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"Cocktail" Therapy for Acute Pancreatitis: Combined Therapy of Protease Inhibitor, Xanthine Oxidase Inhibitor and Platelet Activating Factor Antagonist in Rat Caerulein-Induced Pancreatitis

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Abstract

A supramaximal dose of caerulein (5 μg/kg・hr for 3.5 hours) caused an acute pancreatitis with marked hyperamylasemia and intense interstitial edema in rats. In this model of pancreatitis, the redistribution of lysosomal enzyme in acinar cells as well as the increased lysosomal and mitochondrial fragility were also observed.

The combined therapy of a low molecular weight protease inhibitor, FOY, a synthetic platelet activating factor (PAF) antagonist, CV 6209, and a xanthine oxidase inhibitor, allopurinol produced more significant improvements in all the parameters examined than the therapy of any only one of these three agents, each only one therapy exerting a partial significant protective effect.

These results indicate that several factors, such as unknown proteases activities, PAF and oxygen-derived free radicals may be involved in the pathogenesis of pancreatic injuries in this caerulein-induced pancreatitis. These results also suggest that such a combined therapy of different kinds of agents, whose therapeutic mechanisms are also different, is useful in the clinical treatment of acute pancreatitis.

Introduction

It has been reported that edematous pancreatitis with marked hyperamylasemia can be induced by infusing rats with a dose of the cholecystokinin-pancreozymin analogue caerulein in excess of that which causes maximum rates of digestive enzyme secretion from the pancreas[1-5]. In this caerulein-induced pancreatitis, several factors such as oxygen-derived free radicals[6-8], or unknown protease activities[9] have also reported to be closely related to the pathogenesis of pancreatic injuries. Changes in pancreatic microciculation during other models of pancreatitis have also been documented[10-12]. In this caerulein-induced pancreatitis, since increased vascular permeability can be observed in the form of pancreatic interstitial edema, involvement of platelet activating factor (PAF) as a mediator in acute pancreas can not be ruled out. In fact, there has been a report regarding an important

Key words: Caerulein-induced pancreatitis, Cathepsin B, Lysosome, Mitochondria, PAF, Protease inhibitor, Xanthine oxidase.

索引用語：セルレイン誘起膵炎，カテプシンB，ライソゾーム，ミトコンドリア，血小板活性化因子．蛋白分解酵素阻害剤，キサンチン酸化酵素．

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pathogenic role of PAF in pancreatic duct ligation model of pancreatitis[13]. All these reports suggest that several factors are involved in the pathogenesis of acute pancreatitis.

On the other hand, although there have been some reports concerning the partial protective effects of protease inhibitors[14,15], or xanthine oxidase inhibitor[16] against pancreatic injuries in this caerulein-induced pancreatitis, until now, there have been few reports about the protective effect of PAF antagonist, or combined therapy of such a different kind of agent against caerulein-induced pancreatitis. Furthermore, in this caerulein-induced pancreatitis, redistribution of lysosomal enzymes and colocalization of lysosomal enzymes with digestive enzymes have been reported to occur[17-20]. This redistribution, combined with the colocalization of these two types of enzymes in the same subcellular compartment has been also reported to play a triggering role in the development of acute pancreatitis[21,22], since the lysosomal enzyme, cathepsin B can activate trypsinogen[23-25] and trypsin can activate many other pancreatic digestive enzymes.

In this study, we report the results of studies evaluating the protective effects of the combined therapy of a new potent synthetic protease inhibitor, FOY, well known xanthine oxidase inhibitor, allopurinol, and a new synthetic PAF antagonist, CV 6209, against the redistribution of cathepsin B and subcellular organellar fragility induced by a supramaximal dose of caerulein.

Materials and Methods

Animal preparation :

Male Wistar rats (180–250 g; Shizuoka Experimental Animals, Shizuoka, Japan) were used for the caerulein-induced pancreatitis experiments. They were housed in light-dark cycle regulated (light; 5:00–17:00) and temperature controlled (23±3°C) animal quarters, allowed free access to rat chow (Purina Rodent Chow, Purina Mills Inc., St. Louis, MO, U.S.A.) and tap water, and acclimatized to standard laboratory conditions for at least 4 days. The rats were maintained throughout the study in accordance with the guidelines of the Committee on Animal Care of Kyoto University. After a 16-hour fast, a V-3 catheter (Insul-Tab, Inc., Woburn, MA, U.S.A.) was placed in the right jugular vein through to the superior vena cava (S.V.C.) under light intraperitoneal sodium pentobarbital anesthesia (25 mg/kg). Its patency was maintained by a continuous infusion of heparinized (30 IU/ml) 150 mM NaCl solution at a rate of 0.21 ml/hr by using an infusion pump (Harvard Apparatus, Suoth Natick, MA, U.S.A.). The catheter was tunneled beneath the skin on the back, taken out at the base of the tail, and fixed to the cage by a stainless steel coil, through which the catheter was taken out. The rats were housed in shoe-box cages in each animal and allowed 12 hours to recover from the effects of anesthesia and surgery. During this time, each rat was kept free access to tap water and rat chow before the next caerulein infusion. Animals were susequently divided into the following 6 experimental groups (Table 1); (a) 20 control rats (CONT)—infused only with heparinized saline at a rate of 0.58 ml/hr for 3.5 hours, (b) 32 caerulein rats (CER)—infused with heparinized saline as above (0.58 ml/hr for 3.5 hours) but with caerulein added to the infusate such that each animal received 5 μg/kg · hr, (c) 28 caerulein plus FOY rats (FOY)—identical to the CER group but FOY (50 mg/kg) was infused throughout the 3.5 hours of caerulein infusion, (d) 28 caerulein plus alloprinol rats (Allop)—identical to the CER group but allopurinol was injected intravenously (20 mg/kg) just before the caerulein infusion and at 2 hours after the beginning of the caerulein infusion, (e) 28 caerulein plus CV 6209 rats (CV)—identical to the CER group but CV 6209 was injected intravenously (1 mg/kg) just before the caerulein infusion and at 2 hours after the

Table 1 Treatment groups

Group	n	Treatment
CONT	20	Normal saline
CER	32	Caerulein
FOY	28	Caerulein + FOY
Allop	28	Caerulein + allopurinol
CV	28	Caerulein + CV 6209
COMB	36	Caerulein + FOY + allopurinol + CV 6209

beginning of the caerulein infusion, (f) 36 caerulein plus combined therapy rats (COMB)—identical to the CER group but FOY, allopurinol and CV 6209 were given as in the FOY, Allop and CV group. During these infusions, tap water and rat chow were removed from the cages. Caerulein was purchased from Kyowa, Tokyo, Japan (CEOSUNIN Injection) and FOY was a kind donation from Ono Pharmaceutical Company, Osaka, Japan. Allopurinol was purchased from Sigma Chemicals, St. Louis, MO, U.S.A. and CV 6209 was a kind donation from Takeda Chemical Industries, Osaka, Japan.

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

At selected times after 3.5-hour infusions, rats in each group were sacrificed by a large dose of intravenous pentobarbital. After blood samplings from the portal trunk for the determination of serum amylase, cathepsin B and MDH, as a pancreatic digestive, lysosomal and mitochondrial enzyme, respectively, portions of the pancreas were quickly removed and trimmed of fat. About half of the pancreas was used for the quantitiation of pancreatic edema by comparing this weight obtained immediately after sacrificing the rats (wet weight) to that of the same sample after incubation at 150°C for 48 hours in the desiccator (ISOTEMP^{R}, Fischer Scientific, Fair Lawn, NJ, U.S.A.) (dry weight). Another half of the pancreas was homogenized in 5 ml of ice-cold phosphate-buffered saline (pH 7.4) containing 0.5% Triton X-100 (Fischer Scientific) using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Westbury, NY, U.S.A.) for the determination of pancreatic amylase and cathepsin B content. Amylase and cathepsin B activities, as well as deoxyribonucleic acid (DNA) concentration were measured in the resulting supernatant after low speed centrifugation (150 × g at 4°C for 15 min). These two enzymes in the pancreatic tissue were expressed as U/mg DNA.

Histological changes :

For each group, samples of rat pancreas were fixed by immersion in phosphate-buffered 10% neutral formalin. After paraffin embedding, sectioning and staining with hematoxylin-eosin, the sections were examined by a independent observer. Histological changes such as acinar cell vacuolization, interstitial edema and inflammatory cell infiltration were graded on a scale between 0 to 4+ (0; no changes, 4+; maximal changes).

Distribution of cathepsin B and amylase activity in the subcellular fractionation :

Other new rats in each group were used in this subcellular fractionation study. The portions of the pancreas were homogenized in 6 ml of ice-cold 5 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer (pH 6.5) (Sigma Chemicals) containing 1 mM $MgSO_4$ and 250 mM sucrose with 3 up- and -down strokes of the Dounce homogenizer (Wheaton, Millville, NJ, U.S.A.). The homogenate

was separated into its various subcellular fractions by differential centrifugations. The protocol was originally developed by TARTAKOFF and JAMIESON[26] and modified by SALUJA and co-workers[18] for the study of rat pancreas. Briefly, the resulting homogenate was centrifuged (150×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 components measured. This supernatant was again centrifuged (1300×g at 4°C for 15 min) to obtain a zymogen granule-enriched pellet (1.3 KP). The supernatant was centrifuged (12000×g at 4°C for 12 min) to yield a lysosome- and mitochondria-enriched pellet (12 KP). The remaining supernatant was microsomal and soluble fraction (12 KS). The various pellets obtained during fractionations were individually resuspended in 2 ml of 5 mM MOPS buffer. The amylase and cathepsin B activity in each fraction were measured and expressed as a percent of the total activity as an index of the distribution of lysosomal and digestive enzyme in pancreatic acinar cells.

Cathepsin B leakage from lysosomes and malate dehydrogenase (MDH) leakage from mitochondria :

For other new rats of each group, at selected times, the rats were sacrificed and portions of the pancreas were removed, trimmed of fat and homogenized in ice-cold 5 mM MOPS buffer as described above. This homogenate was centrifuged (150×g at 4°C for 10 min) to remove unbroken cells and debris. The resulting supernatant was centrifuged twice (1300×g at 4°C for 15 min and 12000×g at 4°C for 12 min) to obtain a combined lysosome-mitochondria-enriched pellet. This pellet, arbitrarily considered to contain 100% of the lysosomal and mitochondrial enzymes activities, was suspended in 5 mM MOPS buffer and incubated for varying intervals (30, 60 and 90 min) at 25°C in a shaking bath under room air. The samples were then re-centrifuged (12000×g at 4°C for 12 min) to separate the particulate from the soluble lysosomal and mitochondrial enzymes activities, 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. Soluble cathepsin B activity was expressed as a percent of the total cathepsin B activity as an index of lysosomal fragility. For the same samples, MDH activity, as a mitochondrial enzyme, was measured and MDH leakage from mitochondria was expressed in the same way as in the cathepsin B leakage as an index of mitochondrial fragility.

Assays :

Amylase activity was measured by the method of BERNFELD[27] with soluble starch as the substrate, and cathepsin B activity was measured fluorometrically by the method of MCDONALD and ELLIS[28] with CBZ-arginyl-arginine-β-naphthylamide (Bachem Feinchemikalien AG, Budendorf, Switzerland) as the substrate. Deoxyribonucleic acid (DNA) concentration was measured fluorometrically by the method of LABARCA and PAIGEN[29] with calf thymus DNA (Sigma Chemicals) as the standard. Malate dehydrogenase (MDH) activity was measured spectrometrically by the method of BERGMEYER[30].

Data presentation and analysis :

The results reported in this study represent the means±SEM for n determination. Differences between groups were evaluated by ANOVA with the Tukey method except for the histological data. For evaluating the histological changes, Wilcoxon rank-sum test was used and significant differences were defined as those associated with a probability value (p) of less than 0.05 ($p<0.05$).

Results

Portal serum amylase, cathepsin B and malate dehydrogenase levels, and pancreatic water, amylase and cathepsin B content :

In agreement with previous reports, infusion of a supramaximal dose of caerulein (5 μg/kg · hr for 3.5 hours) caused a significant increase in serum amylase levels compared with the control group. A supramaximal dose of caerulein also caused a significant increase in both cathepsin B and malate dehydrogenase levels, considered to represent the increased fragility of lysosomes and mitochondria in this model of pancreatitis (Table 2). Only FOY, or allopurinol or CV 6209 therapy exerted a significant protective effect against these changes, FOY having the most protective effect among these three agents. However, the combination therapy of these three agents exerted the more protective effect than any only one therapy. A supramaximal dose of caerulein caused a significant increase in pancreatic water content, considered to be a pancreatic edema. Only FOY, or allopurinol or CV 6209 therapy exerted a significant protective effect against this pancreatic edema, the combination therapy of these three agents having more significant protective effect than any only one therapy (Table 3). A supramaximal dose of caerulein also caused a significant increase in pancreatic amylase content, considered to represent a congestion of pancreatic digestive enzymes. Only FOY, or allopurinol or CV therapy exerted a significant protective effect against this congestion of

Table 2 Effect of a supramaximal dose of caerulein on the portal serum amylase, cathepsin B and malate dehydrogenase (MDH) levels in rats

Group	n	Portal serum enzyme levels		
		Amylase (U/ml)	Cathepsin B (U/ml)	MDH (U/ml)
CONT	5	8±1*	2.1±0.3*	55±12*
CER	8	35±3	8.7±0.6	182±29
FOY	7	19±3**	4.3±0.5**	96±21**
Allop	7	23±2***	5.0±0.9***	112±20***
CV	7	20±3**	4.7±0.8***	118±24
COMB	9	10±2*	2.8±0.4*	61±18*

The symbol in each group is the same as in Table 1.
*; $p<0.01$, **; $p<0.02$, and ***; $p<0.05$ compared with the CER group.

Table 3 Effect of a supramaximal dose of caerulein on the pancreatic water, amylase and cathepsin B content in rats

Group	n	Pancreatic water content (% of wet weight)	Pancreatic amylase content (U/mg DNA)	Pancreatic cathepsin B content (U/mg DNA)
CONT	5	74±1**	403±33*+	1124±175
CER	8	87±2	685±47	1591±239
FOY	7	79±2***	502±38**	1216±192
Allop	7	81±2***	517±34**	1284±207
CV	7	80±2***	532±37**	1305±184
COMB	9	76±2**	412±29*+	1173±198

The symbol in each group is the same as in Table 1.
*; $p<0.01$, **; $p<0.02$, and ***; $p<0.05$ compared with the CER group, +; $p<0.05$ compared with the Allop and the CV group.

amylase in the pancreas, the combination therapy of these three agents having more significant protective effect than any only one therapy. As regards the pancreatic cathepsin B content, a supramaximal dose of caerulein caused a slight increase, no significant differences observed among these 6 groups.

Histological changes :

In the samples taken from the supramaximally stimulated rats, histological changes such as remarkable interstitial edema, acinar cell vacuolization and mild inflammatory cell infiltration were observed. Only FOY, or allopurinol or CV 6209 therapy exerted a partial significant protective effect against these histological changes, such as FOY in acinar cell vacuolization and interstitial edema, alloprinol in interstitial edema, and CV 6209 in interstitial edema (Table 4). However, the combination therapy of these three agents exerted the most significant protective effect against these histological changes.

Distribution of cathepsin B and amylase activity in the subcellular fractionation :

A supramaximal dose of caerulein caused a remarkable shift of cathepsin B activity from the lysosomal fraction (12 KP) to the heavier zymogen fraction (1.3 KP) (12 KP; 28±2%, 1.3 KP; 48±3%) compared with the control group (12 KP; 56±2%, 1.3 KP; 24±2%) and this shift indicated the redistribution of lysosomal enzyme in pancreatic acinar cells (Fig. 1). Only FOY, or allopurinol or CV 6209 therapy exerted a significant protective effect against this redistribution (FOY: 12 KP; 41±2%, 1.3 KP; 35±2%, Allop: 12 PK; 39±2%, 1.3 KP; 38±2%, CV: 12 KP; 38±3%, 1.3 KP; 40±2%). However, the combination therapy of these three agents had the most significant protective effect (12 KP; 53±3%, 1.3 KP; 26±2%). Approximately 40% of the amylase activity of the control group was located in the zymogen fraction (1.3 KP). Approximately another 40% of the amylase activity remained in the microsomal and soluble fraction (12 KS). This microsomal and soluble fraction activity was considered to represent the amylase activity released

Table 4 Effect of a supramaximal dose of caerulein on the pancreatic histological changes in rats

Group	n	Pancreatic histological changes		
		Acinar cell vacuolization	Interstitial edema	Inflammatory cell infiltration
CONT	5	0^{*+} [0] (0)	0^{*++} [0] (0)	0^{***} [0] (0)
CER	8	3^{+} [2.6±0.2] (2–3)	3^{+} [3.1±0.3] (2–4)	1^{+} [1.3±0.2] (1–2)
FOY	7	1^{***+} [1.4±0.2] (1–2)	1^{**+} [1.4±0.2] (1–2)	0 [0.4±0.3] (0–2)
Allop	7	2^{+} [2.3±0.3] (1–3)	2^{***+} [2.1±0.3] (1–3)	1^{+} [0.6±0.3] (0–2)
CV	7	2^{+} [2.0±0.3] (1–3)	1^{**+} [1.4±0.3] (1–3)	0 [0.4±0.2] (0–1)
COMB	9	0^{*+} [0.2±0.1] (0–1)	0^{*++} [0.3±0.2] (0–1)	0^{***} [0.1±0.1] (0–1)

The symbol in each group is the same as in Table 1. The values are expressed as the mean rounded to the nearest whole number. [ ]; mean±SEM, ( ); ranges of values, *; $p<0.01$, **; $p<0.02$, and ***; $p<0.05$ compared with the CER group, +; $p<0.02$ compared with the Allop and CV group, and $p<0.05$ compared with the FOY group, ++; $p<0.02$ compared with the Allop group and $p<0.05$ compared with the FOY and CV group.

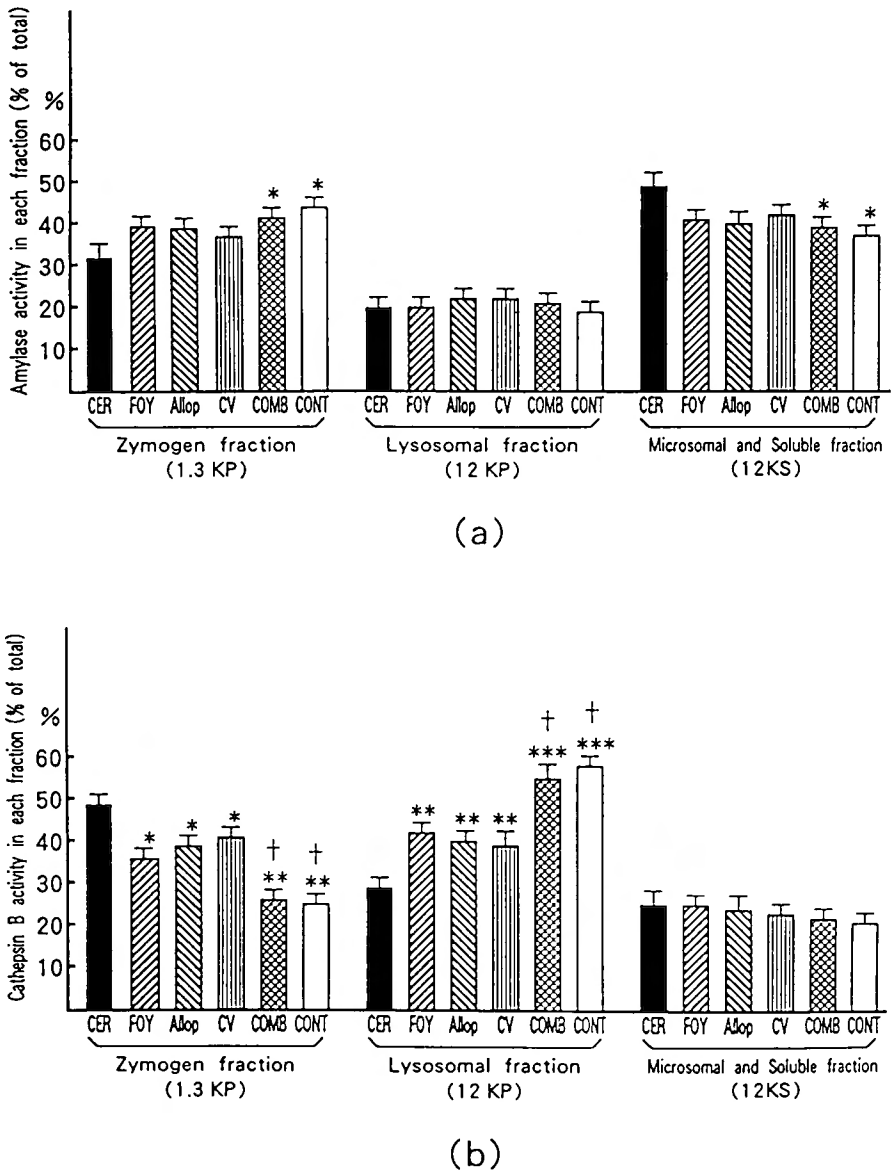

Fig. 1 Effect of a supramaximal dose of caerulein on the distribution of amylase (a) and cathepsin B activity (b) in rat pancreatic acinar cells. CER; caerulein group, FOY; caerulein plus FOY group, Allop; caerulein plus allopurinol group, CV; caerulein plus CV 6209 group, COMB; caerulein plus FOY, allopurinol and CV 6209 group, CONT; only saline group (*; $p<0.05$ compared with the CER group, **; $p<0.02$ compared with the CER group, ***; $p<0.01$ compared with the CER group, $^{+}$; $p<0.05$ compared with the FOY, Allop and CV group).

Table 5 Effect of a supramaximal dose of caerulein on the cathepsin B leakage from lysosomes and malate dehydrogenase (MDH) leakage from mitochondrias in rats

Group	n	Cathepsin B leakage from lysosomes (% of total)			MDH leakage from mitochondrias (% of total)		
		Incubation time (min)			Incubation time (min)		
		30	60	90	30	60	90
CONT	5	6±1	12±2***	21±2**+	8±2	15±2	25±2**
CER	8	10±1	23±2	39±3	13±2	26±3	42±3
FOY	7	8±1	17±2	29±2***	10±2	21±2	32±2***
Allop	7	9±1	19±2	31±2***	10±2	22±2	33±2***
CV	7	9±2	29±2	33±3	11±2	23±2	34±2
COMB	9	7±1	14±2***	23±2**++	9±2	18±2***	28±2**

The symbol in each group is the same as in Table 1.
; $p<0.02$ and *; $p<0.05$ compared with the CER group, +; $p<0.05$ compared with the Allop and CV group, ++; $p<0.05$ compared with the CV group.

from the fragile organelles which ruptured during tissue processing. A supramaximal dose of caerulein caused the amylase activity in this microsomal and soluble fraction to increase significantly (48±3%) compared with the control group (37±2%) and the amylase activity in the zymogen fraction to decrease significantly (32±3%) compared with the control group (44±2%) (Fig. 1). Treatment with only FOY, or allopurinol or CV 6209 exerted a slight protective effect, but not significantly (FOY: 12 KS; 41±2%, 1.3 KP; 39±2%, Allop: 12 KS; 40±3%, 1.3 KP; 38±2%, CV: 12 KS; 42±2%, 1.3 KP; 36±2%). However, the combined therapy of these three agents had the most protective effect (12 KS; 38±2%, 1.3 KP; 41±2%).

Cathepsin B leakage from lysosomes and malate dehydrogenase (MDH) leakage from mitochondrias

A supramaximal dose of caerulein caused an increase in cathepsin B leakage from lysosomes, and this accelerated leakage of lysosomal enzyme is due to the increased lysosomal fragility in caerulein-induced pancreatitis. Only FOY, or only allopurinol therapy exerted a significant protective effect only in the prolonged incubation time (90 min) and only CV 6209 therapy had no significant protective effect (Table 5). However, the combined therapy of these three agents had the most significant protective effect against this lysosomal fragility. A supramaximal dose of caerulein also caused an increase in the MDH leakage from mitochondrias. This accelerated leakage is due to the increased mitochondrial fragility in caerulein-induced pancreatitis. As in the lysosomal fragility, only FOY, or allopurinol therapy exerted a significant protective effect only in the prolonged incubation time (90 min) (Table 5). Only CV 6209 therapy had no significant protective effect. However, the combined therapy of these three agents exerted the most significant protective effect.

Discussion

Many studies in rodents[1–5] have shown that either a continuous intravenous infusion, or a subcutaneous injection of large pharmacological doses of caerulein, or caeruletide produce interstitial edematous acute pancreatitis. This model of acute pancreatitis is characterized by marked elevations in serum levels of pancreatic digestive enzymes and histological changes such as intense interstitial edema, acinar cell vacuolization and mild inflammatory cell infiltration. The rapid onset

and high reproducibility have made this secretagogue-induced model attractive for biochemical and histological studies of factors involved in the early development and progression of acute pancreatitis. This model has also been used recently to evaluate the efficacy of therapeutic agents in the treatment of acute pancreatitis. We used this model in our present study, since it is a mild type of pancreatitis and seems to be useful in studying the various changes and the triggering events in the pathogenesis of acute pancreatitis.

The nature of the caerulein-induced lesion which eventually progresses to remarkable interstitial edema of the pancreas has not been elucidated. However, there is a evidence of altered vascular permeability early in the disease process and there seems to be some possibility about the involvement of PAF in this vascular permeability. In this secretagogue-induced pancreatitis, colocalization of lysosomal hydrolases and pancreatic digestive enzymes in large cytoplasmic vacuoles has been reported to precede the appearance of cell injuries[21,22]. The distribution of cathepsin B is altered so that a smaller fraction of the total is confined to the lysosomes than to the zymogen fractions. Furthermore, an accelerated leakage of cathepsin B from the lysosomes and an accelerated leakage of MDH from the mitochondrias were found during in-vitro incubation.

The lysosomal hydrolase, cathepsin B, can activate trypsinogen[23–25] and trypsin can activate many other pancreatic digestive enzymes. The colocalization of cathepsin B with digestive enzymes in the same subcellular compartment and the leakage of cathepsin B from the lysosomes might therefore result in intracellular digestive enzymes activation and autodigestion of acinar cells, exerting harmful effects on the subcellular organelles such as mitochondria, and may be of importance in the etiology of this form of pancreatitis.

On the other hand, there have been many reports[31–35] regarding the crucial roles of proteases in the pathogenesis of acute pancreatitis both in experimental and clinical settings and a few reports regarding the possible involvement of oxygen-derived free radicals in the disease[6–8]. Thus, it seems to be of interest to evaluate the protective effects of a combined therapy of such a different kind of agent as PAF antagonist, CV 6209, xanthine oxidase inhibitor, allopurinol, and a low molecular weight protease inhibitor, FOY, on the exocrine pacreas, in the early stages of the disease, since, in general, the use of aprotinine and other old types of protease inhibitors showed no significant protective effects in the clinical treatment of acute pancreatitis[36], and many factors seem to be related to the pathogenesis of acute pancreatitis.

In this communication, we report that such a combined therapy of several kinds of agents produced the improvement in almost all of the tested parameters. The therapy with any only one of these agents had partial significant protective effects, and only slight beneficial effects were noted. FOY (gabexate mesilate; Ethyl-4-(6-guanidinohexanoyloxy)benzoate methanesulfonate) is a new sythetic agent, which inhibits a number of key enzymes such as trypsin, phospholipase A_2, kallikrein, plasmin, thrombin and C_{1r} and C_{1s} esterases[37]. The relatively small molecular weight (417 daltons) has suggested that it may penetrate into the pancreatic acinar cells from the vasculature. This encouraged studies evaluating its protective effects against pancreatitis. We used the dose of 50 mg/kg · hr, since the most significant protective effects have been observed previously at this dose in caerulein-induced pancreatitis (data not shown). CV 6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxycarbonyl)aminomethyl]-1-ethylpyridinium chloride) has been reported to inhibit PAF in-vivo and in-vitro[38] and allopurinol is a well-known xanthine oxidase inhibitor, inhibiting the generation of oxygen-derived free radicals. We used the dose of 1 mg/kg of CV 6209, since this dose has been reported to improve the survival rate of PAF-induced death in mice[38] and we

used the dose of 20 mg/kg of allopurinol, since this dose has been reported to be partial significantly protective against caerulein-induced pancreatitis[16].

As a result of this study about in-vivo and in-vitro changes, we conclude that the redistribution phenomenon (and presumably the colocalization of lysosomal hydrolases with digestive enzymes), the accelerated cellular and subcellular organellar fragility are closely related to several factors such as some unknown protease activities, which are susceptible to inhibition by FOY, PAF, which can be inhibited by CV 6209, and oxygen-derived free radicals, whose generation can be inhibited by allopurinol.

The favorable results of FOY, CV 6209 and allopurinol on the cellular and subcellular organellar fragility in the early stages of caerulein-induced pancreatitis may justify an evaluation of such a combined "cocktail" therapy in a clinical study.

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和文抄録

ラットセルレイン膵炎でのプロテアーゼ阻害剤，キサンチン酸化酵素阻害剤と PAF 拮抗剤の併用療法について

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ラットにおいて高濃度のセルレイン（5 μg/kg・hrにて3.5時間）の投与により，高アミラーゼ血症と膵浮腫を特徴とする急性膵炎が誘起され，膵腺房細胞内でのライソゾーム酵素の再分布とともにライソゾームとミトコンドリアの脆弱性も観察された．プロテアーゼ阻害剤の FOY, PAF 拮抗剤の CV 6209 とキサンチン酸化酵素阻害剤であるアロプリノールの3者併用療法はこの膵炎にて，どの3者の単独療法よりも有意に膵保護効果を示した．これらの結果は，セルレイン膵炎の病態にて，何らかのプロテアーゼ活性や，PAF や活性酸素種といった，いくつかの要素が関与していることを示唆するとともに，臨床での急性膵炎治療においてこの様な，いくつかの作用機序が異った薬剤の併用療法の有用性をも示唆するものであった．