Routes of spread of pathogens into the pancreas in a feline model of acute pancreatitis

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Abstract

The routes of spread of pathogens into the pancreas in acute pancreatitis were investigated. Four experiments were performed: (1) cats with and without acute pancreatitis were given 10^7 *Escherichia coli* (*E coli*) intravenously, (2) in cats with acute pancreatitis 10^8 *E coli* was placed in the colon. In half of them the colon was then enclosed in an impermeable bag to prevent transmural spread. (3) *E coli* (10^4) was placed in the pancreatic duct in cats with and without acute pancreatitis. (4) In cats with acute pancreatitis 10^5 *E coli* was placed in the gall bladder. In half of them the common bile duct was ligated to prevent biliary-pancreatic reflux. After 24 hours, intravenous *E coli* infected the pancreas in six of nine cats with acute pancreatitis and three of 10 controls. After 72 hours *E coli* spread to the pancreas from the colon in six of nine cats with acute pancreatitis. This was prevented by enclosing the colon in an impermeable bag ($p=0.02$). In five of six cats with acute pancreatitis and five of six controls *E coli* placed in the pancreatic duct colonised the pancreas within 24 hours. Pancreatic colonisation from the gall bladder occurred in five of six cats with a patent common bile duct and in three of six with an obstructed common bile duct. In conclusion, in cats *E coli* can spread to the pancreas by the blood stream, transmurally from the colon, and by reflux into the pancreatic duct.

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The pancreas is usually sterile and only becomes infected as a complication of associated disease processes such as acute pancreatitis.¹ A comparison of the typical pancreatic pathogens with the type of bacteria found in other sites in health or disease suggests several possible sources including the colon.¹⁻² In previous experiments we showed that pathogens may spread to the pancreas from the colon, gall bladder, and kidney.³ The only possible route of the spread of pathogens from the kidney is by the circulation whereas alternative routes were possible from the other sources. The aim of this study was to investigate routes by which pathogens could infect the pancreas.

Methods

MODEL OF ACUTE PANCREATITIS

The animal model of acute pancreatitis used has been described in detail previously.⁴⁻⁵

and only a brief account will be given here. Fasted adult mongrel cats were used in all experiments. No antibiotics were given at any stage. Cats were anaesthetised with xylazine hydrochloride (1 mg/kg body weight, intramuscular) and sodium pentobarbital (25 mg/kg body weight, intraperitoneal). All operations were performed using an aseptic technique and infusions were made through in line bacterial filters (0.2 μ m pore size).

A femoral vein was cannulated with a heparinised plastic catheter (external diameter 0.965 mm). The abdomen was entered and the pancreatic duct was cannulated in the tail of the gland with a plastic catheter (external diameter 0.61 mm) primed with standard perfusate. Acute pancreatitis was induced by perfusing 0.5 ml of 7.5 mM glycodeoxycholic acid into the pancreatic duct over one hour. Then 0.5 ml of pooled pancreatic juice (activated by prior incubation with enterokinase) was perfused over a second hour. For two hours while the pancreatic duct was being perfused 16,16 dimethyl prostaglandin E_2 (2 μ g/kg body weight/hour) was infused intravenously to cause the development of acute pancreatitis. A similar procedure was performed in control animals except the pancreatic duct was perfused with standard perfusate for two hours and 0.9% isotonic saline was infused intravenously instead of prostaglandin E_2 . Cats were then allowed to recover. Reoperation was performed on anaesthetised animals to remove tissues for microbiology.

MICROBIOLOGICAL METHODS

A clinical strain of *E coli* was used as the marker pathogen. This strain had distinctive antimicrobial sensitivities so that it could be distinguished from endogenous *E coli*. A precise number of *E coli* was used in each experiment. These were counted immediately before administration using a Petroff-Hausser bacterial counting chamber.⁶

At the second operation specimens of pancreas, liver, lung, and kidney were removed. These were washed in sterile saline, blotted dry, homogenised, and then placed in brain heart infusion broth. In addition a venous blood sample was taken for culture to assess the bacteraemia rate, and bile was aspirated for culture in experiment 3. Samples were incubated at 37°C for seven days. Standard microbiological techniques were used to isolate and identify *E coli* and the concentration of *E coli* in the pancreas and bile was estimated using a viable plate count method.⁶

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The number of *E coli* placed into each site and the timing of the reoperation to remove tissues for culture were estimated from preliminary experiments in which groups of cats received different numbers of *E coli* by the different routes.

EXPERIMENTAL PROTOCOL

Four routes were studied.

Experiment 1: The vascular route

Preliminary experiments showed that *E coli* spread to the pancreas within 24 hours after giving 10^7 *E coli* or more as an intravenous bolus.

After the induction of acute pancreatitis 10^7 *E coli* were given as an intravenous bolus in nine cats. Ten control cats (no acute pancreatitis) were treated in the same way. Cats were allowed to recover and 24 hours later reoperation was performed and tissues were removed for culture.

Experiment 2: The transmural route from the colon

Preliminary experiments showed that *E coli* did not spread to the pancreas within 24 hours despite giving 10^{10} *E coli*. Translocation to the pancreas from the colon, however, did occur within 72 hours of placing 10^8 *E coli* into the colon.

In 17 cats acute pancreatitis was induced. At the end of this procedure 10^8 *E coli* was injected through a 30 gauge needle inserted obliquely through the anti-pancreatic wall of the proximal transverse colon. To ensure that leakage from the needle puncture site did not occur a very fine needle was used and a microbiological swab of the puncture site was taken after injecting the *E coli*. This was sterile in all the cats. In eight cats the transverse colon was then enclosed in a plastic bag, which was impermeable to the *E coli* and was not bactericidal for *E coli*. The colon was retained in the bag by three interrupted transmesenteric 3/0 dexton sutures (Figure). Care was taken to ensure the colon was not obstructed or the blood supply impaired. In the remaining nine cats *E coli* was given and the colon replaced in the abdomen. It was not enclosed in a plastic bag. The laparotomy wound was closed and all cats were allowed to recover. Reoperation was performed 72 hours later and tissues were removed for culture.

Experiment 3: The pancreatic duct route

Preliminary experiments showed that *E coli* spread to the pancreas within 24 hours of placing 10^4 *E coli* or more into the pancreatic duct.

Acute pancreatitis was induced in six cats and then 10^4 *E coli* (in 0.1 ml) was placed in the pancreatic duct. Six control cats (no acute pancreatitis) were treated in the same way. The pancreatic catheter was then removed and the open end of the pancreatic duct ligated.

The laparotomy wound was closed and the cats allowed to recover. Reoperation was performed 24 hours later and tissues were removed for culture.

Experiment 4: The effect of common bile duct ligation on the spread of E coli from the gall bladder

Preliminary experiments showed that *E coli* spread to the pancreas within 24 hours of placing 10^5 *E coli* or more into the gall bladder.

Acute pancreatitis was induced in 12 cats. At the end of the procedure cats were randomly divided into two equal groups. In six cats the supraduodenal common bile duct was ligated. In the remaining six cats the common bile duct remained patent. In all cats 10^5 *E coli* was then injected into the gall bladder through a 30 gauge needle. Reoperation was performed 24 hours later and tissues were removed for culture.

Experiments were approved by the office of research and development of the Sepulveda Veterans Administration Medical Center and conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals. Cats were kept in individual cages and looked after in the animal house under the supervision of a veterinary surgeon and qualified technicians. Intramuscular buprenorphine (0.005–0.01 mg/kg body weight, hourly as required, maximum eight doses) was provided for pain relief. At the end of each experiment anaesthetised animals were killed with an overdose of potassium chloride.

STATISTICAL METHODS

The pancreatic colonisation rates were compared using two tailed Fisher's exact test. The concentration of *E coli* in the pancreas and bile was expressed as median (interquartile range) logarithm of the colony forming units (log cfu) of *E coli* per ml of bile or per gram of pancreas and results were compared using the Mann-Whitney U test.

Results

EXPERIMENT 1

An *E coli* bacteraemia caused pancreatic colonisation in 67% (six of nine) of cats with acute pancreatitis and in 30% (three of 10) of control (no acute pancreatitis) cats ($p=0.1$). *E coli* was also isolated from the liver, kidney, and lung in a similar proportion of animals. The concentration of *E coli* in the inflamed gland, 1.6 (3.1) log cfu/g of pancreas, was not significantly different from the concentration in control glands, 1.7 (2) log cfu/g.

EXPERIMENT 2

E coli translocated from the colon to the pancreas in 67% (six of nine) of cats with acute pancreatitis in which the colon was not enclosed in a plastic bag. In cats with acute

Line diagram to illustrate the method of enclosing the colon in an impermeable bag.

pancreatitis, however, in which the colon was enclosed in an impermeable bag, *E coli* were not isolated from any of the pancreatic specimens ($p=0.02$). *E coli* was isolated from the liver in two of the cats and from the lung in one cat in which the colon was enclosed by a plastic bag. The concentration of *E coli* in glands colonised by *E coli* was $2.93 (1.86) \log \text{cfu/g}$ of pancreas. *E coli* was cultured from the circulation in five of nine cats (five of six cats with pancreatic colonisation) in the group where the colon was not enclosed in a bag but in none of the cats in which the colon had been enclosed in a plastic bag.

EXPERIMENT 3

E coli placed in the pancreatic duct caused pancreatic colonisation equally (83%; five of six) in cats with acute pancreatitis and control (no acute pancreatitis) animals. The concentration of *E coli* in the inflamed gland, $2.79 (0.37) \log \text{cfu/g}$ of pancreas, was not different from the concentration in control glands, $2.51 (1.89) \log \text{cfu/g}$. *E coli* was cultured from the blood taken at reoperation (bacteraemia rate) in only one cat with acute pancreatitis and pancreatic colonisation from the pancreatic duct (one of 10 cats with pancreatic colonisation).

EXPERIMENT 4

In cats with acute pancreatitis *E coli* translocated from the gall bladder to the pancreas and other organs despite ligating the common bile duct. Pancreatic colonisation occurred in five of six cats with a patent common bile duct and in three of six cats with an obstructed duct ($p=0.3$). The concentration of *E coli* in the bile was about 10^7cfu/ml of bile in all cats. The concentration of *E coli* isolated from pancreatic specimens in each group was similar: $3.39 (3.12) \log \text{cfu/g}$ of pancreas in cats with a patent common bile duct, $4.34 (1.95) \log \text{cfu/g}$, in cats with an obstructed common bile duct. *E coli* was isolated from blood taken at

reoperation in three of six cats in each group. Among cats with a patent common bile duct with pancreatic colonisation the bacteraemia rate was three of five ($p=0.004$ v the bacteraemia rate, one of 10, when the pancreas was colonised from the pancreatic duct), and three of three cats with pancreatic colonisation despite ligating the common bile duct ($p=0.01$ v the bacteraemia rate, one of 10, when the pancreas was colonised from the pancreatic duct).

Discussion

Pancreatic infection is the principal life threatening complication of acute pancreatitis. Patients with necrotising acute pancreatitis are at increased risk of developing pancreatic infection.^{1 2 7 8} For this reason an animal model of acute necrotising pancreatitis was used.

This was a qualitative study and for this reason the number of *E coli* given to each of the sites was the minimum number associated with translocation to the pancreas as determined by preliminary experiments. Physiological numbers of *E coli* were used in each of the experiments. The median concentration of *E coli* in the circulation after a bolus intravenous injection of 10^7 *E coli* was 68 cfu/ml. This concentration is found in more than 10% of bacteraemias clinically.⁹⁻¹¹ The typical concentration of *E coli* in faeces is 10^8cfu/g . A bolus of 10^8 *E coli* will probably not change the host flora. The concentration of *E coli* isolated from bile in this study (10^7cfu/ml) was similar to that found in patients with bactobilia.¹² If only 0.1 ml of infected bile refluxed into the pancreatic duct the pancreas would be exposed to 10^6 *E coli*. 10^4 *E coli* was placed in the pancreatic duct in this study.

Previously we have shown that *E coli* may spread to the pancreas from the colon, gall bladder, and kidney.³ The only route of spread of pathogens common to each of these sites is by the circulation. Here we showed that a transient bacteraemia caused pancreatic colonisation in both control cats and cats with acute pancreatitis. This is an important finding because bacteraemias may arise spontaneously from the biliary and gastrointestinal tract, from urinary tract or respiratory tract infections, by direct shedding from venous cannulas or during invasive procedures.⁹⁻¹¹ Most bacteraemias are rapidly cleared by the reticuloendothelial system.^{5 13-15} A proportion, however, of circulating bacteria is deposited in organs throughout the body.^{16 17} Indeed, in this study *E coli* was isolated from the liver, lung, and kidney in addition to the pancreas. Bacteraemias are a recognised cause of pyelonephritis, brain abscesses, endocarditis, septic arthritis, and osteomyelitis^{9 16 18 19} and must now be implicated in the pathogenesis of pancreatic infection. Furthermore, in acute pancreatitis the risk of organ colonisation and subsequent infection is increased because the rate of clearance of *E coli* from the circulation is reduced.⁵

The transverse colon is generally considered the most probable source of pancreatic pathogens because of its proximity to the pancreas and because pancreatic pathogens are normal members of the colonic microbial flora.^{2 20-22} Previous studies from this laboratory and others have shown that pathogens can spread from the colon to the pancreas.^{3 23} Pathogens may spread to the pancreas either directly across the bowel wall (transmural route) or by the circulation. It would be very difficult to prove either of these routes. If the transmural route was blocked, however, then any spread that occurred must be by the circulation. In this study we found that enclosing the colon in an impermeable plastic bag to block the transmural route prevented the spread of *E coli* to the pancreas. The bag was not bactericidal and postoperatively there was no evidence of functional or mechanical interference with bowel function. Indeed, if there had been the likelihood of spread from the colon to the pancreas would have been increased not decreased. This suggested that the most important route of spread of *E coli* from the colon was the transmural route at this time interval. Other routes, however, such as by the lymphatic system and the circulation at later time intervals, were not ruled out by this study. In support of the transmural route other animal experiments have shown that bacteria translocate from the colon into the pancreas^{3 23} and nearby intra-abdominal abscesses^{24 25} or into an inflamed peritoneal cavity^{24 26} without seeming to spread by the blood stream.

The mechanisms by which the transmural spread of bacteria occurs are incompletely understood.²⁴ One theory is that colonic macrophages migrate into nearby areas of inflammation. Some of these macrophages may carry viable pathogens, which are then released and in the presence of dead and devitalised tissue can multiply.

Bile is a potential source of pancreatic pathogens that has received scant attention. The spectrum of biliary and pancreatic pathogens are similar and previously we have shown that *E coli* can spread from the bile to the pancreas.³ Of the many possible routes by which biliary bacteria could spread to the pancreas the most likely is biliary-pancreatic reflux. Pancreatic and bile duct pressures vary widely so that although the mean pressure in the pancreatic duct is higher than in the common bile duct²⁷⁻²⁹ there are occasions when the pressure difference is reversed and reflux occurs.³⁰ Here we have shown that pathogens placed in the pancreatic duct may cause pancreatic infection. This finding supports previous studies performed using different animal models of acute pancreatitis³¹ and either a higher concentration of *E coli* or *E coli* in combination with *Bacteroides fragilis*.³²

Ligation of the common bile duct to prevent biliary-pancreatic reflux did not prevent the spread of *E coli* from the gall bladder. This suggested other routes of spread were possible. Biliary tract obstruction, however, may have

artificially promoted the spread of *E coli* by causing biliary hypertension and ascending cholangitis. Cholangiovenous reflux may occur in an obstructed biliary tract.^{33 34} In patients with biliary acute pancreatitis the common bile duct is obstructed, at least transiently, during the passage of a calculus.^{35 36} In these patients bacteria may spread from the biliary tract to the pancreas by the circulation. In support of the cholangiovenous route, the bacteraemia rate among cats with pancreatic colonisation caused by *E coli* from the gall bladder whether or not the common bile duct was ligated was greater than the rate among cats with pancreatic colonisation directly from the pancreatic duct. As the pancreas was colonised in both groups of animals it is probable that the increased bacteraemia rate resulted from the shedding of *E coli* into the circulation from the biliary tract.

In conclusion, in cats pathogens may reach the pancreas by several routes. *E coli* can spread to the pancreas by the blood stream, transmurally from the colon, and by reflux into the pancreatic duct. This suggests it would be difficult to prevent bacteria from reaching the pancreas.

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