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Combined Therapy of a Cephalosporin, Shiomarin and a New Potent Protease Inhibitor, E3123 in Rat Taurocholate-Induced Pancreatitis

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Abstract

The role of infectious factors in the pathogenesis of acute pancreatitis and the protective effect of combined therapy with a new potent synthetic protease inhibitor, E3123, and a new potent synthetic cephalosporin, Shiomarin (SM) were examined in rat acute pancreatitis. Sodium taurocholate injection into the pancreatico-biliary duct of rats caused severe pancreatitis with a high mortality rate, characterized by hyperamylasemia, high amylase activity in ascitic fluid, and hyperendotoxemia and a high serum level of fibrin degradation products (FDP), redistribution of cathepsin B from the lysosomal fraction to the zymogen fraction. In rats with E3123 infusion almost all parameters were improved, including mortality rate, serum and ascitic fluid amylase levels, plasma endotoxin and serum FDP levels, and distribution of lysosomal enzyme. But combination therapy with E3123 and SM was significantly more protective than E3123 therapy alone.

These results indicate that infection plays an important role in the development of severe pancreatitis and that combination therapy with a new synthetic protease inhibitor and a new potent antibiotic may be useful in the treatment of severe pancreatitis.

Introduction

Morphological and biochemical studies have shown that, in the early stage of two forms of experimental pancreatitis, diet-induced[1,2] and secretagogue-induced[3,4] pancreatitis there is colocalization of digestive enzymes with lysosomal hydrolases inside large cytoplasmic vacuoles[5], and lysosomal enzymes are redistributed from the lysosome-rich to the zymogen-rich fraction. Since cathepsin B, a lysosomal enzyme, can activate trypsinogen[6,7] and trypsin can activate the other pancreatic digestive enzymes, the colocalization of digestive enzymes with lysosomal hydrolases could lead to the activation of intracellular digestive enzymes. Thus, lysosomal enzymes seem to play an important role in the pathogenesis of acute pancreatitis. Another model of severe experimental pancreatitis, bile salt-induced pancreatitis, has been described[8,9]. However, there have been no reports so far on the redistribution of lysosomal enzyme or on lysosomal fragility in this model. Moreover, infection has often been reported to affect the mortality rate in severe clinical pancreatitis[10–13], and infection also seems to play an important role in the development of acute pancreatitis.

Key words: E3123, Lysosomal enzyme, Shiomarin, Cathepsin B, Taurocholate-induced pancreatitis.
索引用語：E3123, ライソゾーム酵素, シオマリン, カテプシンB, タウロコール酸誘起膵炎.

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There have been several reports on the protective effects of new potent synthetic low molecular weight protease inhibitors in both clinical and experimental pancreatitis[2,14-16]. E3123 (4-12-succinimidoethylthio-phenyl 4-guanidinobenzoate methanesulfonate) is one of these, and it has been found that it inhibits several key enzymes in pancreatitis[17].

In this study, we used a rat model of severe sodium taurocholate-induced acute pancreatitis to evaluate the protective effects of E3123 in combination with a new potent synthetic cephalosporin, Shiomarin.

Materials and Methods

About 300 male Wister rats weighing about 350 g (Shizuoka Experimental Animals, Shizuoka, Japan) were allowed to become acclimated to standard laboratory conditions for at least 4 days. The rats were maintained throughout the study in accordance with the guidelines of the Animal Care Commitee of Kyoto University, Kyoto, Japan. Experiments were begun after a 16-hour fast, starting at 8:00–10:00 AM to rule out the effects of circadian rhythm on the exocrine pancreas.

The rats were divided into the following 3 groups: a) Sodium taurocholate-induced pancreatitis control group (120 rats) (CONT group)—After a 16-hour fast, general anesthesia was induced by intraperitoneal pentobarbital (25 mg/kg), and a venous line was established through a catheter (V-3 catheter, Insul-Tab, Woburn, MA, U.S.A.) into the superior vena cava via the right external jugular vein. The catheter was tunneled beneath the skin of the back to the root of the tail and fixed to the cage by a stainless steel coil through which the catheter was brought out. Before fixation to the cage, laparotomy was performed through a small upper midline incision, and a duodenal loop was pulled out through the operative wound. A small incision was made in the duodenal wall opposite the papilla of Vater, and a catheter (PE 10, Clay Adams, Parsippany, NJ, U.S.A.) was introduced via the papilla of Vater into the pancreatico-biliary duct. Under very gentle pressure, 0.6 ml of 5% sodium taurocholate in saline (Calbiochem, La Jalla, CA, U.S.A.) was injected. The duodenal incision was closed with 5–0 Dexon, and the abdomen was also closed. After surgery the animals were infused with heparinized (20 U/ml) lactate-Ringer solution at a speed of 0.58 ml/hr with an infusion pump. The rats were not fed but were given free access to tap water. b) E3123 treated group (90 rats) (E group)—Before the injection of 5% taurocholate into the pancreatico-biliary duct, E3123 was infused in a dose of 2 mg/kg·hr for 1 hour; after the injection of 5% taurocholate, E3123 was infused constantly in the same dose at at a speed of 0.58 ml/hr in heparinized saline. c) E3123 plus Shiomarin-treated group (90 rats) (S group)—E3123 infusion were performed as in the E group, but after the injection of 5% taurocholate into the pancreatico-biliary duct, Shiomarin was infused in a dose of 20 mg/kg·hr for 60 minutes in 0.58 ml of saline every 8 hours, starting just after the injection of 5% taurocholate. In addition to these 3 groups, normal rats were also tested as a pure control group.

In 30 rats from each groups the mortality rate was recorded 12, 24, 36 and 48 hours after the induction of taurocholate pancreatitis.

From the same animals 0.4 ml of blood was drawn 12, 24, 36 and 48 hours after the induction of pancreatitis, and serum amylase levels were measured.

At 12, 24 and 36 hours after the induction of pancreatitis, other animals were killed with a large dose of pentobarbital, and the abdomen was reopended. Ascitic fluid was collected in 5 ml disporsable syringes, and its volume and amylase content were determined.

After ascitic fluid had been removed, blood from the central venous line was used for plasma endotoxin and serum fibrin degradation products (FDP) determinations. Whole blood was centrifuged (3000 × g, 10 min, 4°C), and plasma endotoxin levels were measured with a Quantitative Chromogenic Limulus Amebocyte Lystate Assay Kit (QCL-1000, Bioproducts, MA, U.S.A.), endotoxin from Escherichia coli 0127, 1382 (Worthington Biochemicals, Freehold, NJ, U.S.A.), being as the standard. Serum FDP was measured by the Latex Agglugation Test (Boehringer Manheim Yamanouchi, Tokyo, Japan).

Next, portions of the pancreas were removed quickly and one small portion (ca 300 mg) was used for the determination of pancreatic edema by a comparison of the weight immediately after sacrifice (wet weight) with that of the same sample after incubation at 150°C for 48 hours in a dessicator (dry weight).

Other small portions of the pancreas (ca 300 mg) were homogenized in 5 ml of cold phosphate-buffered saline (pH 7.4) containing 0.5% Triton X-100 (Fisher Scientific) in a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, NY, U.S.A.) for the measurement of pancreatic amylase and cathepsin B as a lysosomal enzyme, and both amylase and cathepsin B activity as well as deoxyribonucleic acid (DNA) concentration were measured in the resulting supernatant after low speed centrifugation (150 × g, 15 min, 4°C). Both pancreatic amylase and cathepsin B contents were expressed as U/mg DNA.

Other very small portions of the pancreas from each group at each stage were fixed overnight in phosphate-buffered (pH 7.4) 10% neutral formaldehyde solution. After paraffin embedding, sectioning and staining with hematoxylin-eosin, the sections were examined by a blinded observer, and interstitial edema, acinar cell vacuolization, inflammatory cell infiltration, and acinar cell necrosis were graded on a 0–4+ scale (0, no change; 4+, most change).

The remaining portions of the pancreas (ca 700 mg) were homogenized in 6 ml of ice-chilled 5 mM MOPS (3-(N-morpholino) propanesulfonic acid) (Sigma Chemical, St. Louis, MO, U.S.A.) buffer (pH 6.5), containing 1 mM $MgSO_4$ and 250 mM sucrose. Unbroken cells and debris were removed by low speed centrifugation (150 × g, 4°C, 15 min). Subcellular fractions were prepared by differential centrifugation, as described by TARTAKOFF and JAMIESON[18], with minor modifications for studies of rat tissue[19]. Briefly, the supernatant was centrifuged (1300 × g, 15 min, 4°C) to yield a "zymogen granule" pellet and another supernatant. This supernatant was centrifuged again (12,000 × g, 12 min, 4°C) to yield a "lysosomal and mitochondrial" pellet and a supernatant, which was considered to contain the microsomal and soluble fraction. The amylase and cathepsin B activities in each fraction were measured and expressed as a percentage of the total activity.

Amylase activity was measured by the method of BERNFELD[20]. Cathepsin B activity was measured fluorometrically by the method of MCDONALD and ELLIS[21] with CBZ-arginyl-arginine-β-naphthylamide (Bachem Bioscience, Philadelphia, PA, U.S.A.) as the substrate, and one unit (U) of cathepsin B activity was defined as that which releases 1 nanomole of β-naphythylamine (Sigma Chemical) per min from the substrate at 38°C. Deoxyribonucleic acid (DNA) concentration was measured fluorometrically by the method of LABARCA and PAIGEN[22] with calf thymus DNA (Sigma Chemical) as the standard.

Shiomarin was purchased from Shionogi Pharmaceutical, Osaka, Japan, and E3123 was donated by Eisai Pharmaceutical, Tokyo, Japan.

The results reported in this communication represent the means ± SEM for n determinations. Differences between groups were evaluated by ANOVA and Tukey method. For evaluating the

histological changes, Wilcoxin's rank sum test was used and for the mortality rates, the X^2 method with Yate's correction was used[23]. Significant differences were defined as those associated with a probability value (p) of less than 0.05 ($p<0.05$).

Results

Sodium taurocholate injection into the pancreatico-biliary duct caused severe acute pancreatitis with a survival rate of 96% at 12 hours, 57% at 24 hours, 50% at 36 hours, 43% at 48 hours. Continuous infusion of E3123 in a dose of 2 mg/kg・hr significantly improved the survival rate. Moreover, combination therapy with E3123 and Shiomarin in a dose of 20 mg/kg・hr for 1 hour every 8 hours had the most significant protective effect against this pancreatitis (Table 1).

Sodium taurocholate injection caused a significant increase in serum amylase levels. The administration of E3123 significantly improved this hyperamylasemia. Moreover, combination therapy with E3123 and Shiomarin had the most significant protective effect against hyperamylasemia (Table 2).

Sodium taurocholate caused ascites which increased with time. The administration of E3123 significantly diminished the volume of ascitic fluid, and combination therapy with E3123 and Shiomarin had the most significant protective effect against ascites (Table 3).

In the CONT group, amylase levels in ascitic fluid were significantly higher than in the E group. In the S group ascitic fluid amylase levels were even lower (Table 4).

In the CONT group, plasma endotoxin levels were significantly higher than in the E group. The S group had the lowest levels of plasma endotoxin (Table 5).

Serum FDP levels showed almost the same changes as the endotoxin levels, and taurocholate injection caused a marked rise in serum FDP levels-significantly higher than in normal rats. The ad-

Table 1 Effects of E3123 and Shiomarin on the survival rate in rat taurocholate-induced acute pancreatitis

Group	n	Survival rate (%)				
		Hours after induction of pancreatitis				
		0	12	24	36	48
CONT	30	100	96	57	50	43
E	30	100	97	87**	77**	70**
S	30	100	100	97*	90*	87***

CONT: taurocholate-induced pancreatitis, E: pancreatitis with E3123 infusion at a dose of 2 mg/kg・hr, S: pancreatitis with infusion with E3123 and Shiomarin at a dose of 20 mg/kg・hr. * $p<0.01$, ** $p<0.02$ compared with CONT. *** $p<0.01$ compared with CONT, and $p<0.05$ compared with E.

Table 2 Effects of E3123 and Shiomarin on the serum amylase levels in rat with pancreatitis

Group	n	Amylase levels (U/ml)			
		Hours after induction of pancreatitis			
		12	24	36	48
CONT	6	34±3	36±3	23±2	18±2
E	7	20±2*	23±3*	14±2*	11±2*
S	8	16±2**	17±3**	9±2**	8±2**

* $p<0.05$, ** $p<0.02$ compared with CONT. Amylase level in normal rats (n=5): 6±1 U/ml.

ministration of E3123 had a significant protective effect against these increased levels of FDP. Combination therapy with E3123 and Shiomarin had the most significant protective effect (Table 6).

Sodium taurocholate injection into the rat pancreatico-biliary duct caused a significant increase in pancreatic water content, indicating pancreatic edema, which reached its peak 24 hours after the induction of pancreatitis. The administration of E3123 had a significant protective effect against this pancreatic edema at 12 hours, but not a significant effect at 24 hours or 36 hours. Combination therapy with E3123 and Shiomarin had the most significant protective effect (Table 7).

Sodium taurocholate caused a gradual increase in both pancreatic amylase and cathepsin B content with a peak at 24 hours after the induction of pancreatitis. E3123 administration had a significant protective effect against the congestion of both digestive and lysosomal enzymes in the acinar cells induced by taurocholate injection. Combination therapy with E3123 and Shiomarin had the most significant protective effect (Table 8).

Sodium taurocholate caused marked histological changes in acinar cells, such as interstitial edema, acinar cell vacuolization, inflammatory cell infiltration, and necrosis, with peak changes 24 or 36 hours after the induction of pancreatitis. The administration of E3123 had a partial protective effect against these histological changes. Combination therapy with E3123 and shiomarin had the most significant protective effect against these changes in almost all the parameters examined in this

Table 3 Effects of E3123 and Shiomarin on the ascites volume in rats with pancreatitis

Group	n	Ascites volume (ml/100 gBW)		
		Hours after induction of pancreatitis		
		12	24	36
CONT	6	1.34±0.26	1.66±0.23	1.80±0.31
E	8	0.71±0.17**	0.86±0.20**	1.14±0.17*
S	9	0.57±0.11***	0.63±0.14***	0.74±0.11***+

* $p<0.05$, ** $p<0.02$, *** $p<0.01$ compared with CONT. +; $p<0.05$ compared with E.

Table 4 Effects of E3123 and Shiomarin on the ascites amylase levels in rats with pancreatitis

Group	n	Amylase levels (U/ml)		
		Hours after induction of pancreatitis		
		12	24	36
CONT	6	153±24	162±19	128±14
E	8	94±15*	113±18*	81±12*
S	9	63±17**	70±14**	43±10**+

* $p<0.05$, ** $p<0.02$ compared with CONT. +; $p<0.05$ compared with E.

Table 5 Effects of E3123 and Shiomarin on the plasma endotoxin levels in rats with pancreatitis

Group	n	Endotoxin levels (μg/ml)		
		Hours after induction of pancreatitis		
		12	24	36
CONT	6	0.14±0.04	0.23±0.06	0.26±0.07
E	8	0.05±0.02*	0.08±0.03*	0.11±0.02*
S	9	0.04±0.01*	0.05±0.02**	0.04±0.02**+

* $p<0.05$, ** $p<0.02$ compared with CONT, +; $p<0.05$ compared with E.

experiment (Table 9).

Sodium taurocholate injection into the rat pancreatico-biliary duct caused a significant decrease of amylase activity in the zymogen fraction and a significant increase in the microsomal and soluble fraction at every stage after the induction of pancreatitis suggesting that the fragility of zymogen granules is increased. The administration of E3123 significantly protected against the increased fragility of zymogen granules 24 and 36 hours after the induction of pancreatitis. Combination therapy with E3123 and Shiomarin had the most significant protective effect at all stages examined (Table 10). Sodium taurocholate caused a significant increase of cathepsin B activity in the zymogen fraction and a significant decrease in the lysosomal fraction at all stages after the induction of pancreatitis, showing gradual progress with time and suggesting redistribution of lysosomal enzyme from the lysosomal fraction to the heavier zymogen fraction. The administration of E3123 had a significant protective effect against these redistributions of lysosomal enzyme at all stages examined, and combination therapy with E3123 and Shiomarin had the most significant effect against these changes at all stages (Table 11).

Table 6 Effects of E3123 and Shiomarin on the serum FDP levels in rats with pancreatitis

Group	n	FDP levels (μg/ml)		
		Hours after induction of pancreatitis		
		12	24	36
CONT	6	26±3	34±4	28±2
E	8	15±2*	18±3*	14±2*
S	9	8±2**	10±2**	7±2**+

* $p<0.02$, ** $p<0.01$ compared with CONT, +; $p<0.05$ compared with E. FDP levels in normal rats (n=5): 4±1 μg/ml.

Table 7 Effects of E3123 and Shiomarin on the pancreatic water content in rats with pancreatitis

Group	n	Pancreatic water content (% of total weight)		
		Hours after induction of pancreatitis		
		12	24	36
CONT	6	85±2	87±3	84±2
E	8	79±2*	82±2	80±2
S	9	78±1*	80±2*	79±2

* $p<0.05$ compared with CONT. Water content in normal ras (n=5): 75±1%.

Table 8 Effects of E3123 and Shiomarin on the pancreatic amylase and cathepsin B content in rats with pancreatitis

Group	n	Pancreatic amylase (U/mg DNA) and cathepsin B content (U/mg DNA)					
		Hours after induction of pancreatitis					
		12		24		36	
		Amylase	cathepsin B	Amylase	cathepsin B	Amylase	cathepsin B
CONT	6	392±41	974±105	645±58	1725±164	582±47	1588±149
E	8	376±33	925± 94	428±49*	1258±127*	404±52*	1070±131*
S	9	341±46	948± 87	363±31**	1113±108**	354±44**	983± 95**

* $p<0.05$, ** $p<0.02$ compared with CONT, Normal rats: Amylase, 423±48, cathepsin B, 1094±128.

Table 9 Effects of E3123 and Shiomarin on the histological changes in rats with pancreatitis

Group	Stage: Hours after induction of pancreatitis	n	Histological changes in acinar cells: Interstitial edema	Acinar cell vacuolization	Inflammatory cell infiltration	Necrosis
CONT	12	6	2.3±0.3 (1–3)	1.3±0.2 (1–2)	1.3±0.2 (1–2)	2.3±0.2 (2–3)
	24	6	2.7±0.2 (2–3)	2.3±0.2 (2–3)	2.2±0.2 (2–3)	2.7±0.3 (2–4)
	36	6	2.2±0.2 (2–3)	2.7±0.3 (2–4)	2.3±0.2 (2–3)	3.3±0.2 (3–4)
E	12	8	1.4±0.2 (1–2)	1.1±0.1 (1–2)	1.3±0.2 (1–2)	1.4±0.2* (1–2)
	24	8	2.0±0.3 (1–3)	1.4±0.2 (1–2)	1.4±0.3 (1–2)	2.0±0.3 (1–3)
	36	8	1.1±0.1 (1–2)	2.3±0.3 (1–3)	1.4±0.2 (1–2)	2.4±0.2* (2–3)
S	12	9	1.1±0.1 (1–2)	0.6±0.2 (0–1)	0.8±0.1 (0–1)	0.7±0.2** (0–1)
	24	9	1.3±0.2* (1–2)	1.1±0.1* (1–2)	1.2±0.2 (0–2)	1.2±0.1**+ (1–2)
	36	9	1.1±0.1* (1–2)	1.2±0.1** (1–2)	1.2±0.2 (1–2)	1.4±0.2**+ (1–2)

Histological changes were blindedly graded on a scale from 0 (no changes) to 4+ (maximum changes) and the values were expressed as the means±SEM of the values, ( ): the range of the values, * $p<0.05$, ** $p<0.02$ compared with CONT, +; $p<0.05$ compared with E.

Table 10 Effect of E3123 and Shiomarin on the subcellular amylase distribution in rats with pancreatitis

Group	Hours after induction of pancreatitis	n	Amylase activity (% of total): Fractions: Zymogen	Lysosome	Microsome and Soluble
CONT	12	6	30±3	14±2	56±4
	24	6	26±2	15±2	59±3
	36	6	22±2	16±2	62±3
E	12	8	36±2	15±2	49±3
	24	8	34±2*	16±2	50±2*
	36	8	32±3	15±2	53±2*
S	12	9	38±2	16±2	46±2*
	24	9	37±2	16±2	47±2**
	36	9	35±2	16±2	49±2**
Normal rats		5	41±2**	14±2	45±2*

* $p<0.05$, ** $p<0.02$ compared with CONT.

Table 11 Effects of E3123 and Shiomarin on the subcellular cathepsin B distribution in rats with pancreatitis

Group	Hours after induction of pancreatitis	n	Cathepsin B activity (% of total)		
			Fractions		
			Zymogen	Lysosome	Microsome and Soluble
CONT	12	6	42±3	36±3	22±2
	24	6	49±3*	29±2	22±2
	36	6	54±3	24±2	21±2
E	12	8	30±2*	49±3*	21±2
	24	8	32±2**	45±3**	23±1
	36	8	39±2**	41±3**	20±2
S	12	9	27±2**	53±3**	20±2
	24	9	29±2***	50±2***	21±2
	36	9	33±2***	46±2***	21±2
Normal rats		5	25±2**	57±2**	18±2

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

Discussion

We used taurocholate-induced pancreatitis in this study, because secretagogue-induced pancreatitis is too mild and CDE deit-induced pancreatitis is too severe. Taurocholate injection into the rat pancreatico-biliary duct caused severe acute pancreatitis with hyperamylasemia, marked histological changes, such as interstitial edema, acinar cell vacuolization and necrosis, and a high mortality rate (about 45% at 48 hours after the induction of pancreatitis), as previously reported[8,9]. Furthermore, in this study, as in the secretagogue-induced, CDE diet-induced, and pancreatic duct obstructed models, there was redistribution of lysosomal enzyme from the lysosomal fraction to the heavier zymogen fraction, indicating colocalization of digestive enzyme with lysosomal hydrolase[3,4]. Thus, this colocalization phenomenon seems to play an important role in the pathogenesis of pancreatic injury in this taurocholate-induced pancreatitis, although in the initial stage the direct detergent effect of the bile salt seems to play a crucial role in this model.

Recently there have been several reports on the protective effects of new potent synthetic protease inhibitors in clinical and experimental acute pancreatitis[2,14–16]. E3123, one of these protease inhibitors, has also been reported to inhibit several key enzymes in pancreatitis; trypsin, elastase, phospholipase A_2, and kallikrein[17]. Because of its relatively small molecular weight (508 daltons), we expected that it could move from the vasculature into pancreatic acinar cells, so we studied its protective effects against this taurocholate-induced rat pancreatitis and found significant protective effects against almost all the parameters examined: mortality rate, high amylase levels in the serum and ascitic fluid, volume of ascitic fluid, pancreatic water content and distribution of lysosomal enzyme. Thus, in this study E3123 single therapy had a significant protective effect against taurocholate-induced pancreatitis. It may therefore be useful in the treatment of the clinical disease. There have also been several reports on the importance of infections in the etiology of severe acute pancreatitis[10–13]. But there have been only a few studies on the effect of antibiotic therapy in severe acute pancreatitis, particularly in combination with the new potent synthetic pro-

tease inhibitors. Shiomarin is one of the third generation cephalosporin antibiotics which has broad antibacterial effects against many important organisms. In this study, taurocholate injection into the rat pancreatico-biliary duct caused marked plasma endotoxemia and high serum levels of FDP. E3123 alone had a significant protective effect, but combination therapy with E3123 and Shiomarin reduced even further the high levels of endotoxin and FDP and the mortality rate. These findings seem to support the hypothesis that infectious factors play a crucial role in the development of severe pancreatitis. This combination therapy also had significant protective effects against the histological abnormalities induced by taurocholate-induced pancreatitis.

Combination therapy with E3123 and Shiomarin had more significant protective effects than E3123 alone, against serum and ascitic fluid amylase levels, and distribution of lysosomal enzyme. Combination therapy with this new potent synthetic protease inhibitor and a strong antibiotic seems to be a rational treatment for severe acute pancreatitis, partly because protease inhibitors can inhibit several key enzymes in the acinar cells and the redistribution of lysosomal enzymes and partly because antibiotics may prevent secondary infections in the pancreas. This combination therapy should probably be started as early as possible after the onset of pancreatitis.

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和文抄録

ラット胆汁酸誘起膵炎における抗生剤シオマリンと E3123 の併用療法について

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ラット総膵胆管にタウロコール酸を注入することにより，高アミラーゼ血症，高エンドトキシン血症および高死亡率を判った重症膵炎が誘起された．さらに，この実験膵炎においては，カテプシンB活性の本来のライソゾーム分画よりチモーゲン分画への移動・再分布が観察された．広域抗生剤であるシオマリンと新しい合成プロテアーゼインヒビターである E3123 との併用療法によりこの膵炎での死亡率の著明な改善やカテプシンBの再分布などのパラメーターでの有意な改善が観察された．E3123 の単独療法にても，すべてのパラメーターにて有意な改善が観察されたが，併用療法には劣った．これらの結果は，重症膵炎においては，何等かのプロテアーゼ活性とともに，感染性因子がその病態にて重要な役割を果していることを示唆するとともに，臨床での重症膵炎での抗生剤とプロテアーゼインヒビターの併用療法の有用性をも示唆させた．

さらに，この実験膵炎の発症機構に，ライソゾーム酵素の再分布が深く関与することも示唆させた．