Experimental Acute Necrotising Pancreatitis: Evaluation and Characterisation of a Model of Intraparenchymal Injection of Sodium Taurocholate in Rats

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ABSTRACT

Objectives: To evaluate a simple model that produces progressive dose dependent pancreatitis, by intraparenchymal injection of sodium taurocholate.

Design: Open laboratory study.

Setting: Teaching hospital, Israel.

Materials: Forty eight Wistar rats.

Interventions: Sodium taurocholate was injected, 0.3 ml/100 g body weight, in concentrations of 5% and 10% into the pancreatic parenchyma of 32 Wistar rats, resulting in two distinct groups of severity. In 16 sham controls, saline was injected into the pancreas in similar fashion. Blood samples were withdrawn before, and 6, 24, 48, and 72 hours after induction of pancreatitis.

Results: Six hours after taurocholate injection, there was a sharp increase in the plasma activities of amylase, lipase, and lactate dehydrogenase (LDH). After 24 hours plasma activities of amylase and lipase decreased to near normal values while LDH remained slightly increased for 48 hours and decreased only after 72 hours. At 6 hours after the injection, interleukin-6 (IL-6) concentrations had increased slightly in the 5% group and decreased to the baseline values at 24 hours. In the 10% group, the increase in IL-6 values was significantly greater than in the 5% group ($p = 0.04$), and correlated well with severity of pancreatitis as defined by histology ($p = 0.01$) and mortality ($p = 0.037$). Twenty four hours after injection of taurocholate, morphological changes comprising diffuse necrosis of the pancreas, fat necrosis, and intestinal dilatation secondary to paralytic ileus were severe. Histopathological examination of the pancreas showed good correlation with the clinical findings and with mortality.

No morphological changes were detected when saline was injected into the pancreas (sham control), and only mild rises of IL-6, lipase, amylase, and LDH activities were seen at 6 hours after injection. The mortality, after 10 days, was 80% in the 10% taurocholate group, 30% in the 5% taurocholate group, and 0 in the sham control group ($p < 0.05$).

Conclusion: The intraparenchymal injection of taurocholate is easy to perform and highly reproducible. The histopathological injury is dose-dependent, as is the mortality. We conclude that this model is valuable for the study of new treatments for pancreatitis.

Key words: experimental model, pancreatitis, rats, taurocholate.

INTRODUCTION

Acute pancreatitis may present as a mild self-limiting disorder, or a severe disease which is sometimes fatal. The pathological features range from mild oedema to haemorrhage and necrosis. Although its pathophysiology has been extensively investigated, it is only partly understood (3). No specific treatment to prevent the deleterious effects of the digestive enzymes on the pancreas and peripancreatic tissues has been found so far. As there is a definite correlation between the extent of pancreatic necrosis and the clinical outcome, there is hope that a specific treatment may be found that would alter the course of the disease. New treatments are usually tried in animal models, so numerous experimental models of acute pancreatitis have been developed. The more commonly used models are diet-induced, intravenous or intraperitoneal injections of the secretagogue caerulein, and retrograde intraductal injection of sodium taurocholate (1, 10, 17). Most of these models however, have limitations related to variability and reproducibility. In 1974 a new and simple model of experimental pancreatitis was described (2). A solution of taurocholic acid was simply injected at several sites in the pancreas. Although this model has been used in several studies (5, 8, 9, 14), it has not been adequately evaluated. We therefore developed and characterised the model of intraparenchymal injection of sodium taurocholate in rats.

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Table I. *Histopathological score*

Oedema:	
0	Absent
5	Diffuse expansion of interlobular septa
10	Diffuse expansion of interacinar septa
20	Diffuse expansion of intercellular spaces
Lobar necrosis in 10 high power fields:	
Number of partially necrotic lobes × 5	
Number of totally necrotic lobes × 10	
Inflammation:	
10	Presence of diffuse leucocyte infiltration in more then 5 high power fields
10	Presence of micro-abscesses

The histopathological score was calculated by adding the oedema score to the number of partially necrotic lobes multiplied by 5 and the number of totally necrotic lobes multiplied by 10, in 10 high power fields. If there was diffuse leucocyte infiltration in more then 5 high power fields or micro-abscesses, 10 points were added to each.

MATERIAL AND METHODS

Male Wistar rats (300 to 400 g) were kept as outlined in the "Guide for the Care and use of Laboratory Animals" (NIH Publication #85–23, 1985). General anaesthesia was induced by intraperitoneal injection of 60 mg/kg of pentobarbitone (Sanosi laboratories, Paris, France). After anaesthesia, the femoral vein was cannulated with a 20G polytetrafluoroethylene catheter (Venflon, Helsingborg, Sweden) which was secured with a ligature. After each use, the catheter was flushed with 0.3 ml of heparin solution (1000 units/100 ml normal saline). The abdomen was then shaved and a midline laparotomy incision was made. The stomach, duodenum, pancreas, and spleen were carefully brought out of the abdominal cavity. Pancreatitis was induced by multiple (between 7 and 10) intraparenchymal injections of a total of 0.3 ml/100 g body weight of sodium taurocholate solution, at different concentrations, through a 30 gauge needle. In sham controls, saline was injected into the pancreatic parenchyma in a similar fashion. The injections were made by carefully introducing the tip of the needle into several parts of the pancreas, under magnifying lenses, while avoiding damage to blood vessels and pancreatic ducts. After the injection, the organs were carefully returned to the peritoneal cavity, and the incision was closed with a continuous 3 0 silk suture. The animals were left to recover for one hour, and put back into unrestraining cages, 3 rats/cage, and given unlimited access to water for 24 hours. They were then given unlimited access to standard rat chow.

Study groups: The rats were divided into 3 groups of 16 rats each: sham operated, and saline injected into the pancreas, 0.3 ml/100 g body weight; 5% taurocholate solution injected into the pancreas; 10% taurocholate solution injected in a similar manner.

Biochemical tests: Blood samples were withdrawn from 20 healthy anaesthetised rats, to establish the base-line values of all laboratory measurements. To avoid severe anaemia, the animals in each study group were randomly divided in two subgroups, and blood samples were obtained only twice from each animal during the study. Blood samples (0.8 ml) were withdrawn at 6 and 48 hours ($n = 8$), or at 24 and 72 hours ($n = 8$) after induction of pancreatitis. The blood was replaced by an equal volume of normal saline. The blood samples were immediately centrifuged and the plasma samples were kept at $-70°$ until tested. The blood samples were analyzed for pH, PCO2, bicarbonate, base excess (Acid Base Analyzer, ABL30 Radiometer), and lactic acid. The sera were assayed for glucose, electrolytes, and calcium concentrations, and activities of amylase and lipase (Hitachi Analyzer 737). IL-6 values (Quantikine M, R&D Systems Minneapolis, USA) were measured by an experienced laboratory technician (TS) who was unaware of what injection each rat had had.

Mortality study

Because repeated anaesthesia and blood drawing may have a biasing effect on mortality, 10 additional rats from each group cited above, were studied. Pancreatitis was induced in the same fashion, but without any further manipulation. They were put in the cages, and observed every 8 hours for 10 days and the mortality recorded.

Pathological examination

After the animals had been killed, the pancreas was harvested and trimmed of fat. Its macroscopic appearance was recorded, and the gland was then fixed in 10% formaldehyde, processed in paraffin, and stained with haematoxylin and eosin. Microscopic examination was made by one pathologist (DK) who was unaware of the rat's treatment. The injury was scored by recording the

number of lobes affected by oedema, partial necrosis, total necrosis and leucocyte infiltration (Table I). We also recorded vascular thrombi, bacterial colonies, fat necrosis, and haemorrhages.

Statistical analysis

Results are expressed as mean (SD). One way analysis of variance (F test), non-paired *t* test, chi square test and ANOVA were used (SPSS, SPSS Inc. software, USA). Probabilities of less than 0.05 were accepted as significant.

RESULTS

Taurocholate-induced pancreatitis was characterised 24 hours after induction, by severe morphological changes in the pancreas including haemorrhages and necrosis. In addition there was fat necrosis throughout the peritoneal cavity and pronounced intestinal dilatation. In controls, the injection of saline into the pancreatic parenchyma was not followed by any macroscopic damage and all abdominal organs looked normal.

Six hours after induction of pancreatitis, plasma activities of amylase, lipase, and LDH were significantly increased in both groups. There were also moderate increases in the control group (Fig. 1). No consistent significant difference was seen in activities of lipase and LDH levels between the pancreatitis groups at 6 hours. Amylase values increased from 1700 (188) to 4213 (637) U/L in the 5% group, and from 1830 (157) to 7626 (1666) U/L in the 10% group. The difference between the groups is significant ($p < 0.001$). Lipase values increased from 15 (12) to 1238 (772) U/L in the 5% group and from 16 (10) to 1801 (530) U/L in 10% group. The difference between the groups is significant ($p = 0.02$). LDH values increased from 696 (443) to 5347 (2746) U/L in the 5% group and from 670 (541) to 6926 (2572) U/L in the 10% group but there was no significant difference between the groups. Twenty-four hours after induction of pancreatitis, serum amylase and lipase activities decreased to almost baseline values, while LDH values remained mildly increased up to 48 hours and returned to baseline after 72 hours.

No consistent significant changes were observed in plasma concentrations of urea, glucose, or calcium, or pH in any of the study groups.

There was a mild but significant increase in plasma lactic acid concentration in the surviving animals at 48 hours and it remained high at 72 hours. This mild metabolic acidosis was compensated by hyperventilation with a decrease in PCO2 and an unchanged pH.

At 6 hours after induction of pancreatitis, IL-6 values increased slightly in the controls and the 5% taurocholate group {from 3.9 (4.5) to 42.4 (33.5) pg/L, and from 4.1 (4.7) to 46.4 (28.5) pg/L, respectively} and remained considerably raised up to 24 hours {9.7 (2.7) pg/L and 27.5 (37.4) pg/L, respectively}, returning to baseline values at 72 hours. In the 10% taurocholate group the increase in the IL-6 value was significantly higher than in the other groups {130.4 (30) pg/L, $p < 0.001$ in both cases} (Fig. 2).

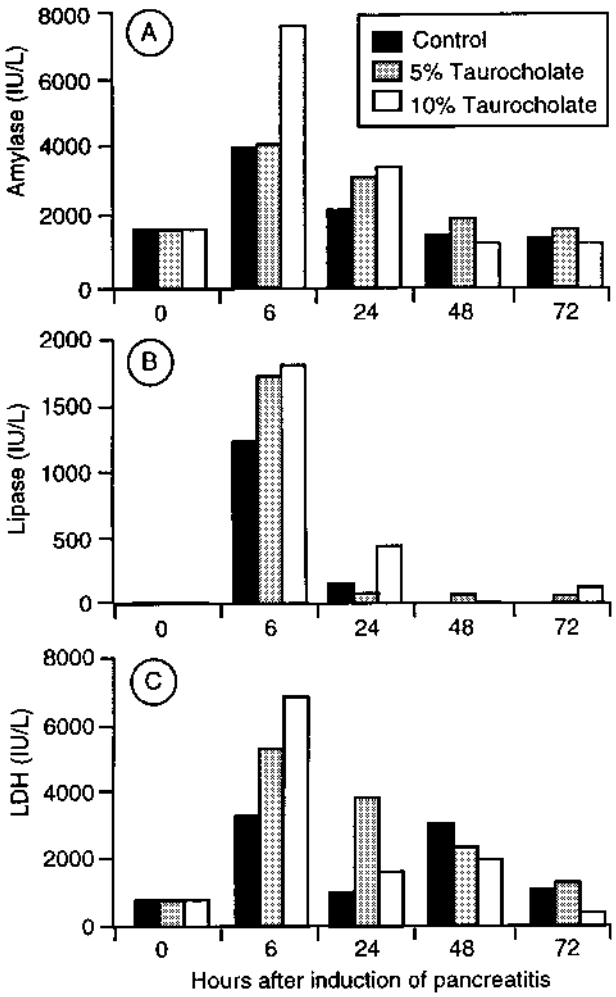

Fig. 1. Plasma concentrations of amylase, lipase, and lactate dehydrogenase after induction of pancreatitis. Enzymes activities are expressed in U/L. Six hours after injection of taurocholate, activities of all three enzymes had increased significantly. At 24 hours plasma lipase and amylase had decreased almost to the baseline values, while lactate dehydrogenase remained mildly raised up to 72 hours. Controls had a small rise in the enzymes values probably related to the mechanical trauma to the gland.

At 24 hours moderate to severe histopathological damage was seen in the two groups with pancreatitis, while in controls, mild oedema was seen in six rats. In the remaining 10 controls, the pancreas looked normal. The histological sections of the rats with pancreatitis showed necrosis and leucocyte infiltration of the

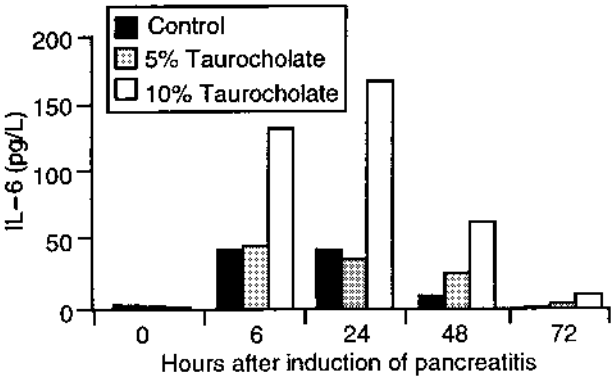

Fig. 2. Two hours after injection of taurocholate, IL-6 values had increased slighly in control and 5% taurocholate groups, and remained significantly higher up to 24 hours, but returned to baseline values at 72 hours. In 10% group the increase in IL-6 values was significantly greater than in the other two groups.

pancreatic lobes to varying degrees, (Fig. 3 and Fig. 4), as well as fat necrosis, haemorrhages, and vascular thrombi in the peripancreatic tissue. Increased concentrations of taurocholate resulted in increased mean damage score which was 3 (2) in the control group, 48 (14) in the 5% taurocholate group and 85 (11) in the 10% taurocholate group ($p < 0.01$). Abundant leucocyte infiltration was associated with higher scores.

The surviving animals were killed after 20 days, and the pancreas examined. Diffuse scarring was noted, replacing areas of necrosis, in the taurocholate groups while the pancreas of the controls looked normal.

Twenty days after induction of pancreatitis, the surviving animals' body weight had decreased from 366 (97) to 330 (12) g while the controls' body weight remained unchanged at 372 (8) g.

None of the rats in the control group died. In the 5% group 5 rats died (30%), and in 10% group 13 died (80%) ($p = 0.012$) (Fig. 5).

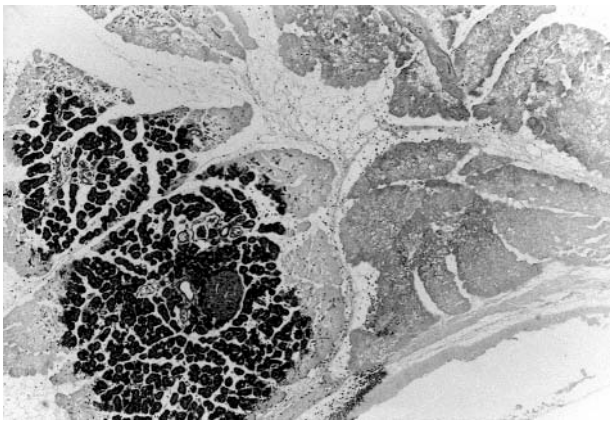

Fig. 3. Twenty-four hours after injection of taurocholate the histological sections of the pancreas showed necrosis and fat necrosis, and the infiltration of leucocytes.

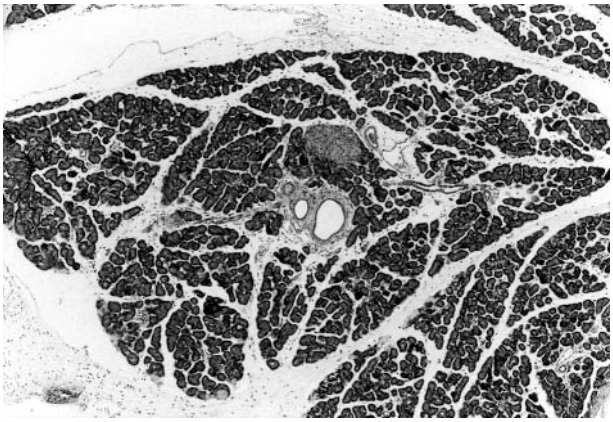

Fig. 4. Twenty-four hours after saline injection, the histological sections of the pancreas showed only mild perilobular oedema and no necrosis.

DISCUSSION

The ideal model of pancreatitis should mimic the clinical signs, time course, and histopathology of the human disease. The most commonly used models are diet-induced, intravenous or intraperitoneal injections of the secretagogue caerulein, and retrograde intraductal injection of sodium taurocholate (1, 10, 17). Most of these models however, have serious limitations related to technical difficulties, time course of the disease, and reproducibility. The over-stimulation technique with caerulein causes only a mild pancreatitis, with no mortality, and variable slight pathological changes. Diet-induced models must be used only in mice, with appreciable technical difficulties, and are almost invariable lethal. The retrograde influx model, despite its popularity, has a variable mortality, and the lesion is confined to the proximal parts of the pancreas. A model combining retrograde taurocholate injection with systemic caerulein infusion has also been described which

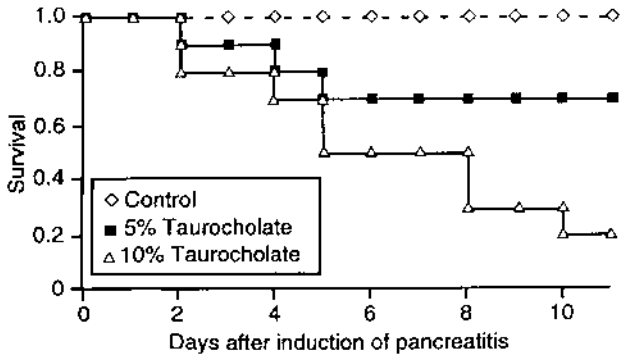

Fig. 5. There was a moderate progressive mortality with increased concentrations of injected taurocholate. After 10 days, the mortality was 0 in sham controls, 30% in the 5% taurocholate group, and 80% in the 10% taurocholate group ($p < 0.01$).

correlates well with the human disease. It is dose-dependent and has good reproducibility, but is time consuming and quite complicated (16).

We developed a simple model of injections of sodium taurocholic acid, at different concentrations, directly into several sites of the pancreatic parenchyma. The technique is easy, is highly reproducible, and results in a macroscopically homogeneous injury to the pancreas, with dose-dependent acinar necrosis and dose-dependent moderate mortality. This dose-dependent damage is useful as the same model can be used to study the mild as well as the fatal necrotising form of the disease, by increasing the concentration of taurocholate, up to the desired degree of damage. The sodium taurocholate causes a detergent effect, with diffuse pancreatic necrosis. The early and late histopathological picture mimics the findings in human disease.

Intraparenchymal injection of sodium taurocholate resulted in a substantial early increase in plasma activities of lipase, amylase, and LDH. The increase in enzyme values seen in the controls probably resulted from the manual handling and puncture of the gland, but did not result in any discernible late damage. It is important to stress, however, that in the clinical setting, amylase and lipase are not considered to be of prognostic significance, and are useful only as markers of the diagnosis of pancreatitis. The increase in these enzymes comes early in the course of the pancreatitis and does not correlate with the amount of damage to the gland or the outcome of the disease (4, 15).

The inflammatory cytokine IL-6, on the other hand, is generated within the pancreas and its concentration in the blood is associated with the degree of inflammation and pancreatic damage (12, 13). Previous studies have shown that IL-6 values correlated well with the severity of the disease (6, 7, 11). In the present study, IL-6 plasma values at 6 hours and 24 hours, correlated with the concentrations of taurocholate injected. The two concentrations that we used also correlated with the pathological score, and with the mortality, much like the human form of the disease.

We conclude that this model of experimental pancreatitis, by intraparenchymal injection in rats, is simple and highly reproducible. It is suitable for the investigation of acute pancreatitis, and will be useful in studies of new methods of treatment.

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