Effect of Bile Induced Pancreatitis on Tobramycin Excretion in Pancreatic Fluid

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The objective of this study has been to lay the groundwork for a re-evaluation of the place of antibiotics in acute pancreatitis. This section has been devoted to determining if antibiotics excreted by the normal pancreas are excreted similarly in acute pancreatitis. Ten mongrel dogs were studied, each acting as its own control. Day 1: Operation—construction of pancreatic fistula. Day 2: Study of antibiotic excretion. Day 14: Operation—induction of acute focal pancreatitis. Day 15: Study of antibiotic excretion. Antibiotic concentrations in pancreatic fluid were studied by injecting tobramycin intravenously (5 mg/kg). Serum levels and excretion of the drug in the pancreatic secretion were then monitored over the next six hours.

Results showed excretion of tobramycin reached bactericidal concentrations in pancreatic fluid from the normal and inflamed pancreas, with no significant differences ($p = 0.2$) between the excretion rates. The place of antibiotics in acute pancreatitis is discussed. Based on usual pathogens isolated in pancreatic abscesses, and their usual sensitivity patterns, tobramycin with Cephamandole are the antibiotics of choice in acute pancreatitis, and a clinical trial is indicated to evaluate their place in reducing complications and deaths in the disease.

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THE PLACE OF ANTIBIOTICS in the treatment of acute pancreatitis is debatable. Some authors[17,20] recommend their use prophylactically, but others claim there is no place for them.[11,15] The ability of antibiotics to penetrate the gland itself is largely unstudied. It is known that some antibiotics are excreted in the pancreatic juice of the normal gland, but it is unknown if this excretion is altered in acute pancreatitis.

Since more information of this type will be needed to evaluate the role of antibiotics in pancreatitis, this experiment has been devised to measure the excretion of Tobramycin in pancreatic secretion, both in the normal gland and in bile induced acute pancreatitis. Tobramycin was studied because of its potential action against the usual pathogens which have been isolated in abscesses secondary to acute pancreatitis. In addition, tobramycin is a "model" drug, in that it is excreted essentially unchanged by the kidney, and its distribution and excretion characteristics can be accurately described both in man and animal by pharmacokinetic analysis. Finally, there are no data on whether or not tobramycin is excreted in pancreatic juice, or on the effect of disease on this excretion.

Materials and Methods

Ten mongrel dogs between 18.4 and 25 kg were used in this study. In order that each dog acted as its own control the following proforma was designed: Day 1: Operation—construction of pancreatic fistula. Day 2: Study of antibiotic excretion. Day 14: Operation—induction of pancreatitis. Day 15: Study of antibiotic excretion.

Pancreatic fistulae were constructed by a modification of the technique initially described by Herrera et al.[10] In brief, the ventral (accessory) duct of the pancreas is ligated and a duodenal pouch constructed incorporating the dorsal (main) duct of the pancreas. Bowel continuity is restored with a slide-to-slide duodenoduodenostomy. A cannula is then inserted into the pouch and bowel, which allows collection of pure activated pancreatic secretion during experiments, but return it to the bowel between experiments.

Acute pancreatitis was induced with the injection of 4 ml of autologous bile into the pancreatic duct.

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Day 1: Food, but not water, was withheld for 18 hours prior to operation. Anesthesia was induced with pentobarbital sodium. Under sterile conditions the abdomen was opened through a midline incision. A pancreatic fistula was then constructed. Fluid was replaced after operation by subcutaneous administration of 5% dextrose/0.2% normal saline (10 ml/kg/eight hours).

Day 2: Tobramycin studies were performed with the dog standing in a Pavlov frame. After irrigating the fistula, cumulative pancreatic juice was collected in successive test tubes placed over the cannula. Blood samples were collected through a long line inserted into the inferior vena cava via a leg vein.

A control sample of pancreatic secretion was collected over a 15 minute period and at the end of that period a blood sample was obtained. Tobramycin (5 mg/kg) was then injected intravenously. Pancreatic secretion was collected over 15-minute periods for the first hour, 30 minute periods for the second hour, and then hourly for four hours. At the end of each collection period, a blood sample was taken. Following the study, the dogs were administered fluids subcutaneously for a further 24 hours, oral fluid for 72 hours, and then a normal diet.

Day 14: Following food restriction for 18 hours, dogs were reanesthetized with pentobarbital sodium. The midline incision was reopened, the duodenal pouch identified, and opened on its antimesenteric border. The dorsal duodenal papilla was located, a cannula inserted, and 4 ml of autologous bile injected. The pouch was then closed. Pancreatitis was confirmed by generalized yellowish staining of the pancreas. The midline incision was closed. Fluid was replaced as following the first operation.

Day 15: Tobramycin (5 mg/kg) was again injected intravenously and samples collected as previously described.

At the end of the experiment the dogs were killed and biopsy specimens of the pancreas were obtained.

The dogs tolerated standing in the Pavlov frame well after both operations.

Assay Methods

Tobramycin concentrations were determined by radioimmunoassay. The amount of the drug in each pancreatic fluid sample was calculated by multiplying the concentration of the drug by the volume of the sample. The excretion of tobramycin in the pancreatic secretion was expressed as the pancreatic fluid excretion rate (micrograms) per hour. Mean concentrations of the samples from the dogs before and after pancreatitis were included in the pharmacokinetic analysis.

Pharmacokinetic Analysis

Tobramycin serum concentration versus time data were fitted to a two-compartment pharmacokinetic model using the computer program NONLIN.[19] The two-compartment model conceives the body to consist of at least two spaces where equilibrium of a substance occurs between the intravascular (central) and the extravascular (peripheral) compartments. The purpose of this obvious oversimplification is to present a standard mathematic model to describe more than one slope in the decline of blood levels after a dose, such as occurs with tobramycin. From the model, dose and two-compartment parameters, the tobramycin volume of distribution in the central compartment, and the steady state distribution volume between the central and peripheral compartments were calculated. The elimination half-life and total clearance were also determined using standard equations.

Results

Two of the ten dogs operated on were rejected from the trial. One developed an intra-abdominal abscess and died, and the other became jaundiced ten days following the first operation (serum bilirubin level = 2.2 mg/100 ml (normal = 0.2 to 1.0 mg/100 ml)). Eight dogs were therefore left for evaluation.

The serum levels and excretion rates of tobramycin are expressed as the mean plus or minus standard deviation of the values of the eight dogs at each phase of the experiment (Figs. 1 and 2). The serum levels of tobramycin declined in two phases over the duration of the experiment, the half-life ranging from 1.4 to 1.8 hours.

In pancreatic fluid the peak excretion rate of tobramycin occurred approximately 40 minutes after drug administration. There were no significant differences (area under curve—Table 1) between serum concentrations or pancreatic excretion rates comparing the two phases of the experiment.

The volume of pancreatic secretion seemed slightly greater in the postpancreatic phase, (15.86 ml/hr) compared with the prepancreatic phase (13.28 ml/hr), but the difference was not significant ($p = <0.5$).

As shown in Table 1, the relevant pharmacokinetic parameters were not affected by bile induced pancreatitis. These parameters indicate that peak (one hour) and trough (six hours) serum concentration in these dogs were similar to those noted in humans receiving recommended doses. The central volume of distribution of tobramycin was also similar to values observed in humans at 0.2 liters/kg of body weight.

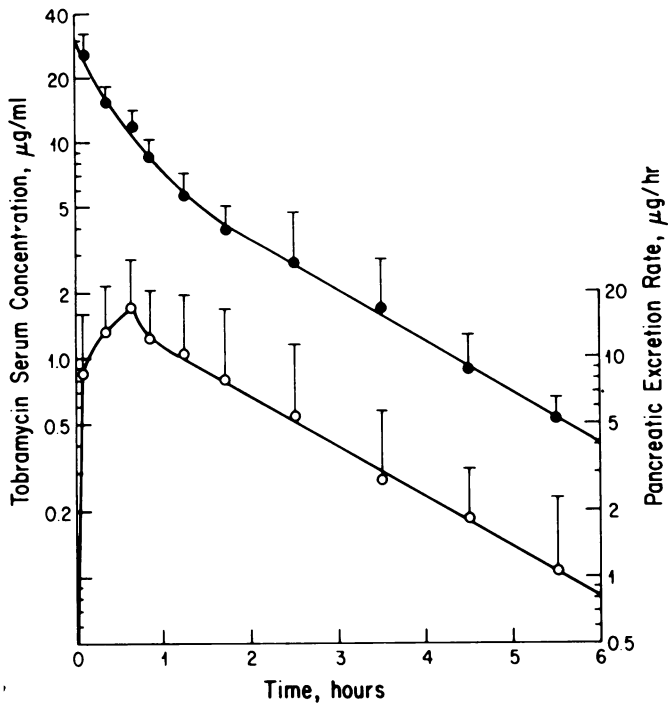

FIG. 1. Prepancreatitis. Serum concentrations and pancreatic excretion rates. ●: serum μg/ml. ○: pancreatic fluid μg/hr.

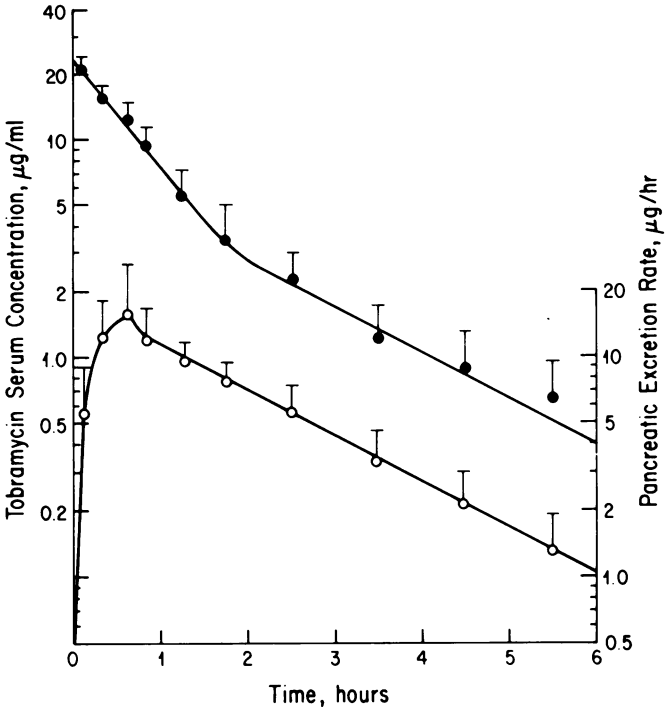

FIG. 2. Postpancreatitis. Serum concentrations and pancreatic excreation rates. ●: serum μg/ml. ○: pancreatic fluid μg/hr.

Histology of the biopsy specimens of the pancreas confirmed acute focal pancreatitis in all dogs.

Discussion

The results demonstrate that tobramycin is excreted in the pancreatic secretion in the normal pancreas and in acute focal pancreatitis, in a concentration directly related to the serum concentration. In addition, we found no significant differences in these values comparing the pre- and postpancreatitis phases. The data indicate that there were no changes in the renal excretion rate after pancreatitis or in the rate of excretion by the pancreas, as evidenced by the parallel curves in Figures 1 and 2.

The dose of tobramycin administered to these dogs produces a serum concentration comparable to that desired in humans, and, thus, the pancreatic fluid excretion rates suggest that the pancreatic fluid concentrations in humans would be sufficient for bactericidal activity (mean inhibitory concentrations: 2–8 μg/ml). However, in advanced stages of pancreatitis, in which there is ischemia, hemorrhage and necrosis, the pancreatic excretion rate would presumably be further reduced and the concentration may be too low to be effective against bacteria.

The place of bacteria in acute pancreatitis is unresolved. It has been suggested that infection may be important in late stages of the disease, and this possibility has been reinforced by advantageous effects of antibiotics in severe forms of experimental pancreatitis.[4,21]

In recent years endoscopic retrograde cholangiopancreatography (ERCP) has become used more widely. Complications following this procedure include acute pancreatitis, reported in 1–4.3% of the cases and pancreatic sepsis of pseudocyst abscess in 0.3% of the cases.[2,12] This latter complication has a mortality rate of 20%.[2] Acute pancreatitis following this procedure may be partially associated with infection, as it has been shown that bacteria in the upper gastrointestinal tract

TABLE 1. *Statistical Analysis and Tobramycin Pharmacokinetics in Eight Dogs, before and after Pancreatitis*

	Prepancreatitis	Postpancreatitis
Area under curve-serum	27.3	25.5
Area under curve-pancreatic excretion rate	32.0	32.1
Dose given (mg/kg)	5.0	5.0
Serum peak concentration —1 hour (μg/ml)	7.5	7.5
Serum trough concentration —6 hours (μg/ml)	0.50	0.40
Half life (hours)	1.4	1.8
Clearance (ml/min)	63.6	62.8
Central volume (liters)	3.5	3.7
Steady state distribution volume (liters)	5.5	5.7

TABLE 2. *Commonest Bacteria Isolated from Pancreatic Abscesses, with Usual Sensitivity Patterns*

	Total Cultures	Sensitivities	
		Tobramycin	Cephamandole
Escherichia coli	100	+	+
Aerobacter	54	+	+
Staphylococcus	39	+	+
Proteus	37	+	+
Enterococcus	22	±*	±*
Streptococcus	21	−	+
Pseudomonas	21	+	−
Klebsiella	19	+	+

* Sensitive *in vitro* but clinical response may be variable.

can be disseminated by the examination, and that bacteria may also be introduced from the outside of the scope.[6] Recently, it has been suggested that an aminoglycoside should be added to contrast agents used for the examination.[13]

Although the incidence of pancreatic abscess secondary to acute pancreatitis is only between 1.0 and 2.8%,[7,8] the mortality rate of this complication is between 37 and 57%.[3,16] In acute pancreatitis it has been found that bacteria are present in the pancreatic secretion in approximately 33% of the cases.[9] Culture specimens of pancreatic abscesses show a preponderance of gram negative bacteria (Table 1).[1,3,5,7,8,14] Several reports suggesting that antibiotics are ineffective in preventing pancreatic abscesses, have too few patients to show statistical significance, or have used antibiotics which are either not excreted in the pancreatic juice or which have the wrong sensitivity.[11,15,18]

A suitable combination of antibiotics against the bacteria most often incorporated in a pancreatic abscess would appear to be Cephamandole and tobramycin (Table 2), if these drugs were excreted in adequate amounts in the pancreatic fluid. Cephamandole is the cephalosporin of choice because of enhanced activity against aerobacter species. These organisms are often cephalosporin-resistant to standard cephalosporins such as cephalothin or cephazolin sodium.

This experiment shows that tobramycin is excreted in bactericidal concentrations not only in the normal gland but also in acute focal pancreatitis. It is known that cephalosporins are excreted in bactericidal concentrations in the normal gland (unpublished observations), and that their elimination from the body is largely renal. It may, therefore, be postulated that Cephamandole would be excreted similarly to tobramycin in the pancreatic fluid in experimental acute pancreatitis.

In 1973 Kodesch and Du Pont[15] reported a retrospective assessment of antibiotics in 100 cases of acute pancreatitis. Forty patients had antibiotics of which 24 usually had kanamycin sulfate or gentamycin sulfate in combination with a cephalosporin or a penicillin. Accepting that this combination does not appear to be as effective as the combination of an aminoglycoside with Cephamandole, there are certain similarities in the sensitivities, and it is interesting to review their results. In those receiving antibiotics, there were no cases of pancreatic abscess, but in the 60 with no antibiotic prophylaxis there occurred one pancreatic abscess, one perirectal abscess, and two cases of bacterial peritonitis, one of which developed a pelvic abscess. With regard to intra-abdominal abscesses the numbers were not large enough to statistically evaluate the effectiveness of the antibiotics on a complication which has an incidence of under 3%, however, it is indicative of a trend that antibiotics may have helped.

A controlled clinical trial is indicated using Cephamandole in combination with tobramycin to assess their prophylactic value in reducing the incidence of severe forms of acute pancreatitis, pancreatic abscess, and pancreatitis secondary to ERCP.

Summary

The excretion of tobramycin in pancreatic secretion has been studied in both the normal pancreas and in acute focal pancreatitis, in dogs with experimental pancreatic fistulae. The experiment shows: 1) Pancreatic fluid excretion rates are proportional to the tobramycin serum levels. 2) tobramycin is excreted in bactericidal concentrations both in the normal pancreas and in acute focal pancreatitis. The place of antibiotics in acute pancreatitis is discussed. A controlled clinical trial is indicated to evaluate the place of tobramycin in combination with Cephamandole in the prevention of severe complications of acute pancreatitis.

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Discussion

Dr. Lloyd M. Nyhus (Chicago, Illinois): The use of antibiotics in the treatment of pancreatitis, as has been said, has remained controversial, even though it has been widely recommended as recently as this past year. Prospective randomized studies dealing with this subject indicate that antibiotics are of no value in the treatment of pancreatitis. These studies, however, have been criticized because only patients with relatively mild alcoholic pancreatitis were included, and the argument remains unsettled.

Parenthetically, Dr. Schenk, the pancreatitis, acute, focal, produced in the animals in your experiments, seem rather mild. What would be the excretion of Tobramycin in fulminating hemorrhagic pancreatitis in your animals? It seems to me it's in this area that we have most interest.

The theoretical prerequisites for the effectiveness of an antibiotic are that the antibiotic reach the area of infection and that the bacteria involved are sensitive to it. The first prerequisite has been proven in the paper given today, at least as far as Tobramycin is concerned.

The second prerequisite, however, remains somewhat in doubt. Even though most studies, including our own, of pancreatic abscesses have not shown any anaerobic bacteria, it seems hard to conceive that this would be the only intra-abdominal infection without participation of this organism.

Before the antibiotic protocol for the treatment of pancreatitis is designed, we should have better microbiologic studies of the infectious complications of this disease.

It is of interest that in our patients with pancreatic abscess, treated preoperatively with aminoglycoside and clindamycin, not a single anaerobic culture was positive. This certainly suggests that clindamycin was at an effective level in the pancreatic tissues and secretions of these patients, thus lending credence to the observations of our essayists, at least as seen in their dog model.

In this context, what is the possible role of anaerobes in pancreatic abscesses, and do you know if antibiotics effective against these organisms are also excreted in the pancreatic juice?

Second, concerning the recommendations—they are only recommendations—for the clinical use of Cephamandole, in addition to Tobramycin, what is the rationale for recommending a combination of Cephamandole and Tobramycin, since Cephamandole does not increase the spectrum of bacteria covered by Tobramycin significantly, and especially as it has very limited activity against anaerobic bacteria?

Dr. William A. Altemeier (Cincinnati, Ohio): The type of experimental pancreatitis produced in their animals allows the excretion of Tobramycin in the pancreatic fluid, but I too raise the question of what will happen in the case of apoplexy of the pancreas, or the hemorrhagic, diffuse, severe type of pancreatitis? Will these glands secrete Tobramycin in the same degree?

Some of the older members of this Association will recall the experiences we had before 1940, before we had serum amylase determinations to help us in the diagnosis of acute pancreatitis and, in particular, hemorrhagic pancreatitis. At that time, it was not uncommon to operate upon patients with severe hemorrhagic pancreatitis. The pancreas would be diffusely and completely involved, and there were several operations which were done, one of which included gridironing the peritoneum over the surface of the pancreas and draining this fluid through flank incisions. At the same time, we took cultures.

I was impressed at that time with the frequency of the hemolytic Streptococcus being present in the fluid about the pancreas, and this has been the basis for my use of aqueous penicillin G for treatment in the early course of the disease.

There is increasing evidence that some cases of pancreatitis may be due to viral infection. This brings into focus the question of the value of Tobramycin in the early stages of this type of pancreatitis. We must conclude, however, that it will be of value in the patients who have survived the acute stage, and who have developed pancreatic abscesses.

Dr. Alexander and I reported 32 cases of pancreatic abscess in 1963, and we studied very carefully the bacteriology of the pus in those instances. Since then, this study has been continued and anaerobes do participate in the development and progress of pancreatic abscesses, particularly the anaerobic Streptococcus and Bacteroides fragilis.

In conclusion, I would echo some of the comments which have been made, that we need additional and detailed bacteriologic studies of the acute phase of pancreatitis (particularly the severe acute case), as well as of established cases with abscess.

Dr. H. Harlan Stone (Atlanta, Georgia): In the prevention or treatment of any type of infection, the use of a specific antibiotic is determined according to three factors: first, safety of drug; second, the appropriate antibacterial spectrum for the bacteria present in the infection; and third, which Dr. Schenk and his coworkers have addressed themselves to, the delivery of antibiotic to the site, whether