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Digestive and Liver Disease 38 (2006) 423–428
Digestive and Liver Disease
www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Early bacterial infection of the pancreas and course of disease in cerulein-induced acute pancreatitis in rats

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Received 13 February 2006; accepted 27 February 2006
Available online 18 April 2006

Abstract

Background. Bacterial infection of the pancreas aggravates the course of acute pancreatitis. Since bacterial translocation from the gut is likely to be an early event, in an animal model of pancreatitis, we investigated the effect of early bacterial supra-infection of the pancreas on the course of the disease.

Methods. Six hours after the induction of acute pancreatitis in male Wistar rats ($n = 180$) by supramaximal stimulation with cerulein (or placebo in a control group), the animals were operated and a suspension of *Helicobacter pylori*, *Escherichia coli* or saline were introduced either in the pancreatic duct or interstitium (12 groups of 15 rats each); after 24 h, animals were killed and the following parameters analysed: macroscopic and histologic appearance of the pancreas (score), wet-to-dry weight ratio, pancreas trypsinogen activation peptide level, serum amylase, interleukin-6 and phospholipase A2 activity.

Results. All parameters were increased in rats with cerulein-induced pancreatitis in comparison to placebo. Interstitial and intraductal application of bacteria increased the pancreatic damage. This effect was more evident with the application of *E. coli* in both cerulein and placebo groups. Application of *E. coli* but not of *H. pylori* determined pancreatic activation of trypsinogen, increased mortality and induced the production of interleukin-6.

Conclusions. Bacterial invasion of the pancreas worsens the histologic and clinical picture of disease and induces a systemic inflammatory response.

Keywords: Acute pancreatitis; Bacterial infection; Cerulein pancreatitis; Pancreatic necrosis; Systemic inflammatory response syndrome (SIRS)

1. Introduction

Infected pancreatic necrosis is the major cause of morbidity and mortality associated with acute pancreatitis (AP). Experimental studies indicated that pancreatic infection in necrotising AP is likely to be due to translocation of bacteria from the gut to lymph nodes, peritoneal fluid and blood, then from these sites to the pancreas itself. Infection of pancreatic necrosis usually occurs in humans after the first week of disease. However, animal models of AP show that bacterial translocation from the gut is probably an earlier event, since as early as 24 h after necrotising AP induction, peritoneal fluid, lymph nodes, blood and distant organs became infected by enteric bacteria [1]. Once the enteric bacteria reach the pancreatic tissue, they could trigger intrapancreatic activation of pancreatic enzymes as well as various chemical mediators, thereby bringing about severe pancreatic necrosis and inflammatory reaction and resulting in self-perpetuating inflammatory state. In experimental studies, Keynes [2] has demonstrated that, when pancreatic infection occurs, oedematous pancreatitis progresses to necrotising pancreatitis. Intraperitoneal administration of LPS in rats with cerulein-induced pancreatitis increases significantly the levels of TNF

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doi:10.1016/j.dld.2006.02.019

and mortality of the animals, as well as determines necrosis, bleeding and vacuolation in the liver as a consequence of a systemic inflammatory response syndrome (SIRS) [3].

In human AP, irrespective of the aetiological factors, once the inflammatory process has started, the further course of the disease is largely unpredictable. The underlying pathogenic steps that may ultimately lead to pancreatic necrosis and systemic morbidity are still not well understood. During the last decade, much evidence has been accumulated indicating inflammatory mediators playing a key role in the evolution of disease.

In the present study, we test the hypothesis that early bacterial invasion of the pancreatic tissue may worsen the course of AP and lead on one side to the progression of oedematous to necrotising pancreatitis, and on the other to the release and activation of the mediators of the SIRS.

2. Material and methods

2.1. Animals

Male Wistar rats, weighing 250–300 g, were used. The study was approved by the Animal Ethical Committee of the University of Magdeburg. Animals were allowed to acclimatise for 7 days prior to experiment and kept in a climate-controlled environment with 12-h light/12-h dark cycle. One hundred and eighty rats were allocated to 12 groups of 15 rats each: controls and oedematous pancreatitis; these groups were further subdivided into those in which *H. pylori*, *E. coli* or saline were introduced into the pancreas duct or interstitium. Group description is given in Table 1.

2.2. Study design

Oedematous pancreatitis was induced by five intraperitoneal injections of cerulein 40 μg/kg over a period of 4 h. The control groups received 0.9% saline in the same way. After the last injection, animals were anaesthetised with the administration of ketamina (80 mg/kg, i.m.) and xylazin (12 mg/kg, i.m.) and underwent upper midline laparotomy. In groups receiving intraductal administration of bacteria or saline, the biliopancreatic duct was cannulated transmurally with a 27 G needle. The biliary duct was closed with a Yasargil-microclip placed distally to the pancreas at the hilum of the liver. Thereafter, 120 μl of a Brucella broth suspension of *H. pylori* (CagA and VacA positive, 0.2 optical density at 546 nm), or of *E. coli* ('Nissle 1917') at the same concentration, or an equal volume of 0.9% saline was infused under constant pressure at the rate of 6 ml/h. After the administration of the suspension, the microclip and the needle were removed and the abdominal wall was closed. In groups receiving interstitial administration of bacteria or saline, 40 μl of the bacterial suspension, or placebo in the same amount were injected with a 27 G needle in three different parts of the pancreatic gland. Similarly, after the injection of the suspensions the abdominal wall was closed. All procedures were performed aseptically. After the operation, animals were allowed to stabilise and fasted overnight with unlimited access to water. At 18 h after operation, the rats were anaesthetised as before, and subjected to laparotomy with a sterile technique. Presence of ascites, as well as the macroscopic appearance of the pancreas were recorded. Briefly, oedema, parenchimal necrosis, fatty tissue necrosis and haemorrhage were graded (scales 0–3) in a blinded fashion [4]. Samples of blood were collected from abdominal vena cava. The whole pancreas gland was removed. Parts of the gland was taken for histological examination. These were fixed in formalin 5%. The remainder of the tissue was homogenised in Tris buffer and stored at −80 °C for subsequent determination.

Table 1
Groups description and number of animals

Group	Pre-treatment	Intervention	Number of animals
1	Placebo	Interstitial saline	15
2	Placebo	Intraductal saline	15
3	Placebo	Interstitial *H. pylori*	15
4	Placebo	Intraductal *H. pylori*	15
5	Placebo	Interstitial *E. coli*	15
6	Placebo	Intraductal *E. coli*	15
7	Cerulein hyperstimulation	Interstitial saline	15
8	Cerulein hyperstimulation	Intraductal saline	15
9	Cerulein hyperstimulation	Interstitial *H. pylori*	15
10	Cerulein hyperstimulation	Intraductal *H. pylori*	15
11	Cerulein hyperstimulation	Interstitial *E. coli*	15
12	Cerulein hyperstimulation	Intraductal *E. coli*	15

2.3. Biochemical analysis

Whole blood was immediately centrifuged and serum was stored at −80 °C until further assayed. Serum amylase activity was measured using a commercial kit (Boehringer Mannheim, Germany). Serum interleukin-6 (IL-6) levels were measured by an enzymatic immunoassay (Amersham Buckinghamshire, England). Total catalytic phospholipase (PL) A2 activity was measured with an *E. coli* bioassay, based on the hydrolysis of phospholipids from C_{14} oleic acid-labeled *E. coli* membranes in the sn-2 position [5].

2.4. Tissue characteristics

The formalin fixed tissue was embedded in paraffin, cut in 5 μm thick section, and stained with haematoxylin and eosin. Histological changes were graded in a blinded manner using the method developed by Spormann et al. [4]. Briefly, oedema and inflammatory infiltration were graded using a scale from 0 to 4; haemorrhage and parenchymal necrosis were graded using a scale from 0 to 7.

Pancreatic water content was determined by calculating the ratio of the initial weight of a piece of pancreas (wet weight) to its weight after incubation at 95 °C for 24 h (dry weight).