The Relative Safety of MRI Contrast Agent in Acute Necrotizing Pancreatitis

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Objective
To validate the safety of gadolinium-diethylenetriamine pentaacetic acid (GD-DTPA) by measuring its effect on pancreatic capillary perfusion and acinar injury in acute pancreatitis.

Background
Contrast-enhanced computed tomography (CECT) is proposed as a gold standard for early evaluation of acute necrotizing pancreatitis. However, iodinated contrast media used for CECT have been shown in these circumstances to reduce pancreatic capillary flow and increase necrosis and mortality. Recent reports suggest that post-GD MRI provides images comparable to CECT in the assessment of severe acute pancreatitis.

Methods
Necrotizing pancreatitis was induced in 14 Wistar rats by intraductal glycodeoxycholic acid (10 mM/L) and intravenous caerulein (5 μg/kg/h) over 6 hours. Intravital microscopic quantitation of pancreatic capillary blood flow was performed using fluorescein isothiocyanate-labeled erythrocytes after induction of pancreatitis and 30 and 60 minutes after an intravenous bolus of either Ringer’s solution or GD-DTPA (0.2 mL/kg).

Results
The two study groups were comparable with regard to mean arterial pressure, heart rate, arterial blood gases, hematocrit, amylase, lipase, and trypsinogen activation peptide production throughout the experiment. GD-DTPA did not reduce capillary flow (1.93 ± 0.05 nL/capillary/min) compared to animals infused with Ringer’s solution (1.90 ± 0.06 nL/capillary/min).

Conclusions
Intravenous injection of GD-DTPA does not further impair pancreatic microcirculation or increase acinar injury in acute necrotizing pancreatitis. Because of this advantage over CT contrast medium, further development of MRI as a staging tool in acute pancreatitis seems desirable.
Fulminant acute pancreatitis is characterized by a marked reduction of capillary flow in regions of the pancreas destined to become necrotic.\textsuperscript{1,2} Contrast-enhanced computed tomography (CECT), which can distinguish between well-perfused and poorly perfused areas, is currently the standard imaging method for differentiating between mild edematous pancreatitis, which resolves spontaneously, and severe necrotizing disease with its high morbidity and mortality.\textsuperscript{3-7} CECT thus may identify early patients at high risk for complications and allows selection of patients for aggressive interventional therapies or special pharmacologic treatments in development. Combined with improved intensive care treatment, CECT is claimed to have had a positive impact on the outcome of patients with necrotizing pancreatitis.\textsuperscript{8}

Our group, however, has demonstrated that the intravenous contrast medium used for CT, whether ionic and nonionic, causes significant additional reductions of capillary flow, especially in areas where flow is already impaired,\textsuperscript{9} lowers pancreatic tissue oxygenation,\textsuperscript{10} and increases acinar necrosis, trypsinogen activation peptide (TAP) production, and mortality.\textsuperscript{11} Ischemia is considered to be a major factor in the progression to necrotizing disease.\textsuperscript{1,2,12-15} Therefore, the additional iatrogenic impairment of the microcirculation by contrast medium in the early phase of pancreatitis may promote the evolution of borderline ischemic pancreatic tissue into necrosis.\textsuperscript{9-11} The suggestion to reconsider or even avoid CECT early in the clinical course of pancreatitis\textsuperscript{9-11} has been strongly resisted on the grounds that an alternative nonsurgical method to define pancreatic ischemic areas and necrosis was unavailable.\textsuperscript{8}

More recently, the technique and image quality of magnetic resonance imaging (MRI) have greatly evolved and improved. New clinical trials have demonstrated results in diagnostic imaging of acute pancreatitis using MRI with the contrast medium gadolinium (GD)-DTPA equivalent to those obtained with CECT.\textsuperscript{16-18} If MRI is to be proposed as an alternative to CECT, it will be critical to evaluate the effect of GD on the pancreatic microcirculation and to validate its safety in the setting of severe pancreatitis with an already impaired perfusion.

**MATERIALS AND METHODS**

Male Wistar rats (250–350 g) were housed in rooms maintained at 21 ± 1 C using a 12-hour dark cycle. Animals were fasted overnight before the experiment, with free access to water. Care was provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication #85-23, 1985). The study was approved by the Regierungspfarräsidium Karlsruhe, Germany, committee on animal care.

Surgical anesthesia was induced with vaporized ether and maintained by an intraperitoneal injection of pentobarbital (10 mg/kg Nembutal; Pharmazeutische Handelsgesellschaft, Garbsen, Germany) and an intramuscular injection of ketamine (40 mg/kg Ketanest; Parke, Davis & Co., Berlin, Germany). The right internal jugular vein was cannulated (Luer Lock, ID 0.5 mm; B. Braun AG, Melsungen, Germany). Another catheter of the same type was placed in the left carotid artery for blood sampling and blood pressure and heart rate measurements. All catheters were tunneled subcutaneously to the suprascapular area and brought out via a flow-through tether, which subsequently permitted free movement of the rat. Anesthesia was induced again at 5 hours for the remaining 2 hours of the observation period by intravenous administration of pentobarbital (1–3 mg/kg) and was repeated when needed, as assessed by changes in blood pressure, heart rate, and clinical signs indicating pain.

**Induction of Pancreatitis**

A detailed description of the induction technique used is given elsewhere.\textsuperscript{19,20} Briefly, the biliopancreatic duct was cannulated with a 24-gauge Teflon catheter (Critikon, Tampa, FL), bile and pancreatic juice were drained by gravity for 5 minutes, and the main duct was clamped below the liver to facilitate the subsequent intraductal infusion. Control animals (n = 7) received intraductal saline followed by a 6-hour intravenous saline infusion (group 1). Experimental necrotizing pancreatitis was induced in 14 animals (groups 2 and 3). Freshly prepared glycodeoxycholic acid (GDOC; Sigma, Deisenhofen, Germany, #G-3258) in glycerol-glycine–NaOH buffered solution (pH 8.0, room temperature) at a concentration of 10 mmol/L was infused in a pressure (30 mmHg)- and volume (1.2 mL/kg)-controlled fashion. Subsequently, these 14 animals received a continuous intravenous infusion of caerulein at 5 mg/kg/hr (Takus; Farmitalia, Carlo Erba GMBH, Freiburg, Germany) over 6 hours. Caerulein was reconstituted in normal saline and infused at 8 mL/kg/hr as baseline hydration. Additionally, sodium bicarbonate (0.2 mL/100 g) was included in the infusion.

**Administration of Test Solutions**

Six hours after induction of pancreatitis and after baseline measurements of pancreatic microcirculation were taken, a bolus of either Ringer’s solution (group 2, n =
7) or the contrast medium GD-DTPA (Magnevist; Schering AG, Berlin, FRG, group 3, n = 7) was injected intravenously at 0.2 mL/kg. This dose correlates with the dose given to humans in the clinical setting. Control animals without pancreatitis (group 1) also received 0.2 mL/kg Ringer's solution.

**Intravital Microscopy**

Five hours after intraductal infusion of saline (controls, group 1) or GDOC (pancreatitis animals, group 2 and 3), the abdomen was reopened and the pancreas within the duodenal loop gently exteriorized and placed in an immersion chamber containing Ringer's lactate maintained at 37 ± 1°C by means of a feedback-controlled heating system. Measurements of the pancreatic microcirculation were made at 6 hours after induction (baseline) and at 30 and 60 minutes after the bolus infusion of test solutions.

Quantification of pancreatic capillary blood flow by intravital microscopy was performed as previously described in detail by Mithoefer. Briefly, erythrocytes from donor rats were labeled with fluorescein isothiocyanate (FITC, Isomer I; Sigma, No. F-7250) using a modified procedure according to Butcher and Weissman and Sarelius and Duling. All animals received 0.5 mL/kg FITC-labeled erythrocytes intravenously (hematocrit 50%) before microscopic assessment. Animals were then allowed to stabilize for 30 minutes, and intravital microscopy was performed using a new generation of fluorescence microscope (Leitz, Wetzlar, Germany) equipped with a 25-fold water immersion objective (PL Fluotar 25/075 W; Leitz) and an epi-illumination unit. Epi-illumination was achieved with a short-arc xenon lamp and an excitation filter for FITC-labeled erythrocytes (450–490 nm). Images were transferred to a monitor (PVM/444QM; Sony, Tokyo, Japan) by a low-light camera (Kappa CF 8/1; Kappa, Geichen, Germany) and simultaneously recorded on videotape using a videorecorder (AG 7350; Panasonic, Tokyo, Japan).

Off-line analysis of video recordings was performed by an independent observer and capillary blood flow (nL/capillary/min) was determined. The concentration of fluorescent erythrocytes per unit of arterial blood was measured in all animals at the point of every video recording by counting their number in 50 different fields of a Neubauer chamber and correlating the number of passing FITC-labeled erythrocytes with the labeled fraction of capillary hematocrit. The ratio of capillary to systemic hematocrit is 0.76, so capillary hematocrit was calculated by multiplying the systemic hematocrit by 0.76.

Four different regions of the pancreas with 21 to 32 capillaries per animal were assessed by intravital microscopy. Necrotizing pancreatitis is characterized by the existence of high- and low-flow areas. The cut-off level between these two has been empirically determined at 1.6 nL/capillary/minute. After the administration of CT contrast medium, alteration of tissue perfusion does not occur in high-flow areas but is significantly reduced in areas with a capillary blood flow of less than 1.6 nL/capillary/minute. In this study, we therefore evaluated capillary perfusion separately for high- and low-flow capillaries.

**Monitoring**

Hematocrit was measured at baseline and 6, 6.5, and 7 hours after induction of pancreatitis. The arterial line was connected to a pressure transducer (Servomed; Freiburg, Hellige, Germany) for continuous monitoring of the heart rate and mean arterial blood pressure. Blood gases were analyzed at 6 and 7 hours using an ABL 3 analyzer (Radiometer A/S, Copenhagen, Denmark).

**Quantitation of Pathologic Trypsinogen Activation**

Pathological extraintestinal trypsinogen activation was quantified in plasma (3 and 7 hours after induction of pancreatitis) using an enzyme-linked immunosorbent assay technique that detects free TAP. TAP occurs when inactive trypsinogen is cleaved and converted to active trypsin. Under physiologic conditions, trypsinogen is activated by enterokinase within the gut and TAP is rapidly degraded by proteases so that it is not detected in plasma. In pancreatitis, intrapancreatic trypsinogen activation leads to pathologic TAP generation within the pancreas, and TAP appears in the circulation. The plasma level of TAP correlates well with pancreatic necrosis and mortality and can be used as an index for the severity of the disease. All samples for TAP assay were collected in EDTA (edetic acid) (0.20 Mol/L, 50 mL/0.5 mL blood sample), frozen at −80°C, and assayed within 8 weeks.

**Amylase and Lipase**

Amylase and lipase were measured 3 and 7 hours after intraductal induction of pancreatitis (Hitachi automatic analyzer, Boehringer Mannheim, Mannheim, Germany).

**Statistical Analysis**

Animals were excluded from the study before randomization when one of the following criteria was present: cardiorespiratory derangement as indicated by mean arterial pressure less than 80 mmHg, \( \text{PO}_2 \) less than 80 mmHg, \( \text{PCO}_2 \) more than 50 mmHg, or pH less than 7.3 or more than 7.5. Three animals were excluded according to the
Table 1. HEART RATE, MEAN ARTERIAL PRESSURE, AND HEMATOCRIT

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Control (n = 7)</th>
<th>Ringer's Solution (n = 7)</th>
<th>Gadolinium (n = 7)</th>
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</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>6* 308 ± 16</td>
<td>325 ± 12</td>
<td>317 ± 11</td>
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<tr>
<td>MAP (mmHg)</td>
<td>7† 313 ± 14</td>
<td>330 ± 13</td>
<td>325 ± 12</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>6* 136 ± 4</td>
<td>131 ± 3</td>
<td>129 ± 3</td>
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<tr>
<td></td>
<td>7† 135 ± 3</td>
<td>131 ± 4</td>
<td>125 ± 3</td>
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<td></td>
<td>0‡ 47.8 ± 0.6</td>
<td>46.6 ± 0.4</td>
<td>46.3 ± 0.6</td>
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<tr>
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<td>6* 47.0 ± 0.3</td>
<td>53.0 ± 0.5§</td>
<td>52.1 ± 0.6§</td>
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<tr>
<td></td>
<td>7† 47.2 ± 0.5</td>
<td>52.3 ± 0.5§</td>
<td>52.7 ± 0.6§</td>
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</table>

Data are mean ± SEM.

* Heart rate, mean arterial pressure (MAP), and hematocrit 6 hours after intraductal injection of saline (control group) or induction of pancreatitis (before bolus injection of Ringer or Gadolinium).
† After 7 hours (end of experiment).
‡ Baseline measurement.
§ Significant (p < 0.001) hemoconcentration after induction of pancreatitis in both groups compared with baseline and with control animals.

Described criteria; another animal was excluded because of occlusion of the arterial line. Thirty animals were entered into statistical analysis. Data are presented as mean ± standard error of the mean. Continuous variables (variables that can theoretically assume every possible value; e.g., hematocrit) were tested using the Student's t test. Data without a normal distribution (TAP in plasma) were tested for significance using the Mann-Whitney rank sum test. Differences between groups were considered statistically significant at p < 0.05.

**RESULTS**

**Hemodynamic and Laboratory Parameters**

Mean arterial pressure and heart rate were continuously assessed from the sixth hour on. Table 1 presents data at two representative time points: at 6 hours after intraductal injection of saline (group 1) or GDOC (groups 2 and 3), before the test solutions were injected, and at the end of the experiment (7 hours). There was no significant difference at any time point of the experiment between the control group and the pancreatitis animals, which received either Ringer's solution or GD-DTPA. The hematocrit (see Table 1) indicated a similar significant degree of hemoconcentration in both pancreatitis groups (p < 0.001 vs. controls) despite vigorous intravenous fluid therapy.

The classic markers for pancreatitis—amylase and lipase—were significantly increased after 3 hours in the two pancreatitis groups (p < 0.001 vs. controls; Table 2). In addition, levels of TAP in serum were also increased at 3 hours (p < 0.001 vs. controls), indicating severe pancreatic injury (see Table 2). None of the three markers differed between the two pancreatitis groups before the administration of Ringer’s solution or GD-DTPA, indicating that both groups had similar severity of pancreatitis. One hour after the administration of the test solutions, there still was no significant difference between these two groups (see Table 2).

**Pancreatic Microcirculation**

The following volumetric capillary blood flow results refer to serial measurements in the same capillaries of four different fields of the pancreas (7 animals per group) followed up throughout the entire observation period. The mean capillary flow (investigated capillaries n = 127) in the saline control animals was 2.12 ± 0.07 nL/min at 6 hours and did not change significantly over the duration of the experiment. Saline control animals had a homogeneous perfusion of the pancreas, without any low-flow areas. In animals with severe necrotizing pancreatitis, capillaries with low and high blood flow characteristically can be differentiated.9

There were 96 high-flow and 57 low-flow capillaries evaluated in the Ringer’s solution group and 128 high-flow and 71 low-flow capillaries in the contrast medium group. In high-flow capillaries with a blood flow greater than 1.6 nL/capillary/minute, no relevant changes in pancreatic microcirculation occurred 30 and 60 minutes after injection of either test solution compared to the baseline measurements at 6 hours after induction of pancreatitis (Fig. 1). The high number of low-flow capillaries in both pancreatitis groups is consistent with the expected impairment of the pancreatic microcirculation seen in severe necrotizing pancreatitis. There was no further diminution of capillary perfusion in the low-flow capillaries after the administration either of Ringer’s or GD-DTPA (Fig. 2).
DISCUSSION

Because necrosis is a primary risk factor for septic complications and a major determinant of overall mortality in acute pancreatitis, CECT has been advocated shortly after hospital admission to demonstrate presumably irreversible necrosis of the pancreas, as well as ischemic areas that presumably will become necrotic. Prompt identification of patients at high risk to develop severe necrotizing acute pancreatitis may be important to institute preventive therapy, perhaps to include peritoneal dialysis, or antiproteases or anticytokine drugs, or isovolemic hemodilution with dextran, that may not be justified in patients with mild, self-limited disease.

The potential value of early staging of severe acute pancreatitis notwithstanding, serious concerns have been raised in regard to the administration of radiographic contrast material as used for CT imaging in the presence of a pancreas made more vulnerable by ischemia. Our prior experimental studies have repeatedly demonstrated that ionc and nonionic contrast media, as used for CT evaluation in acute pancreatitis, upgrade the severity of necrotizing acute pancreatitis by aggravating the already existing impairment of pancreatic capillary blood flow. Moreover, a recent retrospective analysis of the effect of CECT in patients with acute pancreatitis concludes that CT con-

<table>
<thead>
<tr>
<th>Table 2. LABORATORY MARKER FOR PANCREATITIS</th>
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<tr>
<td>Time (hr)</td>
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<tr>
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</tr>
<tr>
<td>Amylase (U/L)</td>
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<tr>
<td>Lipase (U/L)</td>
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<tr>
<td>TAP in plasma (nmol/l)</td>
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Data are mean ± SEM.

1 TAP = trypsinogen activation peptide.
2 All data were assessed 3 hours after induction of pancreatitis [before bolus injection of Ringer (control group) or gadolinium].
3 At 7 hours (end of experiment).
4 Significant (p < 0.001) increase of the biochemical markers after induction of pancreatitis (Ringer’s and gadolinium group) compared with control animals.

Figure 1. Intravital microscopy assessment of pancreatic capillary blood flow (nl/capillary/min) in high-flow capillaries. Baseline measurements were made at 6 hours and then repeated 30 and 60 minutes after injection of 0.2 mL/kg Ringer’s solution (control group, n = 96) or gadolinium (n = 128). Data are presented as mean ± standard error of the mean.

Figure 2. Pancreatic capillary perfusion (nl/capillary/min) in low-flow capillaries. Data are presented as in Figure 1.
Contrast agents might worsen or prolong attacks of acute pancreatitis. The natural conclusion from these studies—that CECT perhaps should be avoided in the first days of severe pancreatitis—has been strongly opposed, partly because an alternative diagnostic method for guiding medical and surgical strategy was lacking. However, with the rapid evolution of MRI technology, MRI with GD-DTPA contrast may provide such an alternative.

The potential for MRI of the pancreas in acute pancreatitis was first shown in 1984, but respiratory motion and spatial resolution were still limiting factors. Technical innovations, including a dramatic increase in speed and the possibility of suppressing the high signal of fat, led to the standard use of T₁-weighted fat-suppressed rapid gradient echo and T₂-weighted spin echo sequences in pancreatic imaging. Recent reports demonstrate that MRI with GD-DTPA is as good as CECT in differentiating viable pancreatic parenchyma from areas of pancreatic necrosis and in assessing the location and extent of peri-pancreatic inflammatory changes and fluid collections. Further improvements of MRI are confidently expected. Because MRI also requires a potentially harmful contrast medium for optimal imaging of the pancreas, the safety of GD in necrotizing pancreatitis needs to be proven. In this study, we used the same model of severe necrotizing disease that we have previously used to show the adverse effects of ionic and nonionic CT contrast media. In contrast to the findings with CT contrast agents, GD-DTPA caused no further impairment of pancreatic perfusion, even in low-flow capillaries, and the biochemical markers of severity were not increased. Thus, GD seems to be a safe contrast medium for the early diagnosis of acute necrotizing pancreatitis, even in the presence of a significant hemocoencentration.

The adverse consequences seen after administration of CT contrast agents in the studies of Foitzik et al. and Schmidt et al. were thought to be a consequence of a local impact of the contrast medium on the pancreatic microcirculation, not hypovolemia or systemic hypotension. Some iodinated contrast media have been shown to induce blood cell aggregation and changes in blood cell morphology, causing increased rigidity and impaired oxygen release. Activation of the complement system by contrast agents could aggravate leukocyte–endothelial interaction, which is thought to play a key role in the development of microcirculatory disturbances. Furthermore, contrast media have a relatively high viscosity, which may add to microcirculatory derangements. A direct toxic effect on injured pancreatic acinar cells is also possible. All of these toxicities appear to be avoided when GD is used instead of contrast media for CT imaging, at least under the conditions of this study. In addition, with MRI radiation exposure is reduced or eliminated, and the need for large volumes of renotoxic material is avoided, especially because repeated examinations may be needed within a short time. MRI contrast agents have one-tenth the risk of allergic reactions and can be used safely in patients with impaired renal function.

We conclude that MRI, as the quality of its images improves, its costs decrease, and its availability increases, may supplant CECT as a safer means for evaluating severe acute pancreatitis in its early evolving phase.

References