Diet Stimulation as a Synergistic Factor of Aggravation in a Pancreatic Bile Duct Ligation-Induced Rat Pancreatitis Model

Koji Yoshinaga, Masataka Washizuka, and Yoshihide Segawa*

Department of Applied Research, Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512-1 Oshikiri, Kohnan-machi, Ohkato-gun, Saitama 360-0111, Japan. Received May 11, 2000; accepted July 17, 2000

We evaluated the association between aggravation of pancreatitis and multiple factors enhancing pancreatic exocrine secretion using a rat model of pancreatic bile duct ligation (PBDB)-induced pancreatitis. Under fasting and non-fasting conditions, a PBDB group, a second group treated by hepatic bile duct ligation (BDL) and a third group treated by pancreatic duct ligation (PDL) were compared in terms of serum amylase (S-amylase) activity. The S-amylase activity in the PBDB group was higher than in the sham group. In the PDL group, the increase in S-amylase activity was lower than in the PBDB group. In the BDL group, no increase in S-amylase activity was observed. Diversion of pancreatic and/or bile juice in these groups resulted in no increase of S-amylase activity. Truncal vagotomy or injection of an anticholinergic drug or a cholecystokinin (CCK)-receptor antagonist inhibited pancreatic exocrine secretion and S-amylase activity in the non-fasting PBDB group but not in the fasting PBDB group. These results suggest that retention of pancreatic juice in the pancreatic duct is necessary for the increase of S-amylase activity, and that dietary stimulation and impaired duodenal inflow of bile and pancreatic juice commonly enhance pancreatic exocrine secretion, acting synergistically as aggravating factors in pancreatitis. CCK and the vagus nerve system appear to be involved in enhancing pancreatic exocrine secretion with diet stimulation as an aggravating factor.

Key words pancreatic bile duct ligation (PBDB); pancreatitis; cholecystokinin; serum amylase; fasting, non-fasting

Acute pancreatitis is caused by autodigestion of the pancreas by pancreatic enzymes and is aggravated by enhanced pancreatic exocrine secretion.1-3 Pancreatic bile duct ligation (PBDB)-induced pancreatitis in rats, which is induced by ligation of the hepatic bile duct and the pancreatic duct at its entrance into the duodenum, is a pathological model of clinical pancreatic duct obstruction due to pancreatic stones or edema. In this model, pancreatitis is aggravated by enhanced pancreatic exocrine secretion by dietary stimulation, acetylcholine or gut hormones. Therefore, this PBDB model is considered to be important in clarifying the mechanism of aggravation of pancreatitis.2,3,4

Recent studies using the PBDB pancreatitis model have shown that impaired inflow of bile and pancreatic juice into the duodenum due to PBDB causes enhancement of pancreatic exocrine secretion by a regulation mechanism as luminal feedback.4-7 However, there have been no detailed studies on associations among aggravating factors in PBDB pancreatitis.

In this study, we evaluated the association among various factors involved in PBDB pancreatitis and found that retention of pancreatic juice in the pancreatic duct is necessary for the development of pancreatitis, and that dietary stimulation and impaired duodenal inflow of bile and pancreatic juice enhance pancreatic exocrine secretion, acting as synergistic aggravating factors on pancreatitis. Cholecystokinin (CCK)8 and the vagus nerve system appear to be involved in enhancement of pancreatic exocrine secretion due to dietary stimulation.

MATERIALS AND METHODS

Animals Male CD:SD (IGS) rats aged 7-10 weeks (Charles River Japan Inc., Kanagawa, Japan) were maintained at the experimental animal facility of our institution in polycarbonate cages (270x422x180 mm, Natsume, Tokyo, Japan) with beta chips (Charles River Japan Inc., Kanagawa, Japan) on the floor. Temperature was maintained at 23 ± 3 °C and humidity at 55 ± 10% under ventilation by the return method, with illumination from 7:00 to 19:00. Solid stock food (CRF-1: Charles River Japan Inc., Kanagawa, Japan) and tap water were given ad libitum. The experiments in this study were conducted in accordance with “the Guidelines of Zeria Pharmaceutical Animal Care and Use Committee.”

Reagents The CCK-receptor antagonist, Z-203, sodium (s)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isooquinolyl)-carbonyl] amino-6-methoxy-2-oxo-1-H-indole] propanoate, MW 521.48 was diluted with physiological saline (1%), product synthesized by Zeria Pharmaceutical Co., Ltd. (Tokyo, Japan) before use. Atropine (Sigma Chemicals, St. Louis, MO, U.S.A.) was dissolved in physiological saline before use.

Induction of PBDB Pancreatitis Experiments were performed in fasting and non-fasting groups. In the fasting group, rats were fasted for 16 h before initiation of experiments, with free access to water. In both groups, the abdomen was opened under ether anesthesia, and the hepatic bile duct and the pancreatic duct at its entrance into the duodenum were completely ligated with braided silk 5-0 (Matsuda Medical Engineering, Tokyo, Japan) (PBDB group). After suturing of the abdomen, the rats were placed back into maintenance cages. Neither the hepatic bile duct or the pancreatic duct were not ligated in the sham group. In both the fasting and non-fasting groups, some rats were treated only by common bile duct ligation, pancreatic juice being allowed to flow into the duodenum unhindered (BDL group). Others were treated only by pancreatic duct ligation, with polyethylene tube (Intramedic® PE, Becton Dickinson, Franklin Lakes, NJ, U.S.A.) cannulation between the common bile duct and duodenum, allowing flow of bile into the duodenum (PDL group). In addition, in some PBDB rats, bile and pancreatic juice were allowed to flow via the pancreatic duct at
its duodenal entrance by cannulation (diversion-PB group); and in some PDL, rats in which pancreatic juice was allowed to flow via the pancreatic duct at its duodenal entrance by cannulation (diversion-P group).

**Vagotomy** Truncal vagotomy was performed immediately before PBDL. The abdomen was opened under ether anesthesia, and the vagal nerve running through the anteroposterior wall of the esophagus immediately below the diaphragm was transected according to the method of Li et al.⁹

**Effects of Drugs on PBDL Pancreatitis** Immediately after PBDL, atropine (0.3—3 mg/kg) was subcutaneously injected 6 times at 1 h intervals. Z-203 (0.01, 0.03, and 0.1 mg/kg)⁹—¹² was injected into the caudal vein.

**S-Amylase Activity** Blood was collected at designated intervals, especially at 6 h after operation in each group according to previous reports⁴,⁷ and the serum amylase (S-amylose) activity was measured using a Amylase B Test Wako kit (Wako Pure Chemical Industries, Osaka, Japan).

**Effects of Pancreatic Exocrine Secretion on Aggravation of Pancreatitis** A median incision was made in the rat abdomen under urethane anesthesia (Sigma Chemicals, St. Louis, MO, U.S.A. 1.3 g/5 ml/kg, i.p.), and polyethylene tube cannulation (Intramedic⁶, PE10, Becton Dickinson, Franklin Lakes, New Jersey, U.S.A.) was performed via the pancreatic duct at its entrance into the duodenum. The common bile duct was ligated on the liver side and pancreatic juice collected into a 1.5-ml centrifugation tube (Treff AG, Degersheim, Switzerland). After operation, pancreatic juice was collected for 1 h. The amount of pancreatic juice and the content of protein and amylase activity in the pancreatic juice were then measured. The BCA reaction reagent (Pierce, Rockford, IL, U.S.A.) was used for measurement of protein and the Amylase B-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for measurement of amylase activity. In addition, the effects of vagotomy, atropine, and the CCK₄-receptor antagonist on pancreatic exocrine secretion were evaluated.

**Statistical Analysis** All data are shown as the means± standard error. Statistical analysis was performed using Super ANOVA v. 1.11 software (Abacus Concepts Inc., Berkeley, CA, U.S.A.). The significant differences were tested by use of Dunnott’s r-test for multiple group comparisons and Student’s t-test for comparison between two groups. p<0.05 were regarded as significant.

**RESULTS**

**Serial Changes in S-Amylase Activity in the PBDL Pancreatitis Model** In the non-fasting PBDL group, S-amylose activity began to increase 1 h after ligation and reached a peak after 6 h; the peak value was about 10 times that in the sham group. Subsequently, the increased S-amylose activity gradually decreased until 24 h after ligation. In the fasting PBDL group, the S-amylose activity 6 h after ligation was about 3 times that in the sham group (Fig. 1).

**Aggravation Factors in the PBDL Pancreatitis Model** S-Amylase activity 6 h after the operation was compared among the BDL, PDL and PBDL groups. No increase in S-amylose activity was observed in the non-fasting and fasting BDL groups. The non-fasting and fasting PDL groups showed 60.5% and 42.5% inhibition of the increase in S-amylose activity, respectively, compared with the PBDL group (Fig. 2A). In the non-fasting diversion-PB or diversion-P group, no increase in S-amylose activity was observed.

![Fig. 1. Changes in S-Amylase Activity after PBDL Treatment under Non-fasting or Fasting Condition](image1)

The data represent the mean±S.E.M. of five rats.

![Fig. 2. A) Comparison of S-Amylase Activity in the PBDL, PDL and BDL Groups under Non-fasting or Fasting Condition](image2)

The data represent the mean±S.E.M. of seven to eight rats. ##: Significantly different from the sham group at p<0.01 (Student’s t-test); **p<0.05; ***p<0.01; significantly different from the PBDL group.

**B) Effect of Diversion of Pancreatic Juice to the Duodenum by Cannulation on S-Amylase Activity in the PBDL and PDL Groups under Non-fasting Conditions**

The data represent the mean±S.E.M. of seven to eight rats. ##: Significantly different from the sham group at p<0.01 (Student’s t-test); Significantly different (***p<0.01) from the PBDL group or the PDL group.
Fig. 3. Effect of Truncal Vagotomy, Atropine and CCK₁-Receptor Antagonist Treatment on S-Amylase Activity of the PBDL Group under Non-fasting Conditions

The data represent the mean±S.E.M. of six rats. #: Significantly different from the sham group at p<0.01 (Student's t-test); * p<0.05, ** p<0.01: significantly different from the PBDL group.

Fig. 4. Effect of Truncal Vagotomy, Atropine and CCK₁-Receptor Antagonist Treatment on S-Amylase Activity in the PBDL Group under Fasting Conditions

The data represent the mean±S.E.M. of six rats. #: Significantly different from the sham group at p<0.01 (Student's t-test).

(Fig. 2B).

Involvement of the Vagus Nerve and CCK₁ Receptors in the PBDL Pancreatitis Model. Truncal vagotomy or atropine injection significantly inhibited the increase in S-amylase activity 6 h after ligation in the non-fasting PBDL group (Figs. 3A, B). Injection of Z-203 (0.03–0.1 mg/kg) as a CCK₁-receptor antagonist into the caudal vein significantly and dose-dependently inhibited the increase in S-amylase activity 6 h after ligation under non-fasting condition (Fig. 3C). In the fasting PBDL group, vagotomy or injection of atropine or Z-203 (Fig. 4) 6 h after ligation did not affect the increase in S-amylase activity.

Effects of Non-fasting on Pancreatic Exocrine Secretion

Pancreatic exocrine secretion was compared between the fasting and non-fasting groups (Table 1). Compared with the fasting group, the non-fasting group showed increased in the amount of pancreatic juice, subsequent amylase activity and protein content, indicating enhanced pancreatic exocrine secretion. In the non-fasting group, vagotomy or injection of the CCK₁ receptor antagonist, or Z-203 (0.1 mg/kg, i.v.) significantly inhibited the increases in amylase activity and protein content. Injection of atropine (3 mg/kg, s.c., 1 h intervals) significantly increased the amylase activity and protein content (Table 1). In the fasting group, neither vagotomy nor injection of atropine or Z-203 affected the amount of pancreatic juice, amylase activity or protein content (Table 2).

Table 1. Effects of Truncal Vagotomy, Atropine and CCK₁ Receptor Antagonist Treatment on Pancreatic Exocrine Secretion under Non-fasting Condition

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Weight (mg/h)</th>
<th>P-Amylase (IU/h)</th>
<th>Protein (μg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>Vehicle</td>
<td>28 ± 3</td>
<td>129 ± 16</td>
<td>291 ± 35</td>
</tr>
<tr>
<td>Non-fasting</td>
<td>Vehicle</td>
<td>42 ± 3*</td>
<td>476 ± 174*</td>
<td>1021 ± 309*</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>34 ± 3</td>
<td>185 ± 23*</td>
<td>508 ± 59*</td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>3 mg/kg</td>
<td>112 ± 26*</td>
<td>349 ± 80*</td>
<td></td>
</tr>
<tr>
<td>Z-203</td>
<td>0.1 mg/kg</td>
<td>159 ± 30*</td>
<td>391 ± 43*</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the mean±S.E.M. of 10 to 12 rats in each group. # p<0.01 vs. Vehicle group. Weight: the weight of the pancreatic juice. P-Amylase: the amylase activity in the pancreatic juice. Protein: the protein levels in the pancreatic juice.
Table 2. Effects of Truncal Vagotomy, Atropine and CCK, Receptor Antagonist Treatment on Pancreatic Exocrine Secretion under Fasting Condition

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Weight (mg/h)</th>
<th>P-Amylase (IU/h)</th>
<th>Protein (μg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>32±4</td>
<td>162±22</td>
<td>413±44</td>
</tr>
<tr>
<td>Vagotomy</td>
<td></td>
<td>36±4</td>
<td>193±26</td>
<td>420±69</td>
</tr>
<tr>
<td>Atropine</td>
<td>3 mg/kg</td>
<td>31±5</td>
<td>185±28</td>
<td>427±58</td>
</tr>
<tr>
<td>Z-203</td>
<td>0.1 mg/kg</td>
<td>39±5</td>
<td>209±17</td>
<td>517±26</td>
</tr>
</tbody>
</table>

Results are expressed as the mean±S.E.M. of 10 to 12 rats in each group. No significant difference was observed. Weight: The weight of the pancreatic juice. P-Amylase: the amylase activity in the pancreatic juice. Protein: the protein levels in the pancreatic juice.

DISCUSSION

In this study, we evaluated the association among various factors considered to be involved in PBDL pancreatitis and found that several factors enhanced pancreatic exocrine secretion due to synergistically aggravating pancreatitis. We confirmed the enhancement of pancreatic exocrine secretion induced by diet stimulation via CCK and the vagus nerve system.

In the PBDL pancreatitis model, there are three types of impairment due to the ligation of pancreatic and bile ducts, i.e., impaired bile inflow into the duodenum, impaired inflow of pancreatic juice into the duodenum, and retention of pancreatic juice in the pancreatic duct. Impaired duodenal inflow of bile only can be selectively induced by BDL in which only the hepatic bile duct is ligated. Impaired duodenal inflow of pancreatic juice and retention of pancreatic juice in the pancreatic duct can also be selectively induced by PDL in which a bile bypass is added to PBDL.

Under non-fasting and fasting conditions, comparison among the PBDL, BDL, and PDL groups showed that factors resulting from these operative procedures act as synergistic aggravating factors in the PBDL pancreatitis model (Fig. 2A). The S-amylase activity in the PBDL group was higher than in the PDL group without impaired bile inflow into the duodenum, which suggests impaired bile inflow into the duodenum as an aggravating factor (Fig. 2A). In the BDL group with only impaired bile inflow into the duodenum, no increase in S-amylase activity was observed (Fig. 2A), indicating that impaired duodenal inflow of pancreatic juice and its retention in the pancreatic duct is involved in the development of pancreatitis. In both the PDL and PBDL groups, diversion of pancreatic juice into the duodenum resulted in no increase in S-amylase activity (Fig. 2B). This finding suggests that pancreatitis does not develop only in the presence of impaired duodenal inflow of pancreatic juice, and that retention of pancreatic juice in the pancreatic duct is necessary for the increase of S-amylase activity in the PBDL pancreatitis model.

Concerning the association between bile and pancreatic juice, Samuel et al., who infused bile and pancreatic juice obtained from rats into the duodenum of other rats treated by PBDL, suggested that impaired duodenal inflow of bile and pancreatic juice acts as a synergistic aggravating factor in the development of pancreatitis. In the PDL and PBDL groups, the S-amylase activity under non-fasting conditions increased much more compared with the fasting group. As is shown in Fig. 5, non-fasting and impaired duodenal inflow of bile and pancreatic juice are suggested to be synergistic factors aggravating hyperamylasemia in pancreatitis.

The above aggravating factors in the PBDL pancreatitis model, i.e., impaired duodenal inflow of bile and pancreatic juice and non-fasting conditions, commonly enhance pancreatic exocrine secretion. Pancreatic exocrine secretion in rats is regulated by bile and duodenal protease. Pancreatic exocrine secretion has been reported to increase when the supply of protease is interrupted because of impaired duodenal inflow of bile or pancreatic juice, or when protease activity is consumed by digestion of dietary protein. Therefore, under fasting conditions, it is thought that pancreatic exocrine secretion in the PBDL pancreatitis model is enhanced by impaired duodenal inflow of bile and pancreatic juice. Under non-fasting conditions, it is suggested that pancreatic exocrine secretion in the PBDL pancreatitis model is enhanced by the synergistic action of ligation and non-fasting (Table 1). The differences in pancreatic exocrine secretion between the fasting and non-fasting PBDL groups shown in Tables 1 and 2 are due to differences between ligation alone and ligation combined with non-fasting. This difference is estimated to reflect in S-amylase activity in the fasting and non-fasting PBDL groups.

A close association between enhanced pancreatic exocrine secretion and aggravation of pancreatitis was observed in this study. This association is also supported by the findings that both pancreatic exocrine secretion and S-amylase activity are inhibited by Z-203 as a CCK, receptor antagonist,31—32 truncal vagotomy, or atropine as an anticholinergic drug in the non-fasting PBDL group but not in the fasting PBDL group (Fig. 3, Fig. 4). The differences in the effects of Z-203, truncal vagotomy, and atropine between the fasting and non-fasting groups indicate that all three treatments are ineffective against the effects of ligation under fasting conditions but effective under non-fasting conditions. Recently, enhancement of pancreatic exocrine secretion induced by dietary stimulation or CCK has been reported to be mediated by the vagus nerve system.31—33 The results of this study connect the inhibitory action on pancreatic exocrine secretion to inhibitory action on the increase in S-amylase activity, and suggest that controlling CCK and the vagus nerve system as main factors enhancing pancreatic exocrine secretion can inhibit aggravation of pancreatitis.

In conclusion, non-fasting conditions and impaired duodenal inflow of bile and pancreatic juice enhance pancreatic exocrine secretion, acting as synergistic aggravating factors on PBDL-induced pancreatitis. CCK and the vagus nerve system appear to be involved dominantly in the enhancement of pancreatic exocrine secretion by non-fasting. These results suggest that the decrease of pancreatic secretion due to dietary stimulation may contribute to suppression of aggravation of pancreatitis.

REFERENCES

3) Banerjee A. K., Galloway S. W., Kingsnorth A. N., Br. J. Surg., 81,