EXPERIMENTAL MODELS OF ACUTE PANCREATITIS -CLOSED DUODENAL LOOP MODEL

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Abstract: Randomized controlled studies of severe human acute pancreatitis can be performed only with restriction, especially in children. Some pathophysiological or therapeutic aspects should be first verified through animal experiments. Animal test results can be transferred to clinical practice if the results are based on trials with established models, standardized methods and a study design imitating the clinical situation. This study aims to review the different experimental models proposed for acute pancreatitis, focusing on the experience of the researcher's team with the "closed duodenal loop" model. A closed duodenal loop model was prepared in male Wistar rats. The duodenum was ligated 2cm distal and proximal to the junction of the hepatopancreatic duct. All rats developed severe acute pancreatitis. The histopathological alterations of the pancreas consisted in edema, parenchymal necrosis, thrombosis and hemorrhage. All animals died within 2-5 days from generalized sepsis. Animal experiments are of great value, especially in acute necrotizing pancreatitis because randomized controlled studies are problematic. The most common experimental models of acute pancreatitis are secretagogue hyperstimulation (cerulein pancreatitis), choline-deficient, ethionine-supplemented diet (CDE), duct obstruction/ligation, closed duodenal loop, duct perfusion. Closed duodenal loop model is easy enough from the technical point of view and presents histological characteristics that resemble those of human pancreatitis, but it has the drawback of early mortality.

INTRODUCTION

Acute pancreatitis (AP) varies in etiology, severity, mode of progression and requires a complex treatment. Because changes in evolution of AP are dramatic, its pathophysiologic analysis from clinical cases is difficult. Thus animal models are indispensable for pathophysiological studies, mainly in cell and molecular biology (1). Also, the effectiveness of therapeutic procedures should be examined after testing each substance in the context of legally required study phases, and ideally in the form of randomized controlled clinical studies. In AP this is problematic, due to variations in the causes, degree of severity and disease course, the low incidence of severe forms, the disease onset (usually referred to as the onset of abdominal pain; it cannot be determined exactly). The anatomical site of the pancreas in the retro peritoneum makes this organ rather inaccessible for detailed investigations, so that the treatment of AP is extremely complicated and requires close interdisciplinary work (2).

It has thus become necessary to test new therapeutic procedures and pathophysiological studies in AP first in animal models. The advantages of animal experiments are: standardization, achieved to an extent which cannot be approached clinically; all tests can be carried out in a large number of test animals of the same age and sex, with the same disease severity at the same time after disease onset.

Although animal experiments have contributed to a better understanding of the pathogenesis of AP, using animal models in therapeutic studies is still controversial due to the limited applicability of the results in clinical practice. Examples include cholecystokinin antagonists and protease inhibitors which had a positive effect on the disease course in animal but failed to prove their effectiveness in clinical practice (3).

An ideal experimental model of AP should comply with the following requirements:

1. To show the features of severe necrotizing AP: intra- and extraparenchymal necrosis, signs of systemic inflammation and multiorgan dysfunction syndrome with reproducible mortality;
2. Biphasic disease course: initial systemic inflammatory reaction syndrome (SIRS) with primary multiple organ dysfunction syndrome (MODS), then secondary inflammatory reactions or exacerbation due to infections with secondary MODS; (4-5)
3. Response to therapy;
4. Monitoring: standardized, reproducible and verifiable severity of AP and possibility of continued recording of vital and organ function (2).

It goes without saying that none of the existing models fulfils all these criteria.

Experimental models of acute pancreatitis in rats

There are numerous models of AP and modifications of these, which are differentiated according to the:

1. induction technique (invasive and non invasive models);
2. cause (biliary, obstructive, alcoholic toxic, traumatic, ischemic) or
3. degree of severity (mild/ oedematous or severe/necrotizing) (6-7).

Several laboratory animal species have been used for experimental models of AP.
Although mice have small dimensions and weigh only 20g, there are a lot of papers on murine models, for the invasive diagnostic and therapeutic measures, especially in those with genetic mutation(8). Cats, dogs, sheep or pigs models are expensive and imply ethical objections (9). Rabbits have proved high individual variability of the disease severity even with good standardized induction techniques. Opossum models are good enough but these animals are not easily available (10). For the reasons given, rats are basically ideal test animals for AP.
The common experimental models of AP are:

1. The choline-deficient, ethionine-supplement diet models (CDE) – test animals (mice, rats) develop severe hemorrhagic AP with a big mortality. There are other concomitant causes of death independent of AP, changes in the liver and central nervous system, which contribute to multiple organ failures. This model has a variable disease course, partly due to the animal weight and intake of food (11).
2. Cerulein-pancreatitis – AP is induced by supramaximal stimulation of the pancreas with the cholecystokinin analogue cerulein. Cerulein is injected intraperitoneally (12), subcutaneously or in the femoral vein (13). Cerulein pancreatitis is frequently mild and edematous.
3. Obstruction models – AP is induced by the ligature of the pancreatic duct or bile duct. Reflux of bile and pancreatic secretions alone (without additional exocrine hyper stimulation) leads only to mild pancreatitis without extensive necrosis of infectious complications (14).
4. Duct perfusion models – AP is induced by anterograde and retrograde infusion of bile salts (taurocholate). These models have been used extensively for studying the effect of various therapeutic agents (cholecystokinin antagonists, somatostatin, fibrinolitic agents and cytokine antagonists (15). The induction technique requires a high level of standardization when performing ductal perfusion and the severity of the disease may be too fulminant. This model can be improved by additional noxae not necessarily considered to be involved in causing human AP.
5. Closed duodenal loop (CDL) models – AP is triggered by a reflux of bile and pancreatic secretions following duodenal ligation distal and proximal to the junction of the hepatopancreatic duct (2).
6. Models which expose the animal to the same noxae that trigger AP in man- alcohol, bile duct obstruction, pancreatic ischemia, traumatic damage, toxic substances. This model was not encouraging because the animals showed neither the typical morphological nor the clinical signs characteristic to severe human AP.

Closed duodenal loop models in rats

CDL model of AP is reported to present histological characteristics that resemble those of human pancreatitis (16) and has become one of the most widely used experimental models of AP. The medical literature includes clinical reports of AP associated with duodenal obstruction (17-18).

The factors involved in the development and progression of pancreatitis in the CDL model include: reflux of duodenal fluid, containing activated pancreatic fluid, increased pressure within the pancreatic duct, reflux of bile, infection and impaired pancreatic blood flow. The disease severity depends largely on the perfusion of the closed intestinal loop.(2) The secretory reflux in the closed loop is bacterially contaminated and the intestinal motility and microflora are changed by the lack of bile and pancreatic juice in the intestinal segment distal from the ligated intestinal loop (19). Rats die within 2-3 days from generalized therapy-refractory sepsis.

MATERIALS AND METHODS

Animal preparation: Male Wistar rats, weighing 200-250 g, were used in all experiments. The rats were kept at constant temperature with free access to water and standard rat pellets.

Experimental protocol: The animals received no nourishment other than water ad libitum for a period of at least 18 hours preoperatively.

They were given general anesthesia (induction with ether, then maintenance with atropine 0,1mg/kg, ketamine 1mg/kg and pethidine 1mg/kg, administered intraperitoneally; drug doses were repeated at 30-40 min).

Laparotomy was performed by upper midline incision. To prepare the duodenal loop the duodenum was carefully pierced through and ligated around its entire circumference with 5-0 silk. Locations of ligatures were at 2cm orally and anally of the duodenal entry of the biliopancreatic duct. **(Fig.1)**

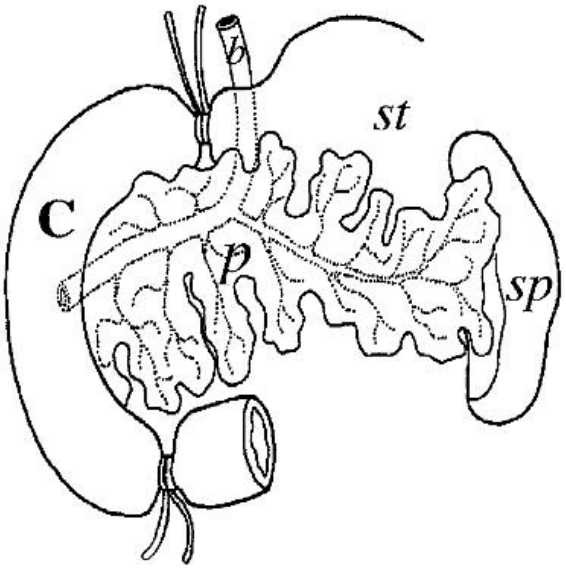

Fig.1 The operative schema: p=pancreas, st=stomach, sp=spleen

In the control group, only laparotomy was performed **(Fig.2-3)**

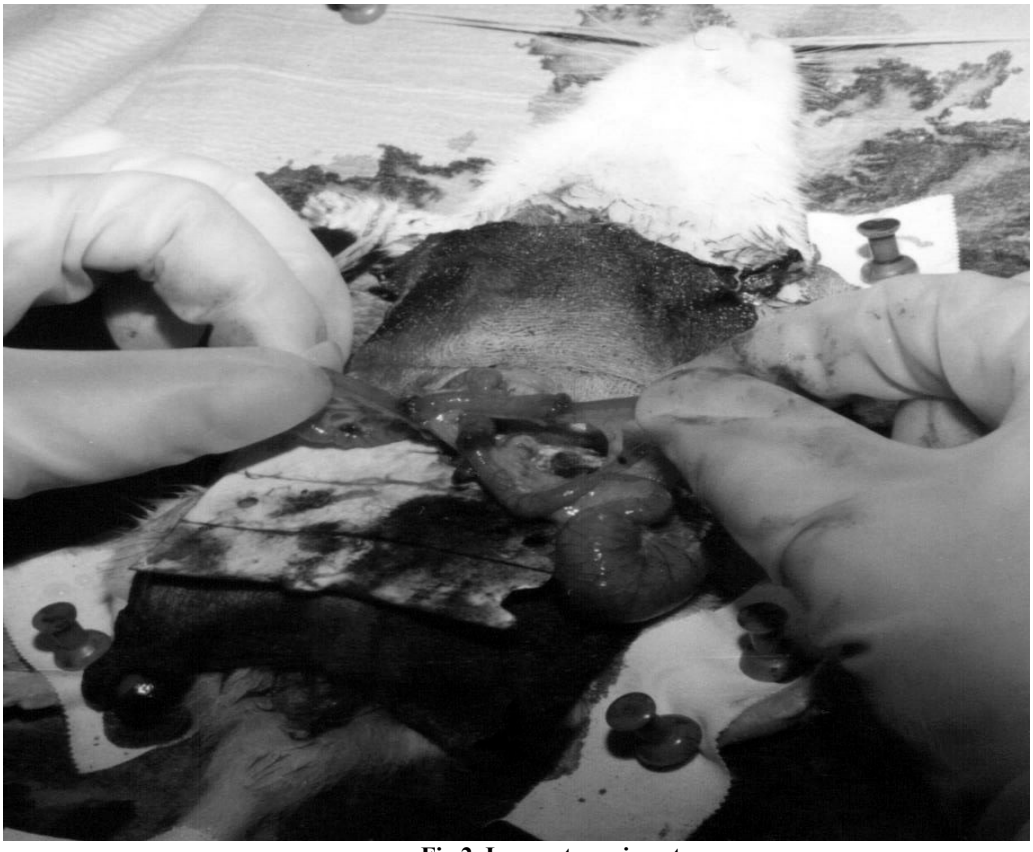

Fig.2 Laparotomy in rats

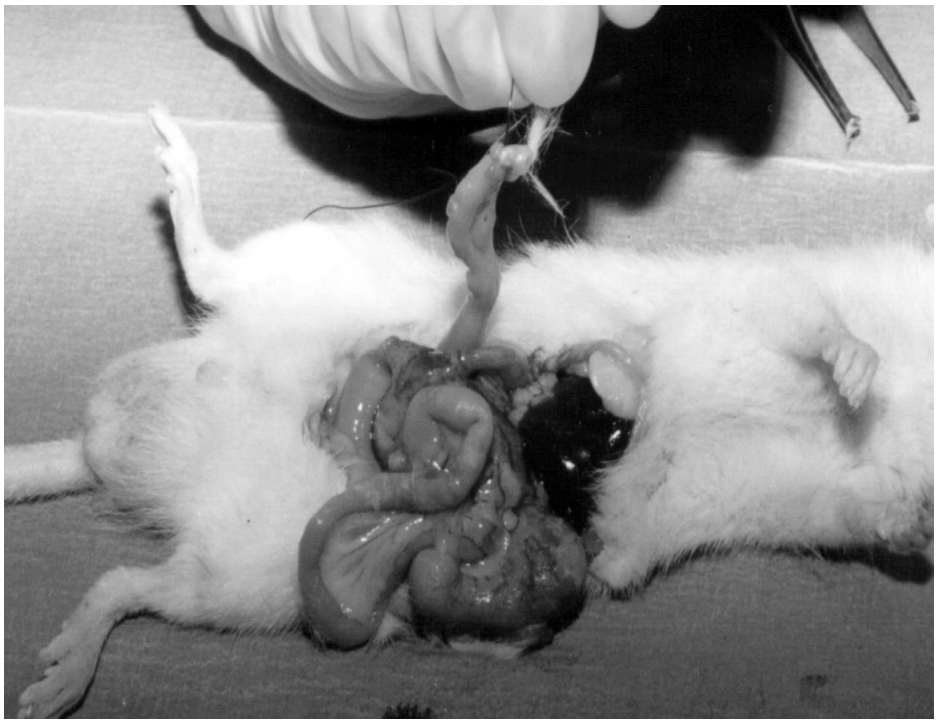

Fig.3 Laparotomy in rats

After surgery, the animals received no nourishment other than water during their survival.

RESULTS

Pancreatic histological changes (compared with a normal pancreas Fig. 4): After the occurrence of death, small samples from the pancreas were fixed by an immersion in Formalin. After paraffin embedding, sectioning and staining with hematoxylin-eosin, the sections were examined light-microscopically. Pancreatic histological changes included interstitial edema, inflammatory cell infiltration (Fig.5), focal necrosis (Fig.6) and hemorrhage (Fig.7).

The average survival period was 24 hours (12- 48hours)

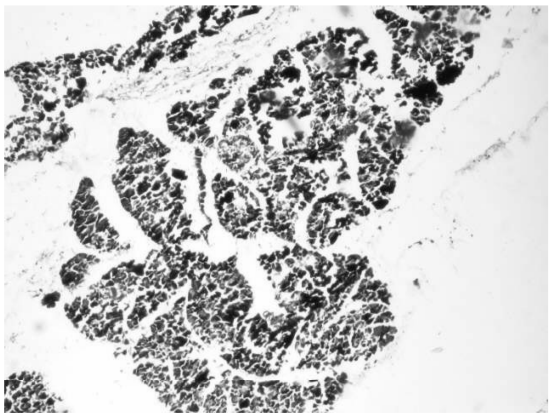

Fig. 4 normal pancreas

Fig. 5 inflammatory cell infiltration

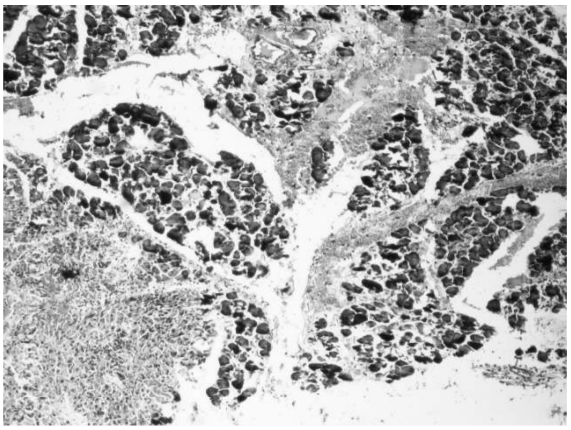

Fig. 6 pancreatic necrosis

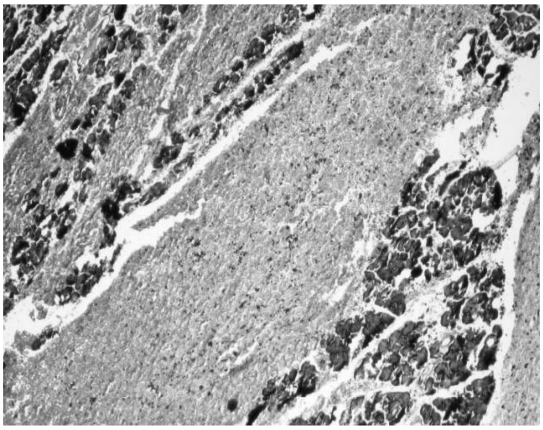

Fig.7 pancreatic hemorrhage

CONCLUSIONS

CDL model in rats leads to the development of AP with marked interstitial edema, focal necrosis and hemorrhage.

The appearance of these lesions and high mortality of the animals are remarkably reproducible.

This model of AP is morphologically and biochemically similar to clinical severe AP.

In this model, the exocrine pancreas was vulnerable to activation of digestive enzymes both inside and outside the acinary cell. The reflux of bile and duodenal contents into the pancreatic duct and subsequent intrapancreatic activation of digestive enzymes have been suggested to play an important role. The high pressure in the duodenum causes a high pressure on the intrapancreatic-duct and the subsequent congestion of pancreatic acinary cells. These pancreatic injuries induce overproduction of many kinds of cytokines and induce systemic inflammatory response with high mortality.

The CDL model has the drawback of early mortality induced by factors such as retention of gastric juice, impaired duodenal blood flow and bacterial infection. To avoid retention of gastric juice, a lot of strategies have been proposed, such as preparation of a gastrojejunal anastomosis, insertion of a tube within the duodenum and temporary release of the obstruction after preparation of CDL, but these surgical invasions have the potential to bias the experimental results.

CDL model is simple to prepare and easy enough from the technical point of view.

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