Bile induced acute oedematous pancreatitis in rats: non-parallel changes in pancreatic morphology and amylase release in vitro

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Abstract
Pancreatic morphology and amylase release from isolated pancreatic acini in response to caerulein was studied in the course of an experimental bile induced acute pancreatitis without acinar necrosis. The inflammation was induced by retrograde microinfusion of 25 µl bile into the rat bile pancreatic duct. A dissociation between functional and structural findings was seen. One hour after the bile injury, there was a transient change in acinar cell function. The response to stimulation by caerulein was reduced by 50%, whereas the sensitivity to caerulein was normal. There was oedema and an initial leucocyte infiltration of the gland. Six hours, one day, three days, and seven days after the bile injury, there was an acute interstitial oedematous pancreatitis with a modest polymorphonuclear leucocyte infiltration of the interstitium and widespread acinar vacuolisation. Morphological changes were most pronounced at the third postoperative day. Acinar amylase release, however, was normal both in terms of sensitivity and responsiveness to stimulation. These results show that bile injury may result in an initial disturbance of acinar cell function. Normal acinar amylase release prevailing in the course of the inflammation suggests that disturbance of the acinar amylase secretory response is not a primary stimulant of the inflammation before the development of acinar necrosis.

Methods

MATERIALS
The following were purchased: highly purified collagenase and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) from Boehringer (Mannheim, Germany), triton X-100 from Ferak (Berlin, Germany), caerulein (Takus) from Farmitalia (Freiburg, Germany), Monotest alpha-amylase and Monotest lactic dehydrogenase-UV from Boehringer (Mannheim, Germany).

ANIMAL MODEL
Fasting female Wistar rats (250–300 g) were anaesthetised with sodium hexobarbitone (80 mg/100 g bodyweight intraperitoneal) and the abdomen was opened along the midline. The tip of a Hamilton microsyringe was passed through the intact antimesenteric wall of the duodenum into the distal bile pancreatic duct and secured by an encircling suture. A W 101 manometer with attached BMT 401 biomonitor (Messgerätewerk Zwönitz, Zwönitz, Germany) was introduced into the system by a three way tap, for constant monitoring of duct pressure, which was always below 20 cm H₂O. Infusion into the duct was delivered by a perfusion pump at a constant rate of 1 µl/min. During infusion, the duct was ligated near the hilus of the liver. Twenty five µl of the solution under study were infused
into the rat bile pancreatic duct. This volume is known to be accepted by the rat pancreas without the development of unphysiological gross ductal rupture. Three experimental groups were studied:

**Group 1:** duct infusion of bile — bile was obtained as described elsewhere and contained (mM/L) glycocholate 66-9, taurocholate 16-2, glycochenodeoxycholate 76-3, glycochenodeoxycholate 54-4, taurochenodeoxycholate 14-5, taurodeoxycholate 6-8, phospholipids 6-2, and cholesterol 7-2.

**Group 2:** duct infusion of physiological saline (controls).

**Group 3:** no duct infusion (sham).

The syringe and the clamp were then removed. The animals were returned to their cages. Rats were anaesthetised again and killed by cardiac section one hour, six hours, one day, three days, and seven days after the completion of the bile pancreatic duct infusion. Pancreatic specimens were prepared as described below.

**MORPHOLOGY**

For light microscopy, pancreatic specimens were fixed with 10% formaldehyde, embedded in paraffin wax, stained with haematoxylin and eosin, and examined in a blinded fashion. Pancreatic tissue was graded for acute pancreatitis according to the presence of oedema, interlobular and intralobular neutrophil infiltration, sialangiitis — that is, inflammatory cell infiltration of the pancreatic duct system, fatty tissue necrosis, acinar vacuolisation, and acinar necrosis. Each of these factors was marked on a scale of 0–3 (absent to severe) and the mean value was calculated.

For electron microscopy, one millimetre cubes of pancreatic tissue were prepared as described in detail elsewhere. Tissue was fixed with cold 2.5% glutaraldehyde and 0.1 M sodium cacodylate buffer, postfixed in 1% OsO4, and embedded in Epon. Thin sections were stained with uranyl acetate followed by lead citrate and examined in a blinded fashion using a BS 613 transmission electron microscope (Tesla, Czechoslovakia).

**ENZYME RELEASE OF ISOLATED PANCREATIC ACINI**

Isolated rat pancreatic acini were prepared essentially as described by Williams et al using collagenase digestion and gentle shearing forces. Acini (0.5 ml) were suspended in an incubation solution (1.5 ml), which contained 0.1 M HEPES enriched with minimal Eagle’s medium amino acid supplement in the presence of various doses of caerulein and incubated for 30 minutes. Then 0.2 ml of the suspension was centrifuged at 10 000 rpm for one minute in a microcentrifuge and the supernatant assayed for amylase and lactic dehydrogenase activity. Lactic dehydrogenase, an intracellular, non-secretory enzyme, was assayed to distinguish a detergent action of bile from a specific releasing effect. Amylase activity was assayed using p-Nitro-phenyl-a-D-maltoside as a chromogenic substrate. Before incubation, some aliquots of acini (0.5 ml) were added to 1.5 ml incubation solution, followed by an immediate centrifugation. The activities of amylase in these aliquots were taken as the initial time zero value. This value was subtracted from values obtained after incubation. At the same time, some aliquots of acini were homogenised by adding 0.2 ml 5% (vol/vol) triton X-100 to the incubation solution followed by assay of total tissue enzyme activity. Enzyme release was calculated by expressing the activity in the medium as a percentage of total enzyme tissue content. Total enzyme content of pancreatic tissue was expressed as total enzyme activity/mg dry weight.

**SURVIVAL**

Survival time was measured over a period of seven days using 15 rats in each of the three experimental groups.

**STATISTICAL ANALYSIS**

The Mann-Whitney rank sum test was used to assess differences between groups. Data are expressed as mean (SEM).

**Results**

**MORPHOLOGY**

**Group 1**

One hour after intraductal bile infusion, a modest oedema was found. Few polymorphonuclear leucocytes were scattered in the interlobular interstitium of the pancreas (Fig 1); the inflammatory score (IS) was 0-4 (p>0.05 v groups 1 and 2).

Six hours, one day, and three days after intraductal bile infusion, acute interstitial oedematous pancreatitis was seen. The inflammation spread from the ductal connective tissue, which showed a severe sialangiitis (Fig 2). There was a modest interlobular and a focal intralobular polymorphonuclear leucocyte infiltration and occasional fatty tissue necrosis. Acinar cell vacuolisation was widespread (Fig 3).
Acute oedematous pancreatitis changes

Figure 1: Widespread microinfusion. Inflammatory cell infiltration of an interlobular duct (haematoxylin and eosin, original magnification ×430).

Autoptastic vacuoles within acinar cells occasionally contained zymogen granules (Fig 4). Cellular organelles believed to be zymogen granules containing electron dense material were found in the interstitial space in a few specimens (Fig 5); zymogen discharge at the basolateral acinar cellular membrane was not seen. Focal necrosis of acinar cells was an exceptional finding. The IS after six hours, one day, and three days of bile injection was 0.9 (0-2), 1.2 (0-3), and 1.3 (0-3) respectively (p<0.05 vs groups 1 and 2). Seven days after intraductal bile infusion, the morphological changes declined and a focal residual interstitial inflammation with ductular transformation of the acinar tissue was found (IS: 0.7; p<0.05 vs groups 1 and 2) (Fig 6).

Group 2
One hour after intraductal saline infusion, a modest oedema and a mild interlobular polymorphonuclear leucocyte infiltration of the gland was seen (IS: 0.3 (0-1)). Six hours and one day after the saline infusion, the pancreas showed a residual focal polymorphonuclear leucocyte infiltration of the interstitium (IS: 0-2 respectively). At three days and seven days, the gland was inconspicuous.

Group 3
One hour, six hours, and one day after the sham operation, a mild oedema of the pancreas was seen (IS: 0-2). At three days and seven days, no morphological changes in the gland were found.

EXPERIMENTS IN VITRO

Amylase release one hour after duct infusion
Basal amylase release was similar in all experimental groups studied. In bile injected rats, acinar amylase release was 4-8 (9-6)% of total content and in saline injected controls and sham operated animals basal secretions were 5-0 (0-8)% and 5-2 (0-7)% respectively (Fig 7). Lactic dehydrogenase activity measured in these same conditions was low (data not shown) and showed no significant differences between groups. These findings confirm the integrity of the acini with which we have been working.

When the amylase release was studied as a function of the concentration of caerulein, the sensitivity of the acini from bile injected rats was not modified. In all groups studied, the minimal and the maximal effective concentration of caerulein for amylase release were 1 and 75 pmol respectively. The maximal secretory response to caerulein was, however, significantly reduced in bile injected rats as acini from these animals released only 11-7 (0-9)% amylase (p<0.05 vs groups 1 and 2) – that is, a 2.5-fold stimulation of amylase release, whereas saline injected controls and sham operated animals released 21-8 (2-0)%

Figure 2: Rat pancreas one day and three days after bile microinfusion. Inflammatory cell infiltration of an interlobular duct (haematoxylin and eosin, original magnification ×430).

Figure 3: Rat pancreas one day and three days after bile microinfusion. Widespread acinar vacuolisation (haematoxylin and eosin, original magnification ×315).

Figure 4: Rat pancreas one day and three days after bile microinfusion. Acinar cell with an autophagic vacuole containing degraded cellular organelles including zymogen granules (original magnification ×9450).

Figure 5: Rat pancreas one day and three days after bile microinfusion. Pancreatic interstitium: process of a macrophage (arrowheads) adjacent to an acinar cell containing electron dense material believed to be zymogen granules (original magnification ×9450).
and 23·3 (3·1)% respectively – that is, had a fivefold stimulation of amylase release. Acini from all experimental groups showed reduced amylase release at supramaximal concentrations of caerulein.

**Amylase release six hours, one day, three days, seven days after duct infusion**

Dose response studies of amylase release from acini prepared at later intervals showed that the maximal secretory capacity in bile injected rats had already returned to normal six hours after the bile injury and that both basal and stimulated amylase release were indistinguishable from controls at all intervals studied (data not shown).

**Amylase content of pancreatic tissue**

Total amylase tissue activity/mg dry weight did not change in the course of acute pancreatitis in bile injected rats, and showed no significant differences compared with saline injected and sham operated controls at all intervals studied (data not shown).

**Survival**

In the three experimental groups studied, all rats survived the total period of observation.

**Discussion**

We describe a model of acute oedematous pancreatitis without significant acinar necrosis after bile microinfusion into the rat bile pancreatic duct. The inflammation was induced under circumstances as similar as possible to those that would be likely to occur after biliopancreatic reflux in humans. Firstly, bile was infused into the rat pancreas to avoid unphysiological damaging effects of individual bile salts on the pancreatic duct system. Secondly, the quantity of fluid infused into the rat bile pancreatic duct was <50 μl – that is, in the range of what is accepted by the pancreatic duct system without gross ductal rupture.

Thirdly, duct pressure was monitored and constantly kept within the physiological range. Thus, bile microinfusion into the rat pancreas is probably more similar to the clinical disease than the commonly used rat model of taurocholate induced acute pancreatitis with retrograde injection volumes grossly in excess of possible reflux and without regard to duct pressure, from which most data are derived on the disturbance of acinar cell structure and function in the course of acute pancreatitis.

Supporting this contention, morphological lesions were seen that resemble those seen in early human acute pancreatitis, but do not occur during the rapid, uniform destruction of the gland in taurocholate induced acute pancreatitis, such as spread of the interstitial polymorphonuclear leucocyte infiltration (Fig 1) from the ductal connective tissue (Fig 2) and vacuolisation of acinar cells (Fig 3). Another advantage over taurocholate induced acute pancreatitis is the wide range of disease activity seen. Ultrastructural data suggest that the inflammation stopped not far from conversion to parenchymal destruction. Firstly, there was degradation of zymogen granules within autophagic vacuoles of acinar cells (Fig 4). This is noteworthy because intra-acinar colocalisation of lysosomal and exocrine digestive enzymes is a precondition for trypsin activation by the lysosomal enzyme cathepsin B, which is believed to cause acinar necrosis in some forms of experimental acute pancreatitis. Secondly, acinar organelles were occasionally found in the interstitial space (Fig 5) pointing to a focal disruption of acinar cellular membranes.
Acute oedematous pancreatitis and changes in pancreatic morphology

In the course of bile induced acute pancreatitis, we saw a dissociation between changes in pancreatic morphology and amylase release in vitro. In an initial phase studied one hour after the bile injury, pancreatic acinar cell function was significantly changed: acini had the same sensitivity in terms of minimal and maximal effective concentration of caerulein as controls, but their responsiveness to the secretagogue was reduced by 50%. At the same time, morphological investigation showed only an oedema and an initial leucocyte infiltration of the interstitium that was not significantly different from controls. In a second phase, which lasted throughout the experiment until the regression of the inflammation, amylase release from pancreatic acini was similar to controls both in terms of sensitivity and responsiveness to caerulein stimulation, while an acute interstitial pancreatitis with a modest polymorphonuclear leucocyte infiltration of the interstitium and widespread acinar vacuolisation was present.

Disturbance of pancreatic acinar digestive enzyme release in the initial phase of the experiment occurred before any significant leucocyte infiltration of the interstitium and, therefore, apparently does not result from secretions of activated leucocytes. Bile salts also were probably not harmful because, in contrast, a specific stimulatory effect on digestive enzyme release from pancreatic acini in vitro was seen at concentrations that do not cause acinar necrosis. Perhaps, inflammatory mediators such as prostaglandins might be involved, which have been reported to impair the secretory capacity of isolated rat pancreatic acini in response to cholecystokinin stimulation.

After the initial reduction of the secretory capacity, however, pancreatic acinar exocrine enzyme release was noticeably undisturbed throughout the period of observation. The preservation of normal acinar stimulus-secretion coupling in non-destructive bile induced pancreatitis is in striking contrast with findings in caerulein induced acute oedematous pancreatitis. There, a change in acinar digestive enzyme release is assumed to play a causative part in the initiation of the inflammation, as a persistent impairment of both acinar sensitivity and responsiveness to stimulation was found to be associated with a concomitant leucocyte infiltration of the interstitium. In non-destructive bile induced acute pancreatitis, the association of normal acinar stimulus secretion coupling prevailing in the course of the inflammation and a progressive leucocyte infiltration of the interstitium shows that bile injury of the pancreas results in an inflammatory response within the interstitium, irrespective of acinar digestive enzyme secretion. We conclude that disturbance of exocrine enzyme release of pancreatic acinar cells as judged by their amylase secretory response is probably not the primary stimulant of the inflammation before the development of acinar necrosis in this model.

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