Long-Term Bile Diversion Enhances Basal and Duodenal Oleate-Stimulated Pancreatic Exocrine Secretion in Dogs

MICHINAGA TAKAHASHI, HIROO NAITO, IWAO SASAKI, YUJI FUNAYAMA, CHIKASHI SHIBATA and SEIKI MATSUNO

South Miyagi Medical Center, Ohgawara 989-1253, and
First Department of Surgery, Tohoku University School of Medicine, Sendai 980-8574

Takahashi, M., Naito, H., Sasaki, I., Funayama, Y., Shibata, C. and Matsuno, S. Long-Term Bile Diversion Enhances Basal and Duodenal Oleate-Stimulated Pancreatic Exocrine Secretion in Dogs. Tohoku J. Exp. Med., 2004, 203 (2), 87-95 —— There have been no previous reports whether long-term bile diversion enhances pancreatic exocrine secretion. The aim of this study was to elucidate the effect of long-term bile diversion on pancreatic exocrine secretion. Four mongrel dogs were prepared for chronic gastric and pancreatic fistulas and received intraduodenal sodium oleate infusion (controls). These dogs, then underwent diversion of bile from the intestines by ligating the common bile duct and interposing a segment of jejunum between the gallbladder and the urinary bladder (total biliary diversion [TBD]). After three weeks, the dogs received an identical sodium oleate infusion. TBD augmented basal pancreatic exocrine secretion compared with controls (4.4-fold increase in basal flow volume; 9.0-fold increase in bicarbonate output; and 3.3-fold increase in protein output). Likewise, TBD augmented oleate-stimulated exocrine secretion (2.0-fold increase in cumulative flow volume; 2.6-fold increase in bicarbonate output; and 1.4-fold increase in protein output). TBD also augmented basal and oleate-stimulated plasma cholecystokinin levels. Administration of a Cholecystokinin-A receptor antagonist (loxiglumide) after TBD reduced the flow volume and bicarbonate output to the control levels, and the protein output to less than a half of the control level. Long-term bile diversion enhances basal and oleate-stimulated pancreatic exocrine secretion, at least partly via increased cholecystokinin secretion. —— biliary diversion; chronic pancreatic fistula; pancreatic exocrine secretion; cholecystokinin; loxiglumide

© 2004 Tohoku University Medical Press
Many studies have focused on the interaction between bile and pancreatic exocrine secretion, but whether biliary secretion plays an important role in control of pancreatic exocrine secretion remains inconclusive. Intraduodenal instillation of bile or bile salts increases release of secretin (Osnes et al. 1978; Bondesen et al. 1985; Miyasaka and Kitani 1986; Riepl et al. 1990), and/or vasoactive intestinal polypeptide (Burhol et al. 1980) which might lead to an increase in pancreatic exocrine secretion. In contrast, under conditions of bile depletion such as temporary biliary diversion, obstructive jaundice, or oral administration of the anion-exchange resin cholestyramine which binds luminal bile salts, pancreatic exocrine secretion increased in rats (Brand and Morgan 1982; Nakamura et al. 1990), dogs (Gomez et al. 1986; 1988; Kuroda et al. 1988), and humans (Koop et al. 1988; Koide et al. 1993), presumably secondary to an increase in plasma cholecystokinin (CCK) concentration. However, no previous reports have examined changes in pancreatic exocrine secretion after long-term bile diversion in the conscious state. We have previously demonstrated that dogs subjected to long-term bile diversion to the urinary bladder induced an increase in basal and fat-stimulated plasma CCK concentrations (Takahashi et al. 1996, 2000). Besides, these dogs developed pancreatic hypertrophy showing profuse and dilated rough endoplasmic reticulum in pancreatic acinar cells (Takahashi et al. 2000), suggesting that long-term bile diversion might also induce pancreatic exocrine hypersecretion. The aim of our study was to determine whether long-term bile diversion enhances pancreatic exocrine secretion in dogs. In addition, we wanted to clarify the role of CCK in any changes in pancreatic exocrine secretion by using the CCK-A receptor antagonist, loxiglumide.

**Materials and Methods**

*Animal preparation*

Four mongrel dogs (22.0 to 25.0 kg) were anesthetized with intravenous pentobarbital. A Herrera fistula (chronic external pancreatic fistula) was constructed by inserting a modified Herrera cannula into the duodenum and the duodenal pouch (Herrera et al. 1968). In addition, a Thomas’ cannula was inserted into the body of the stomach along greater curvature and anchored to the left side of the abdominal wall (Fig. 1).

After completing the studies of intraduodenal sodium oleate infusion described below, each dog was reoperated for construction of total biliary diversion (TBD). A 20-cm segment of the mid small bowel was interposed between the gallbladder and the urinary bladder after ligating and dividing the common bile duct distal to the insertion of the cystic duct (Takahashi et al. 1996).

*Experimental protocol*

Dogs were allowed five weeks for recovery after chronic gastric and pancreatic fistulas operation and three weeks after TBD operation. Prior to each study, dogs were fasted for 18 hours with free access to water. Experiments were performed in the conscious state while resting quietly in a Pavlov sling. The gastric cannula was opened and the gastric juice was drained out during the experiment. Pancreatic juice was collected for at least 30 minutes before beginning the duodenal infusion until the volume of pancreatic juice recovered became constant. Thereafter, after a 30-minute basal period, each dog received an intraduodenal (ID) infusion of 115 mM sodium oleate pH 9.0 for 90 minutes at 72 ml/hour (8.5 mmol of oleate/hour). Pancreatic juice was collected over 15 minutes intervals for 180 minutes and was not returned to the duodenum. Pancreatic juice was stored in air tight plastic tubes and kept at −20°C until measurement of bicarbonate and protein concentration. Peripheral blood samples were collected into chilled tubes containing both EDTA and aprotinin from a cannulated foreleg vein during the basal period (samplings at −15 and 0 minutes), and at 15, 30, 45, 60, 90, 120, 150, and 180 minutes after start of oleate infusion. Plasma was separated and stored at −30°C for later peptide measurement. The sodium oleate infusion was repeated at least three times on sepa-
rate days in each dog after the first and second operation, and mean values in each dog were regarded as representative for that dog.

In addition to these experiments, the CCK-A receptor antagonist loxiglumide was administered intravenously at 10 mg/kg per hour (22 μmol/kg per hour) for 120 minutes beginning at the start of the oleate infusion after the TBD operation. The dose of loxiglumide employed in the study strongly inhibits pancreatic and biliary responses stimulated by intravenous CCK-8 in human subjects (Hildebrand et al. 1990, 1991; Meier et al. 1990), and pancreatic protein responses stimulated by intraduodenal perfusion of tryptophan in dogs (Teyssen et al. 1996). Loxiglumide administration was performed at least twice in three dogs, because one of the four dogs died of intestinal volvulus before experiments with loxiglumide could be performed.

The experimental protocol and procedures used in this study were approved by the Animal Care Committee of the Tohoku University School of Medicine.

Analysis of data
Bicarbonate concentrations were determined by the back titration method using hydrochloric acid. Protein concentrations were estimated by the Lowry method (1951), using purified bovine trypsinogen as a standard. Bicarbonate and protein output per 15 minutes were calculated by multiplying their concentrations by the collected volume of pancreatic juice.

Plasma CCK concentrations were measured by a radioimmunoassay using a CCK-8 N-terminal specific polyclonal antibody, OAL-656 from Otsuka Assay Laboratories (Himeno et al. 1983). Plasma samples were extracted with 96% ethanol (1:2 vol/vol) (Iwai et al. 1986). Synthetic CCK-39 coupled to 125I (Bolton and Hunter reagent) was used as a tracer, and pure porcine CCK-33 served as a standard. Details of the procedures have been described previously (Takahashi et al. 1996). All samples were measured in duplicate and analyzed in the same assay.

Cumulative pancreatic secretion was obtained by summing each sequential 15-minute collection of pancreatic exocrine secretion from the beginning of oleate infusion to various times.

All data are shown as mean±standard error (S.E.). Statistical difference was determined by a paired Student’s t-test for basal values (controls vs. TBD), repeated measures analysis of variance (ANOVA) for the average release of CCK and cumulative pancreatic exocrine output (controls vs. TBD), or ANOVA followed by Fisher’s PSLD test which was employed for multiple comparisons (controls, or TBD vs. TBD with loxiglumide). Differences with a p value of less than 0.05 were considered to be significant.

RESULTS

Basal pancreatic exocrine secretion
The TBD dogs had significantly greater basal volume of pancreatic secretion and bicarbonate output than the control dogs. Protein output in the TBD dogs also tended to be greater than controls (p=0.102) (Table 1).

Changes in pancreatic exocrine secretions after ID oleate infusion
Changes in volume of pancreatic flow after the 90-minute ID oleate infusion appeared to be associated with changes in bicarbonate output in the control dogs. Flow volume and bicarbonate output after oleate infusion increased gradually reaching a plateau from 30-120 minutes, and then decreased to the basal amount from 150-180 minutes. In contrast, protein output in the control dogs increased rapidly after oleate infusion and decreased immediately after stopping the oleate infusion (Fig. 2).

The TBD dogs had greater pancreatic flow volumes and bicarbonate output and protein output than the control dogs during all 30-minute periods. Interestingly, flow volume and bicarbonate output in the TBD dogs did not increase during oleate infusion, and pancreatic secretory volume and bicarbonate increased in the period after completion of oleate infusion (90-150 minutes).
Regarding protein output, the TBD dogs showed a lesser relative increase in protein output during the oleate infusion, but produced greater protein output than the control dogs after stopping the oleate infusion.

**Cumulative pancreatic exocrine secretions**
Most of cumulative flow volumes and bicarbonate outputs in the TBD dogs were significantly greater than in controls, largely resulting from the increase in basal secretion. Over the first 120-minute cumulative flow volume and bicar-
Bonate output after TBD were approximately twice as much as controls (Table 1).

The protein output during oleate infusion, however, was not different between the two groups. Protein output in the TBD dogs for the entire 3 hour period of the study was greater than in the control dogs because the increased protein output after the termination of oleate infusion (90-180 minutes)

**Effect of loxiglumide on cumulative pancreatic exocrine secretion**

When loxiglumide was administered intravenously, the cumulative pancreatic flow volume and bicarbonate output for 0 to 120 minutes in the TBD dogs returned to the control levels. In contrast, protein output in the TBD dogs after loxiglumide was inhibited, decreasing to a half of that of the control dogs (Table 2).

**Table 2. Effect of loxiglumide on cumulative pancreatic exocrine output (0-120 minutes) during and after intraduodenal oleate infusion**

<table>
<thead>
<tr>
<th></th>
<th>Flow volume (ml/120 minutes)</th>
<th>Bicarbonate (mEq/120 minutes)</th>
<th>Protein (mg/120 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.3±8.4</td>
<td>3.43±0.65</td>
<td>773±127</td>
</tr>
<tr>
<td>TBD</td>
<td>88.9±14.8</td>
<td>6.68±1.88</td>
<td>952±212</td>
</tr>
<tr>
<td>TBD+loxiglumide</td>
<td>53.5±16.9</td>
<td>3.59±1.45</td>
<td>376±80*</td>
</tr>
</tbody>
</table>

The values are mean±S.E.M. of three dogs, asterisk indicates significant difference (* p<0.05, vs. TBD).

Plasma cholecystokinin

In the TBD dogs, basal plasma CCK levels were greater than controls (22.5±1.7 vs. 13.5±1.1 pmol/liter, p<0.05). The control dogs showed a small increase of plasma CCK levels after ID oleate infusion, reaching a plateau of about 16~18 pmol/liter. In contrast, the TBD dogs showed a gradual increase in plasma CCK levels after ID oleate infusion, reaching a plateau of 32.0±4.8 pmol/liter at 45 minutes after oleate infusion, and then returned to basal levels 30 minutes after completing oleate infusion (Fig. 3).
The average release of CCK from 0 to 90 minutes in the TBD dogs was greater than in the control dogs (28.1±2.0 vs. 17.1±1.0 pmol/liter, \( p<0.01 \)).

**DISCUSSION**

It is generally accepted that secretin and CCK play an important role in pancreatic exocrine secretion, but prior attempts to define the role of intraluminal bile in the control of pancreatic exocrine secretion remain inconclusive.

Our current study clearly demonstrated that TBD dogs had a higher basal CCK level and greater basal pancreatic flow volume, bicarbonate and protein output than the control dogs. Regarding oleate-stimulated pancreatic secretion, a 90-minute intraduodenal infusion of 115 mM oleate solution in the TBD dogs did not induce an increase in flow volume or bicarbonate output during the oleate infusion. Instead, flow volume and bicarbonate output in TBD dogs increased after completion of oleate infusion. Cumulative output for the period 0-180 minutes in the TBD dogs was almost twice of that in the control dogs because of differences in basal pancreatic flow volume and bicarbonate output. Protein output increased during the oleate infusion in the control and TBD dogs, followed by a rapid decrease after stopping the oleate infusion. The decrease in protein output was less reduction in the TBD dogs after 90 minutes.

Our results showed that the TBD dogs had greater pancreatic exocrine secretion than did the control dogs after oleate infusion, and we suspect that the elevated CCK levels in the TBD dogs might have contribute to this phenomenon. Therefore, in order to clarify the role of CCK in the TBD dogs, the CCK-A receptor antagonist, loxiglumide, was employed.

When loxiglumide was administered intravenously, the cumulative pancreatic flow volume and bicarbonate output of the TBD dogs dropped to the levels equivalent to controls, and protein output decreased to even lower levels than in the control dogs. Bicarbonate secretion is considered to be controlled predominantly by secretin while protein secretion is primarily regulated by levels of plasma CCK (Sharp et al. 2002), and secretin...
and CCK also synergistically stimulate pancreatic exocrine secretion (Fölsch and Wormsley 1973; You et al. 1983; Chey et al. 1984). Simultaneous administration of both hormones induces a greater response of pancreatic bicarbonate secretion than administration of each peptide alone. However, this phenomenon was observed only in bicarbonate secretion. According to Chey et al. (1984) and Beglinger (1985), CCK potentiates secretin-stimulated pancreatic bicarbonate secretion, while secretin does not potentiate CCK-stimulated pancreatic trypsin or protein secretion. On the basis of these studies, our study indicates that loxiglumide administration inhibited the component of bicarbonate secretion potentiated by CCK, and reduced the pancreatic volume and bicarbonate output to similar levels of the control dogs. In contrast, because pancreatic protein secretion is regulated by CCK (Kontrek et al. 1988), loxiglumide administration reduced protein output to less than a half volume of the control dogs.

The reason why ID oleate infusion did not increase first 90-minute pancreatic flow volume and bicarbonate output in the TBD dogs remains unclear, however we can speculate on the reason from the point of view of secretin and CCK. Secretin is released by duodenal acidification and bile exclusion diminishes secretin release in conscious pig (Saetre et al. 1998). Basal pancreatic bicarbonate secretion was strongly enhanced in the TBD dogs which would suppress duodenal acidification, resulting in inhibition of secretin release. This inhibition may possibly have caused a decrease in first 90-minute pancreatic flow volume and bicarbonate output in the TBD dogs.

The increased basal serum CCK levels in the TBD dogs may have continuously stimulated and overloading pancreatic centroacinar cells as well as pancreatic ductal cells, resulting in a failure of immediate response of pancreatic flow volume and bicarbonate secretion. The delayed response to CCK, reaching a peak level at over 45 minutes after ID oleate infusion might also contribute to delayed pancreatic exocrine secretion.

Our findings provide evidence that long-term bile diversion enhances basal outputs of pancreatic volume, bicarbonate, and protein. We conclude that these alterations caused by long-term bile diversion result, at least partly, from increased plasma CCK levels, because the CCK-A receptor antagonist inhibited this enhanced pancreatic exocrine secretion. Our current findings are consistent with previous studies about an intraluminal feedback mechanism between intraluminal bile and CCK secretion (Gomez et al. 1986, 1988). But, other gut hormones such as secretin, VIP, neurotensin (Baca et al. 1982; Sakamoto et al. 1988), and PYY (Pappas et al. 1985) are likely involved in regulating exocrine pancreatic secretion as well even after long-term bile diversion. Further defining the role of intraduodenal bile in pancreatic exocrine secretion awaits future studies.

References


