A new model of chronic pancreatitis in rats

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Yamamoto, Mitsuyoshi, Munenori Otani, and Makoto Otsuki. A new model of chronic pancreatitis in rats. Am J Physiol Gastrointest Liver Physiol 291: G700–G708, 2006; doi:10.1152/ajpgi.00502.2005.—This study was designed to examine whether continuous pancreatic ductal hypertension (PDH) plays an important role in the onset and development of chronic pancreatitis (CP). Pancreatic, biliary, and duodenal cannulas were implanted in male Wistar rats. PDH was induced by vertically raising the free end of the pancreatic duct cannula to exert a hydrostatic pressure and maintained for 2 wk. PDH was gradually increased, but when the pancreatic juice (PJ) flow was interrupted, PDH was decreased to restore PJ flow. The induction of PDH resulted in a marked reduction of amylase activity in PJ and an increase in serum amylase activity. At 2 wk after persistent PDH, pancreatic exocrine function was markedly decreased in response to a bolus injection of secretin (100 pmol/kg) compared with the control group. Histological examination revealed interlobular as well as intralobular fibrosis in the form of nodular pancreatitis at 2 wk after the induction of PDH. Immunohistochemistry revealed the expression of fibronectin and collagen types I and III. Quantitative real-time RT-PCR showed an increase in transforming growth factor-β1 mRNA expression in the pancreas during PDH. The present results suggest that PDH plays an important role in the onset and development of CP. Furthermore, our animal model seems useful for investigating the mechanisms of CP in rats.

Materials and Methods

Animals. Male Wistar rats weighing 270–300 g were used and maintained in a temperature (23 ± 2°C) and humidity (55 ± 5%)-controlled room with a 12:12-h light-dark cycle (lights on at 7:00). The experimental protocol was approved by our institutional animal welfare committee, and rats received humane care according to the guidelines of our institution.

Animal preparation. A midline incision of the abdomen was made under pentobarbital sodium anesthesia (50 mg/kg body wt ip). The common bile duct was ligated proximal to the pancreas near the liver, and a cannula was inserted above the ligature to collect pure bile. Another cannula was inserted into the biliopancreatic duct through the ampulla of Vater to collect pure PJ. An additional cannula was inserted into the duodenum to return biliopancreatic juice. These cannulas (Silastic tubing; 0.020 in. inner diameter × 0.037 in. outer diameter) were initially exteriorized through a subcutaneous channel to the back near the tail. After the rats’ abdomen was closed, rats were prepared with a jugular vein cannula and then placed into a modified Bollman-type restraint cage. Surgical procedures were conducted according to the methods of Green et al. (16). During the recovery and experimental periods, rats had full access to food and water ad libitum. PJ and bile were collected and continuously returned to the duodenum by a servomechanism consisting of a collecting tube in a liquid level detector coupled to a peristaltic pump (17).

Experimental protocol. On postoperative day 4, continuous PDH was induced by raising the free end of the pancreatic duct cannula to a vertical position at 25 cm above the pancreas to exert a hydrostatic pressure. A schematic diagram of the experimental model is shown in Fig. 1. Previous studies have reported that the average intrapancreatic duct pressure in patients with CP is 33.1 cmH2O (6), and the pancreatic duct ruptures at a pressure of 50 cmH2O in rats (4). Therefore, the free end of the pancreatic cannula was raised 5 cm each day from 25 to 35 cm but not above 35 cm to prevent acute pancreatic damage. In addition, because complete obstruction of the pancreatic duct subsequently causes apoptosis followed by atrophy rather than fibrosis of the pancreas over days to weeks in rats (19, 52), the free end of the pancreatic cannula was lowered to maintain continuous PJ flow when the PJ flow was interrupted (PDH group). Control rats were subjected to the same operative procedures, but the free end of the pancreatic cannula was maintained at 4–5 cm below the pancreas (control group). PJ and blood samples were taken before and at 12 h and on days 1, 2, 3, 7, 10, and 14 after the induction of PDH. PJ was analyzed for volume, concentration of protein, and amylase and lipase activity. Serum was analyzed for amylase activity. Mucoprotein concentration in PJ was determined on day 14 after the induction of PDH. At 12 h and on days 1, 3, 7, and 14 after the induction of PDH, rats were killed, the pancreas was taken under pentobarbital anesthesia, and plasma cholecystokinin (CCK) levels were determined. Seven to eight rats were used for each time point. The control group consisted of eight rats; rats were killed, and the pancreas was then removed on day 14, at the end of the experimental period. A splenic portion of the pancreas was used for histological examination, and a

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duodenal portion of the pancreas was used for RNA analysis. A duodenal portion of the pancreas, serum, and PJ were frozen at −80°C until assays.

Previous studies have demonstrated that pancreatic proteases in the duodenum exhibit a feedback control of pancreatic secretion (14, 15) via endogenous CCK release (30, 33). In addition, elevated circulating CCK participates in the onset and progression of acute pancreatitis (13, 47, 48). Because return of PJ into the duodenum was markedly reduced to maintain PDH in the present study, it is likely that increased circulating CCK deteriorates pancreatic damage and promotes pancreatic fibrosis. Thus, we continuously infused PJ from a donor rat into the duodenum of a recipient rat during continuous PDH to suppress the increase in circulating CCK and examined whether the progression of fibrosis was prevented in the recipient rat. In the PJ donor rat, the same operative procedure as in the PDH group of rats was conducted, but PDH was not induced. PJ was collected from the donor rat and mixed with bile and PJ from the recipient rat, and the mixture was continuously returned to the duodenum of the recipient rat during continuous PDH for 2 wk (PJ recipient group). The pancreas was taken from the PJ recipient group of rats on day 14 after the induction of PDH.

Physical analysis. The body weights of rats were measured after the induction of anesthesia before surgery and after the experimental period. Food intake was determined daily during the experimental period in the control and PDH groups.

Pancreatic function test. On day 14, pancreatic exocrine function in response to secretin (Peptide Institute, Protein Research Foundation, Osaka, Japan) was evaluated after an overnight fast. PDH was decompressed from 60 min before and during the pancreatic function test. To decompress PDH, the free end of the pancreatic cannula was maintained at 4–5 cm below the pancreas. Secretin at a dose of 100 pmol/kg was given by a bolus injection after the basal PJ flow was obtained for 30 min at 15-min intervals, and PJ was further collected for 60 min at the same interval. The volume of PJ, bicarbonate concentration, and amylase activity in PJ were determined.

Histological examination. After rats were euthanized, a portion of pancreatic tissue was fixed overnight in 4% buffered neutral paraformaldehyde solution, embedded in paraffin, and deparaffinized by standard procedures. Thin sections (5 µm) were stained with hematoxylin and eosin, and Azan-Mallory staining was used for light microscopic examination. All histological samples were examined in a single blinded fashion.

Quantitative analysis for pancreatic fibrosis. Quantitative evaluation of interstitial fibrosis in the pancreatic specimen was performed using an Axioshot microscope (Carl Zeiss, Eching, Germany) connected to an interactive image analysis system (IBAS, Carl Zeiss). Ten nonoverlapping fields per pancreatic specimen with Azan-Mallory staining (n = 7) were randomly selected at ×100 magnification. The area of the total pancreatic specimen and that of blue-stained interstitial fibrosis were determined by the IBAS. The proportion of pancreatic fibrosis was indicated as a percentage of the total specimen using the following equation: area of interstitial fibrosis/total area of specimen.

Immunohistochemistry. For immunohistochemistry, pancreatic tissue sections were immersed in PBS (pH 7.2) for 10 min and then in PBS containing 3% H2O2 for 10 min to quench endogenous peroxidases. After being further incubated in 0.25% casein solution for 10 min, tissue sections were incubated with a specific primary antibody for α-smooth muscle actin (α-SMA; Dako, Carpinteria, CA) at 1:50, collagen types I and III (Rockland, Gilbertsville, PA) at 1:50, or fibronectin (Dako) at 1:500 diluted in PBS for 16 h at 4°C. Primary antibodies were visualized by the labeled streptavidin-biotin method using a commercially available kit (Dako), and all procedures were performed as recommended by the manufacturer.

Detection of apoptotic acinar cells during PDH. Apoptotic cells were quantified immunohistochemically by a novel monoclonal antibody that recognizes exposed single-stranded regions in the DNA of apoptotic cells (Dako). The procedure of immunohistochemistry for single-stranded DNA was almost the same method described for α-SMA, collagen types I and III, and fibronectin. The primary antibody was diluted at 1:100 in PBS. For the acinar cell apoptotic index, 10 nonoverlapping fields/pancreatic specimen (n = 6) were randomly selected at ×200 magnification. At least 300 acinar cells were counted in each field. The apoptotic index represented the number of positive cells per counted cells.

Assays. The protein concentration in PJ was measured by the methods of Lowry et al. (31) with bovine plasma albumin as the standard. The mucoprotein concentration in PJ was determined using the methods of Winzler et al. (54). Amylase activities in PJ and serum were determined by a chromogenic method with a Phadebas amylase test (7) and expressed in Somogy units. The bicarbonate concentration in PJ was determined by a DST 800 multititration system (Radiometer, Copenhagen, Denmark). Lipase activity in PJ was analyzed enzymatically using commercially available kits (Lipase Kit A
For the measurement of the plasma CCK concentration, CCK was extracted from plasma, and a sensitive and specific radioimmunoassay was conducted using the antiserum OAL-656 with CCK-8 as the standard (55).

Fig. 2. A: serial changes in the height of the pancreatic cannula from the pancreas in rats during the experimental period. The height of the free end of the pancreatic cannula represents pancreatic ductal pressure. On postoperative day 3, PDH was induced by vertically raising the free end of the pancreatic duct cannula to 25 cm from the pancreas. The free end of the pancreatic cannula was raised 5 cm each day up to 35 cm, and, when the pancreatic juice (PJ) flow was interrupted, the pancreatic cannula was lowered to preserve pancreatic secretion. B: serial changes in PJ volume. PDH was associated with a marked decrease of PJ volume. C: serial changes in serum amylase activity [in Somogyi units (SU)/dl]. PDH immediately increased serum amylase activity compared with the control value, but the difference was not statistically significant. Values are expressed as means ± SE of 7–8 rats. *P < 0.05 vs. the control value at each time point.

Fig. 3. A and B: serial changes in amylase (A) and lipase (B) activities in PJ. PDH was associated with a significant decrease of amylase and lipase activities in PJ during the experimental period compared with the control group. C: serial changes in protein concentration in PJ. PDH was associated with an initial significant decrease of protein concentration in PJ. After day 3, protein concentration gradually increased but was significantly lower than the control value. Values are expressed as means ± SE of 7–8 rats. *P < 0.05 vs. the control value at each time point.

Table 1. Effect of continuous PDH on body weight gain, daily food intake, and mucoprotein concentration in PJ

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>PDH Group</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Physical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>285.0±11.6</td>
<td>ND</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>23.7±1.2</td>
<td>24.2±1.9</td>
</tr>
<tr>
<td>PJ analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucoprotein concentration, mg/dl</td>
<td>12.6±0.9</td>
<td>18.0±1.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats/group. PDH, pancreatic ductal hypertension; PJ, pancreatic juice; ND, not determined. *Significant difference (P < 0.05) vs. the value at day 0 in the respective group; †significant difference (P < 0.05) between the values in the control and PDH groups at day 14.

“Marupi,” Dainippon Pharmaceutical, Osaka, Japan. For the measurement of the plasma CCK concentration, CCK was extracted from plasma, and a sensitive and specific radioimmunoassay was conducted using the antiserum OAL-656 with CCK-8 as the standard (55).
Table 2. Changes in plasma CCK levels during the experimental period in control, PDH, and PJ recipient groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4±0.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.7±0.6</td>
</tr>
<tr>
<td>PDH</td>
<td>ND</td>
<td>4.1±0.8</td>
<td>7.8±1.7*</td>
<td>5.7±1.4</td>
<td>5.4±1.8</td>
</tr>
<tr>
<td>PJ recipient</td>
<td>ND</td>
<td>2.6±0.6</td>
<td>2.3±0.4</td>
<td>ND</td>
<td>2.2±0.4</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05) vs. the value at day 0 in the control group. n.d.: not determined. Values are means ± SE (in pmol/l); n = 8 rats/group.

*Significant difference (P < 0.05) vs. the value at day 0 in the control group.

Quantitative real-time RT-PCR. The expression level of transforming growth factor (TGF)-β1 was determined by quantitative TaqMan PCR with the GAPDH gene as a reference. For real-time RT-PCR, total RNA was extracted from frozen pancreatic tissue by the acid guanidium thiocyanate-phenol-chloroform method (28), and 2 μg of total RNA were reverse transcribed using random hexamers and TaqMan RT reagents (Applied Biosystems, Foster City, CA). PCRs for TGF-β1 and GAPDH were performed using the TaqMan probe, TaqMan universal PCR master mix, and primer for TGF-β1 as well as TaqMan rodent GAPDH control reagents (Applied Biosystems). PCR products were amplified (50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min) and analyzed on a real-time PCR cycler, the ABI PRISM 7000 sequence detection system (Applied Biosystems). For quantification, the fluorescence intensity was plotted against the PCR cycle number. The amplification cycle displaying the first significant increase of the fluorescence signal was defined as the threshold cycle (CT). The CT value of each sample was compared with CT values of the standardization series, which consisted of cloned PCR fragments. The amounts of TGF-β1 transcripts were normalized to the amounts of GAPDH transcripts in the same cDNA (expressed as fold change per GAPDH).

Statistical analysis. Each experiment was performed in seven to eight rats, and results are expressed as means ± SE. Statistical analysis was performed by ANOVA followed by Fisher’s test using commercial software (StatView, Abacus Concepts/Brain Power, Berkeley, CA). Differences with P < 0.05 were considered as statistically significant.

RESULTS

Pancreatic ductal pressure. Figure 2A shows the serial changes in pancreatic ductal pressure recorded during the 14-day study. After day 2, the hydrostatic pressure was gradually reduced to maintain PJ flow (Fig. 2B).

Physical analysis. Body weights did not change in the PDH group, whereas they significantly increased in the control group up to day 14 (Table 1). Daily food intake in the PDH group was approximately the same as that in the control group on days 0 and 7 but was significantly lower on day 14 compared with that in the control group (Table 1). However, there were no significant differences in average food intake during the experimental period between the control (23.5 ± 4.8 g/day) and PDH (24.4 ± 1.3 g/day) groups. On the other hand, almost all of the rats evacuated muddy or soft feces during continuous PDH. However, such feces were not observed in the control group of rats.

Blood and pancreatic juice analysis. Although serum amylase activity immediately increased after the induction of PDH compared with the control group, the difference was not significant, and it returned to the control level on day 2 (Fig. 2C). PDH caused a marked and significant decrease in PJ flow (Fig. 2B). Amylase and lipase activities in PJ immediately and significantly decreased after the induction of PDH and further decreased during the experimental period (Fig. 3, A and B). The protein concentration in PJ immediately and markedly decreased after the induction of PDH. Thereafter, the protein concentration gradually increased but was significantly lower than the basal level.
than the control value during PDH (Fig. 3C). The mucoprotein concentration in PJ was significantly higher in the PDH group than in the control group on day 14 (Table 1). Plasma CCK levels during PDH were higher than control values, although a statistically significant difference was obtained only on day 1 (Table 2). In contrast, plasma CCK levels in the PJ recipient group were similar to those in the control group during the experimental period (Table 2).

Pancreatic function test. Although PDH was decompressed from 60 min before and during the pancreatic function test, the basal PJ volume (Fig. 4A) and amylase output (Fig. 4C) in the PDH group were significantly lower than those in the control group. In addition, a bolus injection of secretin at a dose of 100 pmol/kg failed to induce a significant increase in PJ volume (Fig. 4A), bicarbonate concentration (Fig. 4B), and amylase output (Fig. 4C) in the PDH group, but the same dose of secretin markedly and significantly increased these parameters in the control group.

Histological findings. On day 1 after the induction of PDH, pancreatic ducts were slightly dilated, but inflammatory cells or fibrosis were rarely noted in the pancreas (Fig. 5A). On day 7, pancreatic fibrosis was observed mainly in the intralobular area and especially in the periductal area (Fig. 5B). On day 14, pancreatic fibrosis was markedly distributed in interlobular or perilobular areas and was also found in intralobular areas. In addition, marked inflammatory cell infiltration, predominantly lymphocytes, was observed in interlobular and intralobular areas (Fig. 5C). Focal fat necrosis (data not shown) and plug formation in the main pancreatic duct (Fig. 5D) were also observed on day 14. In comparison, only minimal histological changes were noted in the control group on day 14 (Fig. 5E). On the other hand, fibrosis was markedly distributed in interlobular and intralobular areas in the PJ recipient group (Fig. 5F).

Immunohistochemistry. On day 14 after the induction of PDH, immunoreactivity for collagen type I was markedly detected around pancreatic ducts but was slightly detected in interlobular and intralobular areas (Fig. 6A). On the other hand, collagen type III (Fig. 6B) and fibronectin (Fig. 6C) were markedly detected around pancreatic ducts and in interlobular areas.
and intralobular areas. The expression of α-SMA, a marker for activated pancreatic stellate cells (PSCs), was mainly distributed in periductal, periacinar, and interlobular areas (Fig. 6, D and E). However, α-SMA-positive cells were rarely detected except in the vessel walls in the control group (Fig. 6F).

Proportion of fibrotic area and quantitative analysis of TGF-β1 in the pancreas. PDH was associated with a progressive and significant increase in the proportion of the fibrotic area compared with the control group (Fig. 7A). Quantitative real-time RT-PCR revealed that PDH markedly increased the expression level of TGF-β1 transcript normalized to the amount of GAPDH in the pancreas. The expression level of TGF-β1 in the PDH group peaked on day 1. Thereafter, although it gradually decreased, it remained at significantly higher levels than the control value during the experimental period (Fig. 7B).

Index of acinar cell apoptosis during PDH. The apoptotic index of acinar cells in the PDH group increased with time and peaked on day 7. Although it decreased on day 14, it was still significantly higher than the value in the control group (Fig. 7C).

DISCUSSION

The major findings of the present study are as follows: continuous PDH 1) markedly decreased pancreatic exocrine function, 2) caused diffuse interlobular and intralobular fibrosis with expression of fibronectin and collagen types I and III, 3) induced mild hyperamylasemia, and 4) reduced the body weight gain and caused muddy or soft feces in the presence of unaltered food intake, indicating malabsorption probably due to reduced pancreatic enzyme secretion. Those histopathological changes are reliable, reproducible, and closely resemble human CP (10, 46). Therefore, our animal model seems suitable for the investigation of the mechanism of CP.

A previous study (6) has demonstrated that the intrapancreatic duct pressure in patients with CP is significantly higher than in normal subjects (mean 33.1 cmH2O; range 15–57 vs.
rats. a pancreatic pancreas. The expression level of TGF-
progressively decrease in PJ in human CP (21–23, 34, 51). Consistent with
such as hexosamine, lactoferrin, and mucopolysaccharide in-
pancreas. view that continuous PDH results in fibrotic changes in the
kidney (25), and lung (26). These results further support our
results in fibrosis in several organs, including the heart (53),
pressure overload activates myofibroblasts or fibroblasts and
present study provides experimental evidence that CP arises
from or leads to PDH. The outcome of patients with CP. These findings suggest that PDH
plays an important role in the pathogenesis of CP. However, it
is still unclear whether CP arises from or leads to PDH. The
present study provides experimental evidence that CP arises
from continuous PDH. In addition, there is also evidence that
pressure overload activates myofibroblasts or fibroblasts and
results in fibrosis in several organs, including the heart (53),
kidney (25), and lung (26). These results further support our
view that continuous PDH results in fibrotic changes in the
pancreas.

Previous studies have shown that nonenzymatic proteins such as hexosamine, lactoferrin, and mucopolysaccharide increase in PJ in human CP (21–23, 34, 51). Consistent with these results, pancreatic digestive enzymes progressively de-
creased, but protein concentration gradually increased, in PJ
during continuous PDH. In addition, mucoprotein, including
hexosamine, significantly increased in PJ in the PDH group
compared with that in the control group. These results suggest
that PDH enhanced nonenzymatic proteins secretion, and such
proteins were attributable to an increase in the protein concen-
tration in PJ. Because nonenzymatic proteins have been shown
to relate with the viscosity of PJ or formation of protein plugs
(22, 23, 51), it is likely therefore that these proteins play some
roles in the progression of pancreatic fibrosis in our model.

Plasma CCK levels during continuous PDH were higher than those in the control group. However, although plasma CCK levels remained at control levels in the PJ recipient group, marked fibrosis was found in the pancreas at 2 wk after the induction of PDH. These results indicate that elevation of circulating CCK might not play a major role in the progression of pancreatic fibrosis in the present model.

Despite decades of research, the pathophysiology underlying alcoholic CP is poorly defined to date. Long-term ethanol feeding alone has failed to induce acute or chronic pancreatitis in animals (1, 9, 43, 44). In addition, CP occurs in only a
limited number of heavy drinkers (20, 37). These findings suggest that alcohol is a very weak inducer of CP, and other factors are necessary for the onset of the disease. Recently, although several studies have investigated the genetic mechanism of alcoholic CP, only a small minority of patients have underlying gene mutations (41, 42, 49). These results suggest that gene mutations seem to be minor pathogenic factors in alcoholic CP. On the other hand, several lines of evidence suggest that PDH largely participates in the onset of alcoholic CP. Sales et al. (39) reported that protein plugs are present in all small pancreatic ducts in the initial lesions of human alcoholic CP, leading to fibrotic replacement of acinar tissue upstream from the occlusion. In addition, the high incidence of pancreatitis and pancreatic-like pain in patients with pancreas divisum may be due to the relatively narrow ampulla of the Santorini duct (18). It has also been reported that if the pancreas divisum is subjected to massive alcohol intake, CP appears in the region of Santorini duct, leaving the region of Wirsung duct intact (8). We therefore postulate that continuous PDH due to functional or mechanical stenosis of the papilla or pancreatic duct may play an important role in the onset and development of alcoholic CP.

Recently, PSCs have been identified and characterized (2,
5). PSCs are activated upon exposure to cytokines such as
TGF-β1 and PDGF and have the capacity to produce extracel-
lar matrix (ECM) proteins (3, 5). In the present study,
α-SMA-positivecells and ECM proteins, including collagen
types I and III and fibronectin, were markedly expressed in the
pancreas in the PDH group. In addition, TGF-β1 transcript was
upregulated in the pancreas after the induction of PDH. Taken
together, it is conceivable that PDH induces the expression of
TGF-β1 and thereby stimulates PSCs to synthesize ECM pro-
teins in the pancreas. On the other hand, TGF-β1 has been
reported to be expressed in human CP (45, 50), but serial
changes of expression levels of TGF-β1 during onset and
progression of CP is still unknown. In the present study,
quantitative analysis using real-time RT-PCR revealed that
TGF-β1 transcript in the pancreas peaked immediately after the
induction of PDH and remained at significantly higher levels
than the control value during PDH.
CP is characterized by an ongoing process of acinar cell destruction and progressive fibrosis. Recently, several studies demonstrated that apoptosis is involved in the atrophic process of human CP (27, 56). In our study, the apoptotic index of acinar cells was significantly higher in the PDH group than in the control group. These results suggest that PDH plays an important role in the activation of acinar cell apoptosis as well as in the formation of pancreatic fibrosis. On the other hand, continuous PDH showed only little effect on the apoptotic index and histological findings of islet cells and serum levels of glucose and insulin (data not shown). These results suggest that the underlying mechanisms of cellular destruction of acinar and islet cells are mediated via different pathways and that PDH does not largely participate in the fibrosis or destruction of islets. The difference in anatomic locations between endocrine and exocrine cells may be one of the reasons. A clinical study (10) also reported that endocrine insufficiency due to the loss of pancreatic islet cells occurs in <40% of patients with CP and is usually a mild and late manifestation of the disease. Although substantial fibrosis was observed in the almost all of the rats and mortality was quite low (under 5%) during the 2 wk of PDH, surgical and maintenance skills and equipments are required for the present experimental model. In addition, it was difficult to maintain this animal model for >3 wk. These aspects may be limitations of this animal model.

In conclusion, we demonstrated in the present study that chronic PDH plays an important role in the onset and development of CP. Because the histopathological changes in the present study closely resembled those of human CP, this model seems useful for investigating the mechanisms of CP in rats.

GRANTS
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