.77, contrast medium: 17.58 ± 1.38 nMol/L) between animals treated with saline or contrast medium, indicating that both groups suffered a comparable degree of pathological trypsinogen activation.

Pancreatic Microcirculation

The following volumetric capillary blood flow results always refer to the same capillaries observed at each time point.

Total Capillary Flow

Total capillary flow (high- and low-flow capillaries) remained constant in control subjects receiving saline (baseline [n = 530]: 1.65 ± 0.04; 30 min [n = 501]: 1.62 ± 0.03; 60 min [n = 538]: 1.62 ± 0.03 nL/min/cap). In contrast, compared with baseline, total capillary flow was decreased significantly in animals treated with contrast medium at 30 and 60 minutes (baseline [n = 498]: 1.50 ± 0.03; 30 min [n = 453]: 1.20 ± 0.04 [$p < 0.0001$]; 60 min [n = 497]: 1.10 ± 0.04 nL/min/cap [$p < 0.0001$]). Additionally, compared with control subjects receiving saline, total capillary flow was significantly lower in contrast medium-treated animals 30 and 60 minutes after administration of the test solution ($p < 0.0001$).

High-Flow Capillaries

There were no relevant changes in pancreatic microcirculation in high-flow capillaries at 30 and 60 minutes; group differences were small (Fig. 2).

Table 1. HEMODYNAMIC AND LABORATORY PARAMETERS BEFORE AND AFTER ADMINISTRATION OF SALINE OR CONTRAST MEDIUM

	Before Infusion		60 Min After Infusion		
	Saline	Contrast Medium	Saline	Contrast Medium	
MAP	120.6 ± 2.1	116.7 ± 1.9	111.2 ± 2.8	100.6 ± 2.6	mmHg
pH	7.42 ± 0.01	7.41 ± 0.01	7.41 ± 0.01	7.42 ± 0.02	mmHg
P_{o2}	120.8 ± 4.2	100.1 ± 3.5	114.8 ± 3.9	112.6 ± 3.3	mmHg
P_{co2}	35.6 ± 1.6	32.7 ± 1.2	26.9 ± 1.9	24.2 ± 1.4	mmHg
BE	−0.5 ± 0.8	−5.8 ± 0.7	−1.7 ± 0.6	−6.7 ± 0.7	mmol/L

Low-Flow Capillaries

There was no change in the flow through low-flow capillaries after saline administration (baseline: n = 270, 30 min: n = 254, 60 min: n = 269). However, a striking decline of pancreatic microcirculation in low-flow capillaries (baseline: n = 288, 30 min: n = 264, 60 min: n = 287) was observed 30 and 60 minutes after contrast medium infusion ($p < 0.007$) (Fig. 3). Compared with control subjects receiving saline, pancreatic microcirculation was significantly lower in animals receiving radiographic contrast medium ($p < 0.009$).

Capillary Stasis

Complete capillary stasis, e.g., transformation of low-flow capillaries to no-flow capillaries, developed in 15.9 ± 3.4% of animals treated with radiographic contrast material as compared with only 3.2 ± 1.2% in control subjects receiving saline ($p < 0.001$, Fig. 4).

DISCUSSION

Based on its ability to demonstrate whether there is microcirculatory perfusion of the tissues, contrast-enhanced CT has become widely used to differentiate between mild edematous and severe necrotizing pancreatitis.[3,30,31] In many centers, when there is a strong clinical suspicion of a necrotizing injury, contrast-enhanced CT is performed shortly after hospital admission to confirm the diagnosis, grade the severity, and obtain a baseline study for later comparison.[2,4,31–33]

The potential value for early staging of severe acute pancreatitis notwithstanding, serious concerns have been raised in regard to the administration of radiographic contrast material in the presence of an inflamed

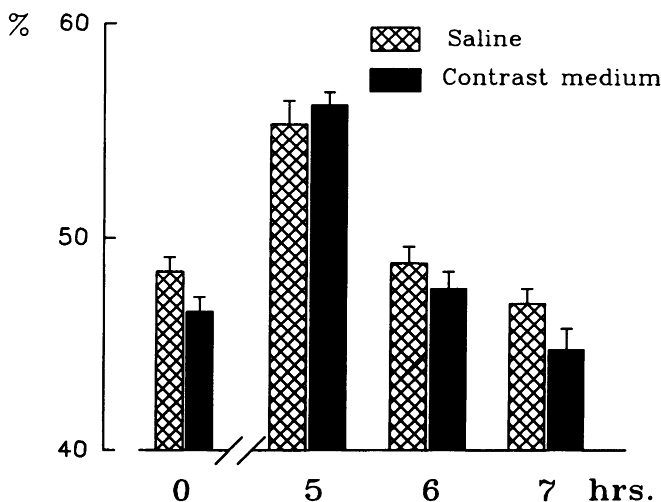

Figure 1. Systemic hematocrit at baseline (0 hrs), before exteriorization of the pancreas (5 hrs), shortly before administration of the test solution (6 hrs), and at the end of the experiment (7 hrs). There were no differences between both study groups at any time during the experiment. Pre-existing hemoconcentration was treated effectively, as indicated by the significant reduction of hematocrit to baseline levels before infusion of the test solution in both groups ($p < 0.0001$).

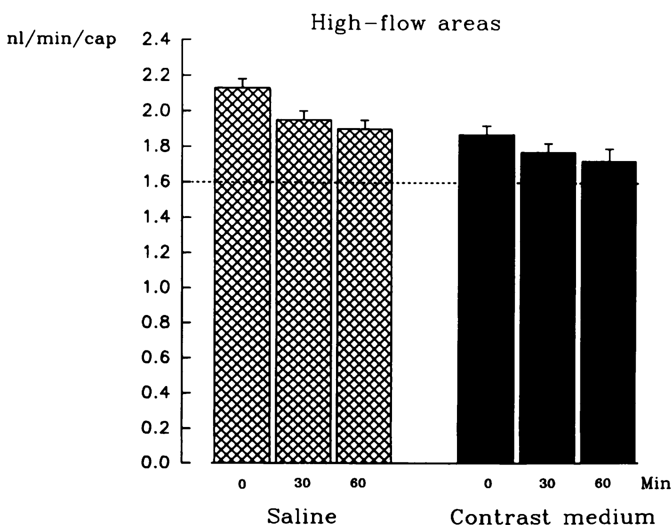

Figure 2. Pancreatic blood flow through high-flow capillaries (>1.6 nL/min) during the experiment. There was no relevant difference between animals receiving contrast medium or saline. No significant difference was noted.

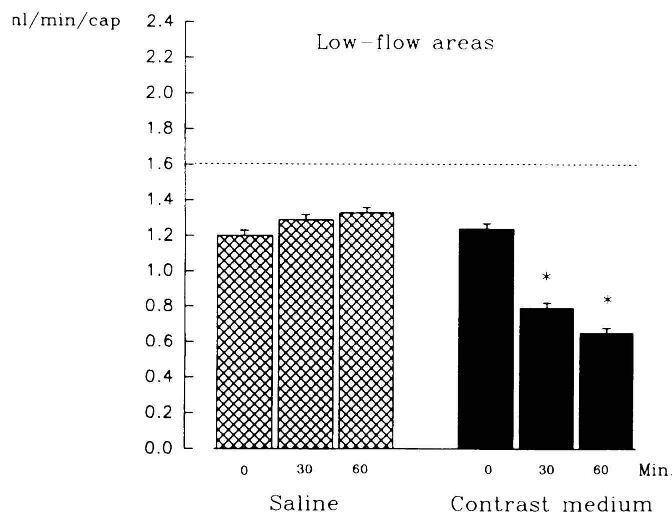

Figure 3. Pancreatic blood flow through low-flow capillaries (<1.6 nL/min) during the experiment. There was a dramatic additional reduction of capillary blood flow in animals treated with radiographic contrast medium. *Indicates significant differences compared with 6 hours and to animals receiving saline 30 and 60 minutes after administration of the respective solute.

and thus, potentially vulnerable pancreas at a time when the degree of pancreatic tissue injury is not yet fully established.[11,14] Ischemia has been proven an important determinant in the progression of acute pancreatitis.[13,34–37] Studies from our own laboratory revealed that the development of necrotizing pancreatitis is associated with a critical reduction of pancreatic capillary flow in conjunction with heterogeneous perfusion in the rat and rabbit.[34,38] This is characterized by high-flow areas representing predominantly edematous changes and low-flow regions of potentially reversible tissue injury adjacent to areas of evolving necrosis.[34,35] Intravenous contrast medium has been shown to be injurious to ischemic renal tissue,[8–10] and the risk of injury is dose dependent.[8] Although the kidney concentrates the contrast medium and may thus be a particularly vulnerable target, contrast medium has been shown to cause direct injury to the pancreas *in vitro*, as indicated by amylase release and histologic changes of exocrine pancreatic tissue.[39] In consequence, we considered it important to test the hypothesis that radiographic contrast media could further aggravate the impairment of pancreatic microcirculation, which characterizes acute necrotizing pancreatitis and that this impairment may be most evident in low-flow capillary regions.

The main finding of this study is that intravenous contrast medium, as used in contrast-enhanced CT, causes a critical reduction of pancreatic capillary blood flow in early necrotizing pancreatitis. There was a significant reduction of overall pancreatic capillary perfusion after contrast medium infusion, even when areas of high-flow and low-flow were analyzed together. If the low-flow capillaries were viewed as a separate group, a concentrated or particular effect was seen in capillaries with low flow. A cut-off of 1.6 nL/min/capillary was empirically chosen as best demonstrating this differentiated effect. This observation implies that such low-flow capillaries are particularly susceptible to further critical reduction of flow after contrast medium is applied. As a result, these low-flow regions frequently were converted to areas with frank capillary stasis, a confirmation and explanation of our recent observation that contrast medium causes reduced oxyhemoglobin levels in pancreatic tissues and increases acinar necrosis in severe pancreatitis, but not in mild or moderate disease.[11,14] Because low-flow regions have been shown to represent areas with severe but potentially reversible tissue damage, the fate of these areas, recovery, or necrosis, may well be determined by the quality and quantity of capillary blood flow.[40]

The undesired action of radiographic contrast medium primarily is at the microcirculatory level rather than the result of systemic hypotension or hypovolemia, because no relevant difference in mean arterial pressure, hematocrit, and arterial blood gases was recorded between saline and contrast medium-treated animals. Furthermore, the observed effects on pancreatic microcirculation occurred despite effective fluid resuscitation and rehydration. We can only speculate about the additional adverse effect radiographic contrast media might have if rehydration was inadequate, as it frequently may be in the clinical setting of patients subjected to a contrast-enhanced CT scan early in the clinical course.

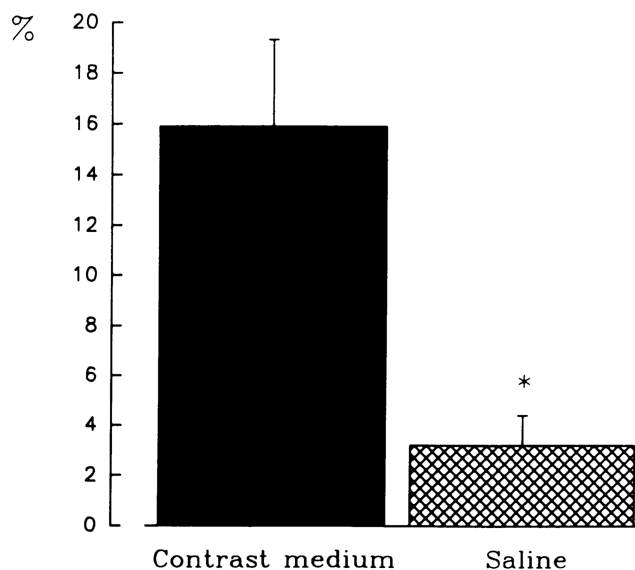

Figure 4. Pancreatic capillary stasis 60 minutes after administration of the test solution. There was highly significantly more capillary stasis after infusion of radiographic contrast medium compared with saline infusion (* $p < 0.001$).

The pathogenesis of contrast medium-induced adverse effect on the microcirculation in severe pancreatitis is not apparent from our experiments. Contrast media have been shown to cause red blood cell aggregation and to change red cell morphology, resulting in increased rigidity and impaired oxygen release.[41–46] Activation of the complement system by contrast media could aggravate leukocyte-endothelial interactions,[47,48] which are thought to be important in the vascular injury of acute pancreatitis.[49] Radiographic contrast media also have a relatively high viscosity, which may add to microcirculatory derangements.[44] Finally, the possibility that contrast medium may have a direct toxic effect on injured acinar cells and that the additional flow impairment is a secondary phenomenon cannot be excluded.

This study demonstrates that radiographic contrast medium further impairs pancreatic capillary flow primarily in areas with pre-existing low-flow conditions and frequently converted poorly perfused regions to frank capillary stasis in experimental necrotizing pancreatitis. Although we cannot directly extrapolate the experimental findings at 6 hours in the rat to the human disease, these observations, in conjunction with previous studies demonstrating impaired pancreatic tissue oxygenation,[14] increased extraintestinal trypsinogen activation, acinar necrosis, and mortality[11] after contrast medium administration, raise concerns about the common practice of using contrast-enhanced CT in the first days after the onset of severe pancreatitis. Pancreatic tissue necrosis in humans seems to evolve within several days after the onset of symptoms.[32] However, surgical debridement is rarely, if ever, indicated in this early period. Therefore, we suggest that contrast-enhanced CT is not necessary and perhaps should not be used in this vulnerable period, when the findings will serve only to satisfy curiosity, not provide clinically necessary data. It seems more rational to us to reserve the application of contrast-enhanced CT to patients whose pancreatitis does not resolve during several days of supportive treatment and in whom the findings of substantial necrosis will provide the basis for debridement. This approach should both reduce the number of unnecessary examinations and reduce the possibility of aggravating the pancreatic injury.

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