
原 著

A New Experimental Model for Gallstone Pancreatitis:
Short-Term Pancreatico-Biliary Duct Obstruction and
Exocrine Stimulation with Systemic Hypotension in Rats

TETSUYA HIRANO and TADAO MANABE

First Department of Surgery, Faculty of Medicine, Kyoto University, Kyoto, Japan

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Abstract

The effects of short-termed (2 hours) obstruction of pancreatico-biliary duct (PBDO) and exocrine stimulation (IDH) by caerulein infusion ($0.2 \mu\text{g/kg.hr}$) with systemic hypotension (SH) (30% reduction of mean arterial pressure for 30 min) on the exocrine pancreas were evaluated in the rat. PBDO and IDH with SH caused more significant rises in portal serum amylase, cathepsin B and malate dehydrogenase levels, and pancreatic water content as well as more significant redistribution of cathepsin B activity from the lysosomal fraction to the zymogen fraction in the subcellular fractionations than only PBDO, or PBDO with IDH, or PBDO with SH group. In addition, more accelerated lysosomal and mitochondrial fragility were observed in the PBDO and IDH with SH group. Moreover, PBDO and IDH with SH caused an activation of larger amount of trypsinogen in the pancreas compared with other groups (PBDO with IDH and PBDO with SH group). These results indicate that present model of short-termed PBDO and exocrine stimulation with systemic hypotension seems to be a pertinent model for gallstone pancreatitis in humans, and that redistribution of lysosomal enzymes and subcellular organellar fragility seem to play an important role in the pathogenesis of pancreatic injuries by PBDO, particularly with exocrine stimulation and pancreatic ischaemia, probably via activation of trypsinogen to trypsin by lysosomal enzyme such as cathepsin B.

Introduction

Both morphological and biochemical studies have shown that, in the early stage, two forms of experimental pancreatitis (diet-induced^{1,2)}, and secretagogue-induced³⁻⁶⁾ share the common attribute of colocalization of digestive enzymes with lysosomal hydrolases inside large cytoplasmic vacuoles⁷⁾,

Key words: Pancreatico-biliary duct obstruction, Lysosome, Mitochondria, Cathepsin B, Intraductal hypertension, Pancreatic ischemia.

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Present address: First Department of Surgery, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606, Japan.

and the subcellular fractionation of acinar cells has demonstrated redistribution of lysosomal enzymes from the lysosome-rich to the zymogen granule-rich fraction. Since cathepsin B, a lysosomal enzyme, can activate trypsinogen^{8,9}, and trypsin can activate many other digestive enzymes, this colocalization phenomenon could lead to the intracellular activation of digestive enzymes and result in an important triggering event in the development of acute pancreatitis inside the acinar cells. Thus, lysosomal enzymes seem to play an crucial role in the pathogenesis of acute pancreatitis¹⁰.

On the other hand, gallstone pancreatitis, which is the most common in humans, seems to be triggered by the passage through, or incarceration of a stone in the terminal bile duct¹¹⁻¹⁴, which is considered to mean the obstruction of both the pancreatic and biliary duct. However, the mechanism whereby pancreatobiliary duct obstruction (PBDO) may induce pancreatitis has not been clarified, and only PBDO by itself could not lead to the severe pancreatic injuries, other factors such as intraductal hypertension and pancreatic ischaemia considered to be needed.

In this study, we examined the in-vitro lysosomal and mitochondrial fragility, subcellular distribution of lysosomal enzyme as well as the possible activation of trypsinogen in the pancreas after short-termed (2 hours) PBDO and exocrine stimulation with temporary pancreatic ischaemia.

Materials and Methods

Animal preparation

Thirty four male Wistar rats weighing about 350 g (Shizuoka Experimental Animals, Shizuoka, Japan) were used. The rats were kept in light-dark cycle regulated (light, 0500–1700 h) and air-conditioned ($23 \pm 3^\circ\text{C}$) animal quarters in our institute before experiments, and were given free access to tap water and diet (Oriental Rodent Chow, Tokyo, Japan). They were allowed to become acclimatized to the standard laboratory conditions for 4 days, and they were maintained throughout the study in accordance with the guidelines of the Committee on Animal Care of Kyoto University. This study was also approved by the committee. Experiments were performed after a 16-hour fast, starting at between 0800 and 0900 h to rule out the effects of circadian rhythms on the exocrine pancreas.

Anaesthesia was induced by intraperitoneal administration of sodium pentobarbital (30 mg/kg) (Nembutal®, Abbott Co., North Chicago, IL, USA), and maintained by periodic intravenous injection of pentobarbital (10 mg/kg). The rats were kept on heating pad at 40°C (KN-473, Natsume Seisakusho, Tokyo, Japan) and under overhead lamps to maintain their core body temperature. Before opening the abdomen, a catheter (Medicut 18-gauge catheter, Sherwood Medical Industries, St. Louis, MO, USA) was placed in the superior vena cava (S.V.C.) via the right external jugular vein. Another catheter (PE-50, Clay Adams, Parsippany, NJ, USA) was placed in the contralateral internal carotid artery and connected to the mercurial manometer (Stentor Engineering Co., Kansas City, MO, USA) for monitoring the arterial pressure.

A midline skin incision was made for laparotomy, and at this point, all the rats were divided into the following five groups (Fig. 1):

- (a) pancreatobiliary duct obstruction (PBDO) group ($n=6$); pancreatobiliary duct (PBD) just adjacent to the duodenum was ligated by 4-0 silk thread for 2 hours.
- (b) pancreatobiliary duct obstruction and exocrine stimulation (PBDO + CER) group ($n=7$); PBD was ligated for 2 hours and immediately after PBDO, caerulein (Ceosunin Injection, Kyowa Hakko Co., Tokyo, Japan) was infused at a dose of $0.2 \mu\text{g/kg.hr}$ for 30 minutes to

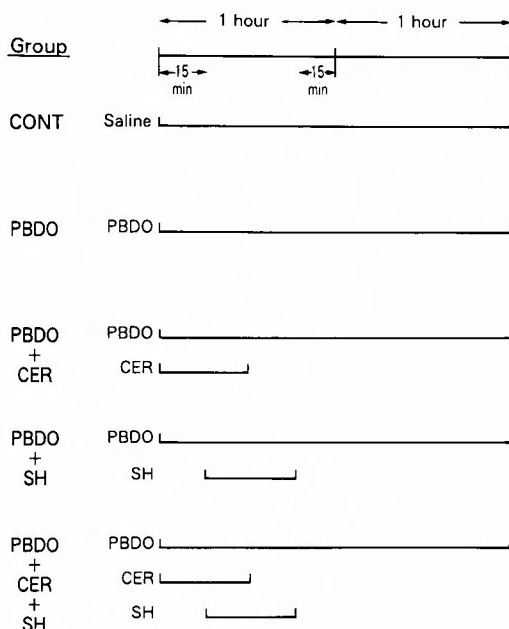


Fig. 1 Experimental groups PBDO: pancreatico-biliary duct obstruction (2 hours), CER: caerulein stimulation ($0.2 \mu\text{g/kg.hr}$ for 30 min), SH: systemic hypotension (30% reduction of mean arterial pressure for 30 min), CONT: control laparotomized group

stimulate the pancreatic secretion and to make an intraductal hypertension.

- (c) pancreatico-biliary duct obstruction and systemic hypotension (PBDO+SH) group ($n=7$); PBDO was made as in the PBDO group, and immediately after induction of PBDO, the mean arterial pressure was reduced to approximately 70% of the control values. This degree of hypotension was achieved in 15 minutes, and maintained for 30 minutes by removing blood from the carotid artery catheter into heparinized syringe. Thirty minutes after the onset of hypotension, normotension was restored by reinfusing the shed blood along with an equal volume of lactate-Ringer solution in 5 minutes.
- (d) pancreatico-biliary duct obstruction and exocrine stimulation with systemic hypotension (PBDO+CER+SH) group ($n=9$); PBDO, CER and SH were made as in the PBDO, PBDO+CER, and PBDO+SH group.
- (e) control laparotomy (CONT) group ($n=5$); PBD near the duodenum was gently manipulated and anaesthesia was maintained for 2 hours.

All the rats were infused with heparinized (30 U/ml) saline at a rate of 0.58 ml/hr during the experiments by an infusion pump (Truth Type B-6, Nakagawaseikodo, Tokyo, Japan). In all the groups, the abdominal wounds were closed after finishing the abdominal procedures.

Portal serum amylase, cathepsin B and malate dehydrogenase (MDH) levels, and pancreatic water content

At the selected times after 2-hour experiments in each group, all the rats were painlessly killed by a large dose of pentobarbital (80 mg/kg). After blood samplings from the portal trunk for the determination of portal serum amylase, cathepsin B and MDH levels, the portions of the pancreas were removed quickly. About 200 mg of the pancreas was used for the quantification of pancreatic

edema by comparing the weight immediately after removal (wet weight) with that of the same sample after incubation at 150°C for 48 hours in a dessicator (Sanyo Drying Oven^R, Sanyo, Tokyo, Japan) (dry weight). Pancreatic water content was expressed as a percentage of the total wet weight.

Pancreatic amylase, cathepsin B, trypsinogen and trypsin content

Another about 200 mg of the pancreas was used for the determination of pancreatic content of amylase, cathepsin B, trypsinogen and trypsin. This portion was homogenized in 3 ml of cold phosphate-buffered (pH 7.4) saline containing 0.5% Triton X-100 (Sigma Chemical Co., St. Louis, MO, USA) in a Polytron homogenizer (Brinkmann Instrument, Westbury, NY, USA), and unbroken cells and debris were removed by low speed centrifugation ($150 \times g$ at 4°C for 15 min). Amylase activity, cathepsin B activity, trypsinogen content and trypsin activity were measured in the resulting supernatant. Deoxyribonucleic acid (DNA) concentration was also measured, and pancreatic content of amylase, cathepsin B, trypsinogen and trypsin were expressed as U/mg DNA.

Pancreatic histological examination

One small portion of the pancreas from the splenic portion was fixed overnight by immersion in phosphate-buffered (pH 7.4) 10% neutral formalin. After paraffin embedding, sectioning and staining with hematoxylin-eosin, the sections were examined light microscopically by an independent observer, who graded acinar cell changes such as interstitial edema, acinar cell vacuolization, inflammatory cell infiltration and acinar cell necrosis on a 0 (no changes) to +4 (maximum changes) scale. One section was made in the center of the specimen from each rat, and whole the section was observed.

Subcellular distribution of cathepsin B activity

Another part of the pancreas (about 300 mg) was used for subcellular fractionation and for the determination of subcellular distribution of cathepsin B activity in acinar cells. The excised, trimmed and homogenized rat pancreas was separated into its various subcellular fractions by differential centrifugation as previously described^{15,16}. Briefly, pancreatic fragments were homogenized in 6 ml of cold 5 mM MOPS (3-(N-morpholino)propanesulphonic acid) (Sigma Chemical Co.) buffer (pH 6.5) containing 1 mM $MgSO_4$ and 250 mM sucrose, with three up- and -down strokes of a Dounce homogenizer (Wheaton, Millville, NJ, USA). The resulting homogenate was centrifuged ($150 \times g$ at 4°C for 10 min) to pellet debris and unbroken cells, which were discarded. The supernatant after this low speed centrifugation was considered to contain 100% of each of the component measured. This supernatant was centrifuged ($1300 \times g$ at 4°C for 15 min) to obtain a zymogen granule-rich pellet (1.3 KP), and this supernatant was centrifuged ($12000 \times g$ at 4°C for 12 min) to yield a lysosome- and mitochondria-rich pellet (12 KP) and a supernatant, which was considered to contain a microsomal and soluble fraction (12 KS). The various pellets obtained during fractionation were resuspended in 2 ml of cold (4°C) 5 mM MOPS buffer, and cathepsin B activity in each fraction was measured and expressed as a percentage of the total activity as an index of subcellular distribution of lysosomal enzymes in acinar cells.

Cathepsin B leakage from lysosomes and malate dehydrogenase leakage from mitochondria

The remaining portions of the pancreas (about 400 mg) were homogenized in cold 5 mM MOPS buffer as described as above. This homogenate was centrifuged ($150 \times g$ at 4°C for 10 min) to remove unbroken cells and debris. The resulting supernatant was centrifuged ($1300 \times g$ at 4°C for 15 min) to pellet a zymogen granule-rich pellet and the supernatant. This supernatant was then centrifuged ($12000 \times g$ at 4°C for 12 min) to obtain a combined lysosome- and mitochondria-rich fraction (12 KP). This pellet, arbitrarily considered to contain 100% of the lysosomal and mitochondria-

drial enzyme activity, was suspended in 2 ml of 5 mM MOPS buffer and incubated for varying intervals (60 and 120 min) at 25°C in a shaking water bath under room air. The samples were then re-centrifuged ($12000 \times g$ at 4°C for 12 min) to separate the particulate from the soluble lysosomal and mitochondrial enzyme activity, each of which was individually measured after separation of the pellet and supernatant. As a lysosomal enzyme, cathepsin B activity was measured both in the pelleted and soluble fraction. Centrifugation and subsequent measurement of particulate and soluble lysosomal enzyme activity identified the rate and extent of in-vitro rupture of lysosomal enzyme containing organelles^{17,18}. Soluble cathepsin B activity was expressed as a percentage of the total activity as an index of lysosomal fragility. For the same samples, as a mitochondrial enzyme, malate dehydrogenase (MDH) activity was measured and MDH leakage from mitochondria was expressed in the same way as in the cathepsin B leakage as an index of mitochondrial fragility¹⁹.

Assays

Amylase activity was measured with blue starch (Shionogi Amylase Test-A, Shionogi, Osaka, Japan) as the substrate by the method of IRIE and co-workers²⁰. Trypsinogen was activated by purified enterokinase (Sigma Chemical Co.) and trypsin activity was measured with p-toluenesulfonyl-L-arginine methyl-ester-HCl (TAME) (Sigma Chemical Co.) as the substrate by the method of HUMMEL²¹, and one unit (U) of the activity was calculated from the standard curve made from purified trypsin (Sigma Chemical Co.). Cathepsin B activity was measured fluorometrically by the method of McDONALD and ELLIS²² with N-carbobenzoxy-arginyl-arginine- β -naphthylamide (Bachem Feinchemikalien AG, Budendorf, Switzerland) as the substrate. MDH activity was measured by the method of BERGMAYER²³, detecting the consumption rate of oxaloacetic acid and reduced diphosphopyridine nucleotide. DNA concentration was measured fluorometrically by the method of LABARCA and PAIGEN²⁴ with calf thymus DNA (Sigma Chemical Co.) as the standard.

Data presentation

The results reported in this study represent the means $\pm$ SEM for *n* determinations. Differences between groups were evaluated by ANOVA with post hoc comparison using the TUKEY procedure, and significant differences were defined as those associated with probability value (*p*) of less than 0.05. For the histological changes, WILCOXON's rank-sum test was used.

Results

Portal serum amylase, cathepsin B and MDH levels, and pancreatic water content

Only 2-hour obstruction of pancreatobiliary duct (PBDO) caused significant rises in portal serum amylase levels (18 ± 2 U/ml) and cathepsin B levels (5 ± 1 U/ml) compared with CONT group (amylase: 8 ± 1 U/ml, cathepsin B: 2 ± 1 U/ml). 2-hour obstruction of pancreatobiliary duct with caerulein stimulation (PBDO+CER), or with systemic hypotension (PBDO+SH) caused more marked and significant rises in serum amylase levels (PBDO+CER: 29 ± 3 U/ml, PBDO+SH: 32 ± 2 U/ml), cathepsin B levels (PBDO+CER: 9 ± 2 U/ml, PBDO+SH: 8 ± 2 U/ml), and MDH levels (PBDO+CER: 93 ± 19 U/ml, PBDO+SH: 108 ± 22 U/ml) compared with CONT group (MDH: 49 ± 12 U/ml). Moreover, PBDO and caerulein stimulation with systemic hypotension (PBDO+CER+SH) caused the most marked rises in serum amylase levels (42 ± 3 U/ml), cathepsin B levels (12 ± 3 U/ml) and MDH levels (146 ± 23 U/ml) (Fig. 2a, b, c).

Only 2-hour PBDO caused a slight, but not significant, increase in pancreatic water content ($77 \pm 2\%$) compared with CONT group ($74 \pm 1\%$). However, PBDO with caerulein stimulation

(PBDO+CER), or with systemic hypotension (PBDO+SH) caused significant increases (PBDO+CER: $85 \pm 2\%$, PBDO+SH: $86 \pm 2\%$) compared with CONT group. Moreover, PBDO+CER+SH caused the most significant increase ($89 \pm 2\%$) (Fig. 3).

Pancreatic amylase, trypsinogen and cathepsin B content

Only pancreatico-biliary duct obstruction (PBDO) for 2 hours caused significant rises in pancreatic amylase and trypsinogen content compared with CONT group, suggesting the congestion of pancreatic digestive enzymes induced by PBDO. PBDO+CER or PBDO+SH also caused more significant rises in pancreatic amylase and trypsinogen content. Moreover, PBDO+CER+SH caused the most marked rises in pancreatic amylase and trypsinogen content. In regard to the pancreatic cathepsin B content, there were no significant differences between these five groups (Tab. I).

Pancreatic trypsin content

Only 2-hour PBDO caused no activation of trypsinogen in the pancreas, but PBDO+CER or PBDO+SH caused a small amount of trypsinogen activation (PBDO+CER: 3 ± 1 U/mg DNA, PBDO+SH: 5 ± 2 U/mg DNA). However, PBDO+CER+SH caused the largest amount of trypsinogen activation (12 ± 3 U/mg DNA) (Fig. 4).

Pancreatic histological changes

Histological examination after 2-hour PBDO showed almost the normal histological findings. Inflammatory cell infiltration was very slight and not significant in any group except in PBDO+CER+SH group. PBDO+CER or PBDO+SH caused significant histological changes such as interstitial edema and acinar cell vacuolization compared with CONT and PBDO group (Tab. II). Moreover, PBDO+CER+SH caused the most significant and marked changes including focal acinar cell necrosis.

Subcellular distribution of cathepsin B activity

Only PBDO for 2 hours caused a significant increase of cathepsin B activity in the zymogen frac-

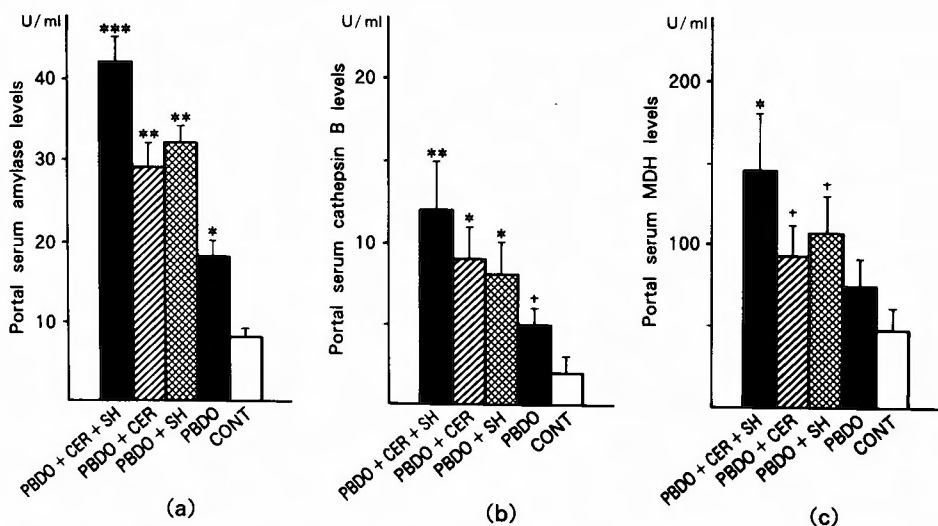


Fig. 2 Effect of short-termed pancreatico-biliary duct obstruction and exocrine stimulation with systemic hypotension on portal serum amylase (a), cathepsin B (b) and malate dehydrogenase (MDH) levels in rats. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$ and + $p < 0.05$ compared with CONT group

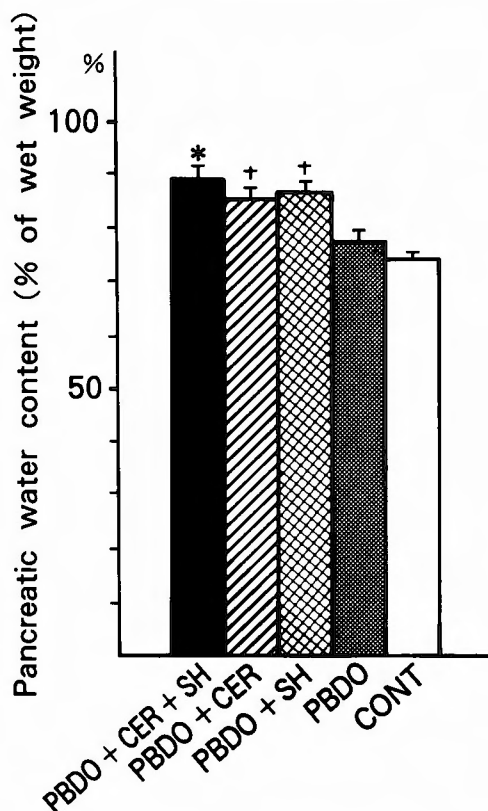


Fig. 3 Effect of short-termed pancreatobiliary duct obstruction and exocrine stimulation with systemic hypotension on pancreatic water content in rats.

⁺p<0.05 and *p<0.02 compared with CONT group

Table 1 Effect of short-termed pancreatobiliary duct obstruction (PBDO) and exocrine stimulation (CER) with systemic hypotension (SH) on pancreatic amylase, trypsinogen and cathepsin B content in rats

Group	n	Pancreatic content (U/mg DNA)		
		Amylase content	Trypsinogen content	Cathepsin B content
PBDO+CER+SH	9	593±52**	132±15**	1525±203
PBDO+CER	7	546±47*	103±12*	1308±156
PBDO+SH	7	539±38*	114±13*	1189±189
PBDO	6	455±43 ⁺	83±10 ⁺	1216±165
CONT	5	342±27	58±9	1059±121

PBDO: pancreatobiliary duct obstruction (2 hours), CER: caerulein stimulation (0.2 µg/kg.hr for 30 min), SH: systemic hypotension (30% reduction of mean arterial pressure for 30 min), All the pancreatic enzyme contents are expressed as U/mg DNA.

*p<0.02, **p<0.01 and ⁺p<0.05 compared with CONT group

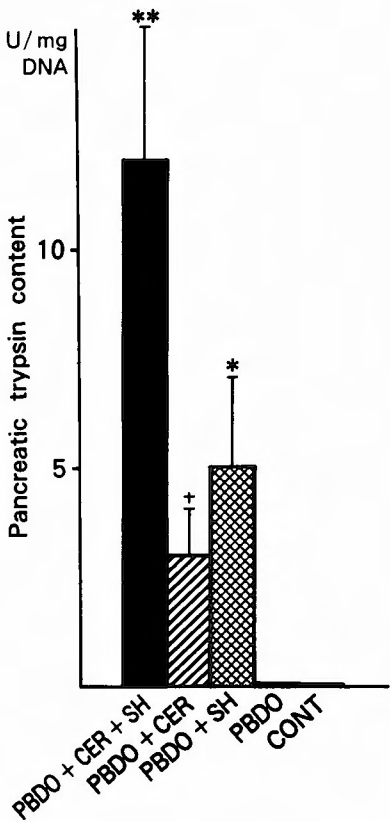


Fig. 4 Effect of short-termed pancreatico-biliary duct obstruction and exocrine stimulation with systemic hypotension on activation of trypsinogen in the rat pancreas
+p<0.05, *p<0.02 and **p<0.01 compared with CONT and PBDO group

Table 2 Effect of short-termed pancreatico-biliary duct obstruction (PBDO) and exocrine stimulation (CER) with systemic hypotension (SH) on pancreatic histological changes in rats

Group	n	Pancreatic histological changes			
		Interstitial edema	Acinar cell vavuolization	Inflammatrory cell infiltration	Acinar cell necrosis
PBDO+CER+SH	9	**3+(2-3) [2.9±0.1]	**3+(2-3) [2.7±0.2]	+1+(1-2) [1.3±0.2]	+1+(0-1) [0.9±0.1]
PBDO+CER	7	*2+(1-2) [1.9±0.1]	*2+(1-2) [1.7±0.2]	0 (0-1) [0.4±0.2]	0 (0-1) [0.1±0.1]
PBDO+SH	7	*2+(1-2) [1.6±0.2]	*1+(1-2) [1.3±0.2]	0 (0-1) [0.3±0.2]	0 (0-1) [0.3±0.2]
PBDO	6	0 (0-1) [0.3±0.2]	0 (0-1) [0.1±0.2]	0 (0-1) [0.2±0.2]	0 (0) [0]
CONT	5	0 (0) [0]	0 (0) [0]	0 (0) [0]	0 (0) [0]

PBDO: short-termed (2 hours) pancreatico-biliary duct obstruction, CER: caerulein stimulation (0.2 μg/kg.hr for 30 min), SH: systemic hypotension (30% reduction of mean arterial presuure for 30 min), The values are expressed as the means rounded to the nearest whole numbers.

(): ranges of scores, []: means±SEM of scores, *p<0.02, **p<0.01 and +p<0.05 compared with CONT group

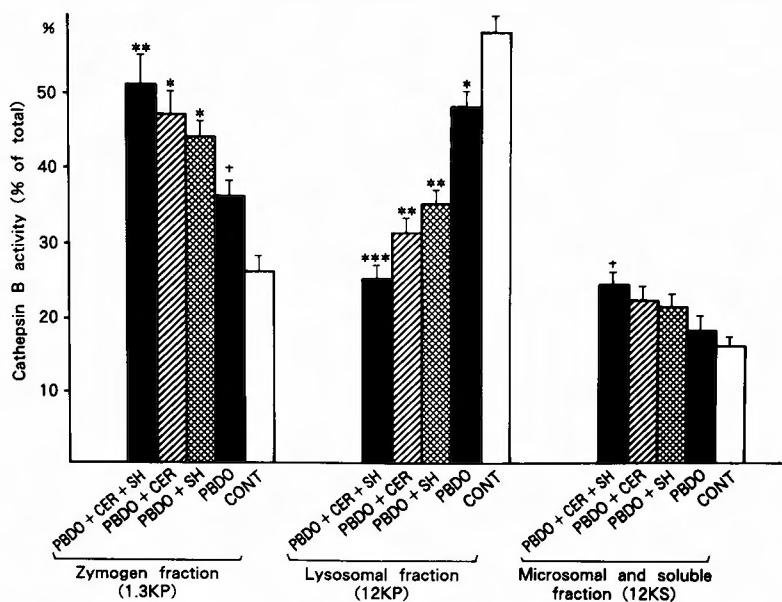


Fig. 5 Effect of short-termed pancreatico-biliary duct obstruction and exocrine stimulation with systemic hypotension on subcellular distribution of cathepsin B activity in the rat pancreas.

+ $p < 0.05$, * $p < 0.02$, ** $p < 0.01$ and *** $p < 0.001$ compared with CONT group.

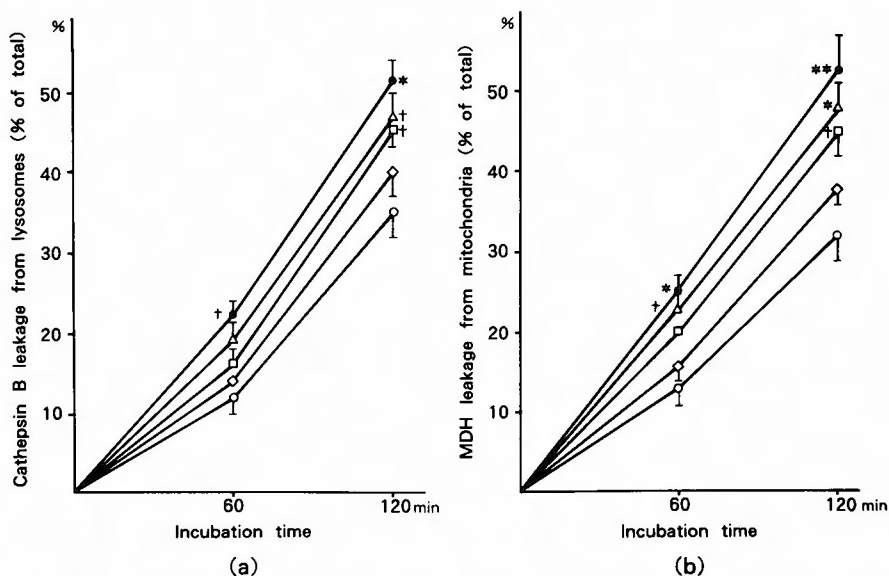


Fig. 6 Effect of short-termed pancreatico-biliary duct obstruction and exocrine stimulation with systemic hypotension on cathepsin B leakage from lysosomes (a) and MDH leakage from mitochondria (b) in rats.

+ $p < 0.05$, * $p < 0.02$ and ** $p < 0.01$ compared with CONT group.

●; PBDO + CER + SH, △; PBDO + CER, □; PBDO + SH, ◇; PBDO, ○; CONT

tion (1.3 KP: $36 \pm 2\%$) and a significant decrease in the lysosomal fraction (12 KP: $46 \pm 2\%$) compared with CONT group (1.3 KP: $26 \pm 2\%$, 12 KP: $58 \pm 2\%$). These changes indicate a shift of cathepsin B activity from the lysosomal fraction to the zymogen fraction, suggesting a redistribution of lysosomal enzyme in the subcellular fractionation of acinar cells. PBDO+CER or PBDO+SH also caused more marked shifts of cathepsin B activity (PBDO+CER: 1.3 KP: $47 \pm 3\%$, 12 KP: $31 \pm 2\%$, PBDO+SH: 1.3 KP: $44 \pm 2\%$, 12 KP: $35 \pm 2\%$). Moreover, PBDO+CER+SH caused the most significant redistribution of cathepsin B activity (1.3 KP: $51 \pm 4\%$, 12 KP: $25 \pm 2\%$). In PBDO+CER+SH group, the cathepsin B activity in the microsomal and soluble fraction (12 KS: $24 \pm 2\%$) was also significantly increased than in CONT group ($16 \pm 1\%$), suggesting the increased fragility of lysosomes in the subcellular fractionation procedure (Fig. 5).

Cathepsin B leakage from lysosomes and MDH leakage from mitochondria

Only PBDO for 2 hours caused no significant increases in cathepsin B leakage from lysosomes (60 min: $14 \pm 2\%$, 120 min: $40 \pm 3\%$) compared with CONT group (60 min: $12 \pm 2\%$, 120 min: $35 \pm 3\%$). However, PBDO+CER or PBDO+SH caused significant increases in cathepsin B leakage, particularly in the prolonged incubation time (120 min) (PBDO+CER: $47 \pm 3\%$, PBDO+SH: $45 \pm 2\%$). Moreover, PBDO+CER+SH caused the most significant cathepsin B leakage (60 min: $22 \pm 2\%$, 120 min: $51 \pm 3\%$), indicating the most accelerated lysosomal fragility. In regard to the MDH leakage from mitochondria, only PBDO for 2 hours caused no significant increases (60 min: $16 \pm 2\%$, 120 min: $38 \pm 2\%$) compared with CONT group (60 min: $13 \pm 2\%$, 120 min: $32 \pm 3\%$). PBDO+CER or PBDO+SH caused significant increases in MDH leakage (PBDO+CER: 60 min: $23 \pm 2\%$, 120 min: $48 \pm 3\%$, PBDO+SH: 60 min: $20 \pm 2\%$, 120 min: $45 \pm 3\%$). Moreover, PBDO+CER+SH caused the most marked MDH leakage (60 min: $25 \pm 2\%$, 120 min: $53 \pm 4\%$), indicating the most accelerated mitochondrial fragility (Fig. 6).

Discussion

Gallstone pancreatitis in humans appears to be precipitated by the passage of a stone through, or its incarceration in the terminal portion of the common bile duct^{11,12)}. The mechanism whereby such a stone might precipitate acute pancreatitis has been the subject of many studies and continues to be an issue of considerable controversy.

The results reported in this communication may provide an important clue to the understanding of the triggering events leading to acute pancreatitis in the pancreatico-biliary duct obstructed animals: common channel theory, since they show that single short-termed (2 hours) pancreatico-biliary duct obstruction (PBDO) caused only marginal pancreatic injuries, and also show that short-termed PBDO with exocrine stimulation, or with systemic hypotension caused more marked and significant pancreatic injuries compared with the single PBDO group. Moreover, short-termed PBDO, when superimposed by both the exocrine stimulation and systemic hypotension, caused the most marked pancreatic injuries including pancreatic focal acinar cell necrosis and intrapancreatic activation of trypsinogen. Although we have not measured the intraductal pressure after the stimulation of caerulein with pancreatico-biliary duct ligation, this dose of caerulein ($0.2 \mu\text{g/kg.hr}$) was found to cause a significant increase in both pancreatic juice volume and digestive enzymes^{25,26)}, and will cause the intraductal hypertension.

In both caerulein-induced^{3,4,6)} and diet-induced pancreatitis¹⁾, marked enlargement of zymogen-containing organelles in the cell apex and colocalization of lysosomal hydrolases and digestive en-

zymes within large cytoplasmic vacuoles have been observed. In this study, too, subcellular fractionation experiments showed that pancreatico-biliary duct obstruction leads to a redistribution of cathepsin B activity and that, as a result, lysosomal hydrolases become localized in a fraction that is rich in digestive enzymes. The colocalization of these two types of enzymes in our present study is probably the result of a defect in the normal sorting events by which digestive enzymes and lysosomal hydrolases are separated from each other as they pass through the Golgi apparatus²⁷).

In addition to within normal pancreatic acinar cells²⁸), in our recent studies, many factors such as pancreatic ischaemia²⁹) and pancreatic direct surface cooling³⁰) seem to easily induce this colocalization phenomenon, and this phenomenon seems to be a main common pathway in the pathogenesis of several types of pancreatic injuries. This colocalization could, under appropriate conditions such as exocrine stimulation and systemic hypotension, result in the intra-acinar cell activation of potentially dangerous digestive enzymes, since lysosomal enzyme, cathepsin B can activate trypsinogen^{8,9}) and trypsin can activate many other digestive enzymes. In this study, PBDO+CER+SH induced about 9% activation of trypsinogen in the pancreas.

Another suggestive finding in our study was that the lysosomal and mitochondrial fragility were accelerated after short-termed PBDO with exocrine stimulation and systemic hypotension (PBDO+CER+SH) compared with PBDO+CER or PBDO+SH group, although each of these latter groups showed a significant increase in the lysosomal and mitochondrial fragility compared with CONT group and only PBDO group. The augmented redistribution of cathepsin B and colocalization of cathepsin B with digestive enzymes in PBDO+CER+SH animals might have a special clinical importance in the etiology of gallstone pancreatitis. If the pancreatico-biliary ductal system is obstructed, the exocrine pancreas is stimulated by food intake, and systemic hypotension is superimposed, there will be more marked redistribution of lysosomal enzymes and colocalization of lysosomal hydrolases with digestive enzymes in acinar cells compared with single pancreatico-biliary duct obstruction, suggesting the more dangerous situation for acinar cells through intracellular activation of trypsinogen or increased subcellular organellar fragility.

At present, we can not explain the mechanism whereby pancreatico-biliary duct obstruction induces the lysosomal and mitochondrial fragility. However, this colocalization phenomenon, and possible intracellular activation of trypsinogen and digestive enzymes, seem to play some role in the pathogenesis of these subcellular organellar fragility.

Although the currently reported studies support the hypothesis that pancreatico-biliary duct obstruction may be important in the pathogenesis of gallstone pancreatitis, it is clear from these as well as other studies that duct obstruction alone is not sufficient to cause the more definite morphological changes in the pancreas. Clearly, other events must occur, such as intraductal hypertension and systemic hypotension in this study, if the changes induced by common channel obstruction are to lead to the injuries seen in more severe pancreatitis. Studies designed to identify and clarify those events are of great importance and the next step to this study, because they are likely to be the ultimate determinants of severity of pancreatitis and to advance our knowledge of the pathogenesis of this disease. Even more important, they may lead to therapeutic advances.

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和文抄録

胆石性膵炎の新しい実験モデル：ラットにおける短期間の 総膵胆管閉塞，外分泌刺激および低血圧について

京都大学医学部 第一外科学教室

平野 鉄也，真辺 忠夫

ラットにおいて2週間の総膵胆管閉塞下，セルレイン投与（0.2 $\mu\text{g/kg}\cdot\text{hr}$ ）による外分泌刺激および脱血による低血圧（平均動脈圧を30%低下）の膵外分泌およびその影響を検討した。このモデルにおいては，門脈血清アミラーゼ，カテプシンBおよび林檎酸脱水素酵素レベルが単に総膵胆管閉塞や閉塞＋外分泌刺激や閉塞＋低血圧群に比べ有意に上昇するのみならず，ライ

ソゾーム酵素のチモーゲン分画への再分布，ライソゾームとミトコンドリア脆弱性の亢進が観察された。これらの結果は，今回のこのモデルがヒトにおける胆石性膵炎の実験モデルとして有用であるのみならず，胆石性膵炎の病態生理におけるライソゾーム酵素の再分布と細胞内小器官の脆弱性の亢進の重要な役割が示唆された。