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Experimental acute pancreatitis in dog

2) Effect of urinary trypsin inhibitor (UTI) on amidase and elastolytic activities of plasma and pancreas tissue

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Key words: Acute pancreatitis; amidase activity; elastase; urinary trypsin inhibitor

SUMMARY

In the dog with acute pancreatitis induced by the duodenal blind loop method, the time course changes in plasma enzyme activities were studied in 10 kinds of synthetic amide substrates. The activities of all enzymes measured 36 hr after preparation of acute pancreatitis were found to be intensified. Particularly, the plasma elastase activity measured by two substrates, MeOSuc-Ala-Pro-Val-pNA and pyro-Glu-Pro-Val-pNA was increased over 5 times ($p<0.001$) as high as that before preparation of the pancreatitis or that of the control (sham operation) group.

On the other hand, the elastase activation in the UTI (i.v.) group was markedly inhibited, e.g., the elastase activity in the UTI 20,000 U/kg group was inhibited strongly by more than 65% ($p<0.001$) compared with that in the control group even though any synthetic substrates were used for measurement 12 hr after preparation of the pancreatitis, and the activity in the UTI 60,000 U/kg group was also inhibited significantly by more than 78% ($p<0.001$) 18 hr after preparation of the pancreatitis. Aprotinin, 120,000 KIU/kg, had no such potent inhibitory effect on activation of elastase.

The activities of chymotrypsin, trypsin and urokinase measured by Bz-L-Tyr-pNA, Bz-L-Arg-pNA and pyro-Glu-Gly-Arg-pNA respectively also tended to be inhibited with UTI ($p<0.05$, measured 12 hr after preparation of acute pancreatitis in the UTI 60,000 U/kg group).

UTI exerted a potent inhibitory effect on activation of elastase present in plasma and pancreas tissue: The Congored-elastinolytic activity in the extract from dog pancreatitic tissue was about 5 times as high as that in the sham operation group (control), but its activation was inhibited by more than 99% ($p<0.005$) with UTI (60,000 U/kg, i.v.).

It appeared that not on already activated enzymes in blood, UTI given i.v. shows a potent inhibitory effect on activation of proteases including elastase in pancreas tissue or on 'escape' of active-form enzymes from pancreas into blood.

INTRODUCTION

Autodigestion of pancreas by activated pancreatic enzymes and escape of pancreatic enzymes from pancreas into blood are important pathologic conditions in acute pancreatitis. Meanwhile, the role of proteases other than trypsin, viz., elastase and phospholipase A_2, which had not been traditionally regarded as imoportant enzymes and had been known to cause damages of cell membrane and tissue, has recently been reconsidered[1,2].

In a previous paper[3], we demonstrated that the i.v. injection of UTI or its injection into the blind loop could increase the survival rate in the dog with experimental pancreatitis induced by the duodenal blind loop method and that UTI strongly inhibited the activation of plasma enzymes as measured by a synthetic substrate, TAMe. However, low molecular synthetic substrates[4], e.g., TAMe or Bz-L-Arg-pNA which has long been used to measure activities of plasma trypsin-like enzymes, are common substrates to various enzymes (proteases) including trypsin, kallikrein, elastase, thrombin and the first component of complement (Cls). Therefore, the specificities of these amides are low. Development of synthetic amide substrates with extremely high specificity(chromozyme) to enzymes consisting of three or four amino acid residues has recently been in progress[5,6].

The purpose of this study was to examine the time course changes in plasma exzyme activities during experimental acute pancreatitis by the chromozyme substrates. In relation to the plasma elastase activity which showed a particularly great change, the pro-elastase activity together with the activities of active-form enzymes in pancreas tissues were measured by natural substrates. As the result, UTI given i.v. was confirmed to inhibit strongly their activation. The detail of it is also described in this paper.

MATEROALS AND METHODS

1. *Drugs*

Gel-filtered human UTI standard (Lot 007 E) without pyrogenic substances, generously supplied by JCR (Kobe, Japan), had molecular weight of 67,000 (by gel filtration) and specific activity of 2,700/mg protein. Aprotinin and trypsin (Type 1) were obtained from Bayer (West Germany) and Sigma (U.S.A.), respectively.

Bz-L-Arg-pNA, Bz-L-Tyr-pNA and MeOSuc-Ala-Ala-Pro-Val-pNA were purchased from Peptide Research Laboratory (Osaka, Japan). Pyro-Clu-Pro-Val-pNA (S-2484), pyro-Glu-Gly-Arg-pNA (S-2444), H-D-Phe-Pip-Arg-pNA (S-2238), H-D-Ile-Pro-Arg-pNA (S-2288), H-D-Val-Leu-Lys-pNA (S-2251) and H-D-Val-Leu-Arg-pNA (S-

2266) were purchased from Kabi (Sweden). All were dissolved in distilled water to make a concentration of 2.5 x 10^{-3}M before use. Congored-elastin (Sigma, U.S.A.) was dissolved in 0.02M borate buffer (pH 8.8) to make a concentration of 1 mg/ml immediately before use.

2. *Animals*

Thirty eight mongrel adult dogs (including 2 females) weighing about 10 kg, previously confirmed to be free from abnormality in coagulation-fibrinolysis system, were fasted for 24 hr before use. Acute pancreatitis was experimentally prepared by the duodenal blind loop method according to the method of Pfeffer et al.[7] as described in our initial report[3]. UTI at 3,000 U/kg or aprotinin at 60,000 KIU/kg was injected intravenously(i.v.) by taking for 4 to 5 successive hr, starting immediately after preparation of the blind loop. Furthermore, UTI (750, 2,500 or 7,500U/kg) or aprotinin (15,000 KIU/kg) was injected i.v. immediately before and after preparation of the blind loop, and at the 6- and 12-postoperative hrs, respectively (total doses: 6,000, 20,000 and 60,000 U/kg for UTI and 120,000 KIU/kg for aprotinin).

3. *Measurement of enzyme activity*

The following substrates were used to measure each enzyme activity by the end-point method modified by Bang and Matter[8]: Bz-L-Arg-pNA for trypsin activity, Bz-L-Tyr-pNA for chymotrypsin activity, MeOSuc-Ala-Ala-Pro-Val-pNA (Sub. 1)[9], Suc-Ala-Ala-Ala-pNA (Sub. 2)[10] and pyro-Glu-Pro-Val-pNA (Sub. 3)[11] for elastase activity. H-D-Val-Leu-Lys-pNA for plasmin activity, pyro-Glu-Gly-Arg-pNA for urokinase activity, H-D-Ile-Pro-pNA for tissue plasminogen activator activity. H-D-Phe-Pip-Arg-pNA for thrombin activity, H-D-Val-Leu-Arg-pNA for kallikrein activity. Each reaction system containing each sample plasma (1 ml) was incubated at 37℃ in each substrate (concentration: 5 x 10^{-4}M in 0.1M phosphate buffer or pH 7.4), and the reaction was stopped by adding 0.2ml of 50% acetic acid followed by quantification fo the produced p-nitroanilide (pNA) at 405 nm.

The Congored-elastolytic activity of elastase was measured by the Shotton's method[12]. A mixture of a sample and 0.02M borate buffer (7.0ml, pH 8.8) was added to 1 ml of a substrate solution, stirred at 37 ℃ for 45 min, and centrifuged at 3,000 rpm for 10 min. Then, the elastolytic activity in the supernatant liquid was measured at 495 nm. As the control, a substrate solution was added to the sample and buffer mixture, centrifuged immediately, and the supernatant was measured. For the measurement of the elastase activity in the tissue sample, 2.5ml of 0.1M phosphate buffer (pH 7.4) was added to 1 g (wet weight) of pancreas tissues (obtained from 3 different sites) upon death owing to pancreatitis or at the time of sacrifice 36 hr after preparation of experimental

pancreatitis for the survival dog, homogenized at 0 ℃ for 5 min, and centrifuged at 40, 000 rpm 10 min, followed by measurement of the supernatant at 495 nm. In the case of measurement of total elastase activity (active form), an equivolume of a trypsin solution (1 mg/ml) was added to 1 ml of the supernatant by the method of Geokas et al.[13], and incubated at 37℃ for 30 min to use as a sample [Pro-elastase actvity= Total elastase activity-Untreated elastase activity].

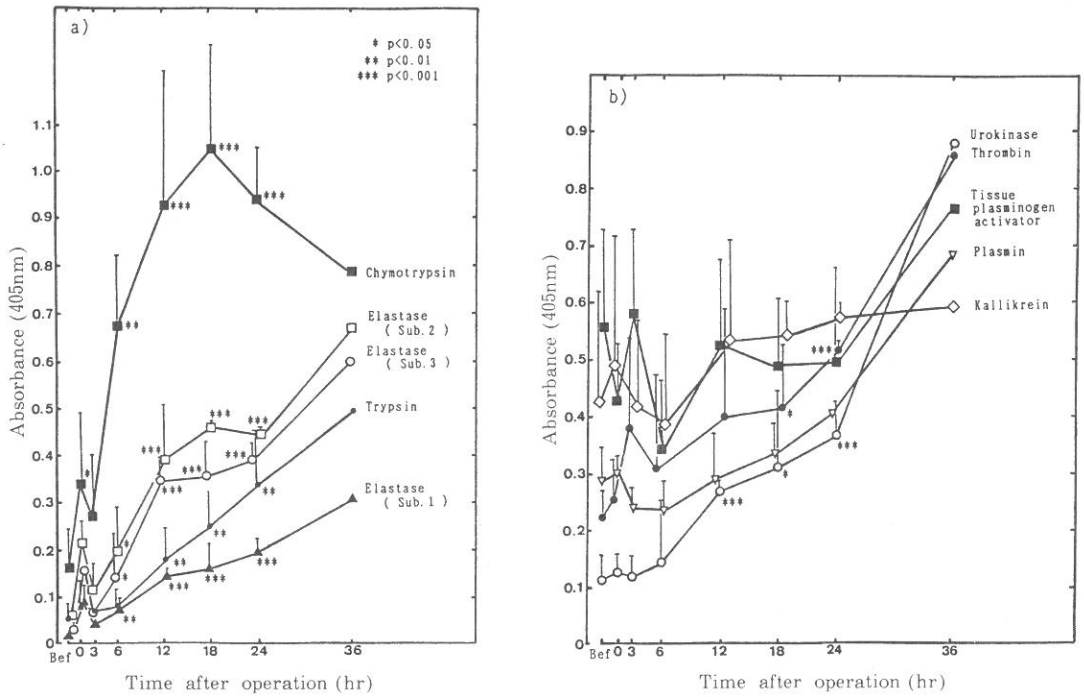

Fig. 1. Onset of acute pancreatitis and plasma protease activities

a) Chymotrypsin: Incubation of 0.1 ml of plasma at 37℃ for 2 hr in the presence of substrate, Bz-L-Tyr-pNA. Elastase:Incubation of 0.1 ml of plasma at 37℃ for 2 hr in the presence of substrate, MeOSuc-Ala-Ala-Pro-Val-pNA(Sub.1) , Suc-Ala-Ala-Ala-pNA (Sub.2) or pyro-Gle-Plo-Val-pNA (Sub.3). Trypsin: Incubation of 0.1 ml of plasma at 37℃ for 2 hr in the presence of substrate, Bz-L-Arg-pNA. In all reaction systems, 1 ml of substrate at concentration of 5 x 10^{-4}M in 0.1 M phosphate buffer of pH 7.4 was used. The reaction was stopped by adding 0.2 ml of 50 % acetic acid, and the absorbance of formed p-nitroanilide (pNA) was measured at 405 nm.

b) Urokinase: Incubation of 0.025 ml of plasma at 37℃ for 1 hr in substrate, pyro-Glu-Gly-Arg-pNA. Thrombin: Incubation of 0.025 ml of plasma at 37℃ for 1 hr in the presence of substrate, H-D-Phe-Pip-Arg-pNA. Tissue plasminogen activator: Incubation of 0.025 ml of Plasma at 37℃ for 1 hr in the presence of substrate, H-D-Ile-Pro-Arg-pNA. Plasmin: Incubation of 0.025 ml of plasma at 37℃ for 1 hr in the presence of substrate, H-D-Val-Leu-Arg-pNA. Kallikrein: Incubation of 0.025 ml of plasma at 37℃ for 1 hr in the presence of substrate, H-D-Val-Leu-Arg-pNA. In all reaction systems, 1 ml of substrate at concentration of 5 x10^{-4}M in 0.1 M phosphate buffer of pH 7.4 was used. The reaction was stopped by adding 0.2 ml of 50 % acetic acid, and the absorbance of formed p-nitroanilide (p-NA) was measured at 405 nm.

RESULTS

1. *Activation of plasma enzymes by pancreatitis*

The time course changes in activities of trypsin, chymotrypsin, elastase, kallikrein, thrombin, plasmin and urokinase in plasma or in tissue plasminogen activator activity of the dog with acute pancreatitis were measured by 10 kinds of synthetic amide substrates (Figs. 1a and 1b). The changes were slight in an incipient stage until 3 hr after preparation of the blind loop, but the activities were significantly increased from 6 hr after preparation of acute pancreatitis. Because the measuring conditions for each enzyme were different, the quantity of

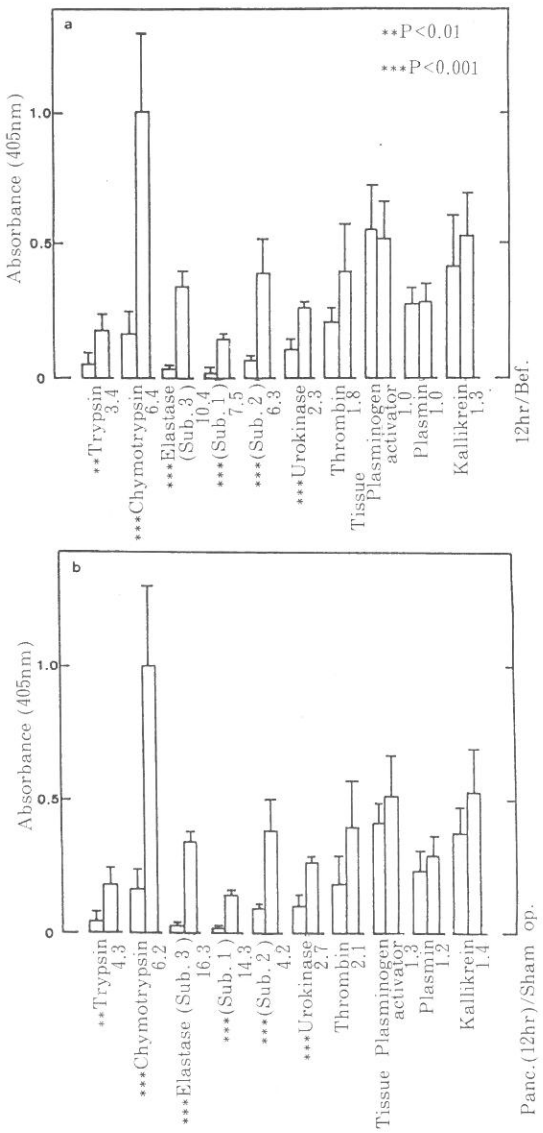

Fig. 2. Activation of plasma enzymes 12 hr after preparation of acute pancreatitis
Plasma enzyme activities were measured 12 hr after preparation of acute pancreatitis measuring conditions were same as those used in Fig. 1, and compared with a) those before preparation of acute pancreatitis and b) those of the sham operation (control) group. The values of horizontal lines represent activation multiples of each enzyme.

plasma and duration of incubation varied consequently. However, the maximal amount of hydrolytic substrate per unit time on calculation (μmoles / 1 hr, 37℃/ml plasma) was in order of urokinase (3.52) > thrombin (3.44) > tissue plasminogen activator (3.04) > kallikrein (2.37) > plasmin (0.68) > chymotrypsin (0.57) > trypsin (0.25) > elastase (0.13/Suc-Ala-Ala-Ala-pNA, 0.12/pyro-Glu-Pro-Val-pNA, 0.06/MeOSuc-Ala-Ala-Pro-Val-pNA).

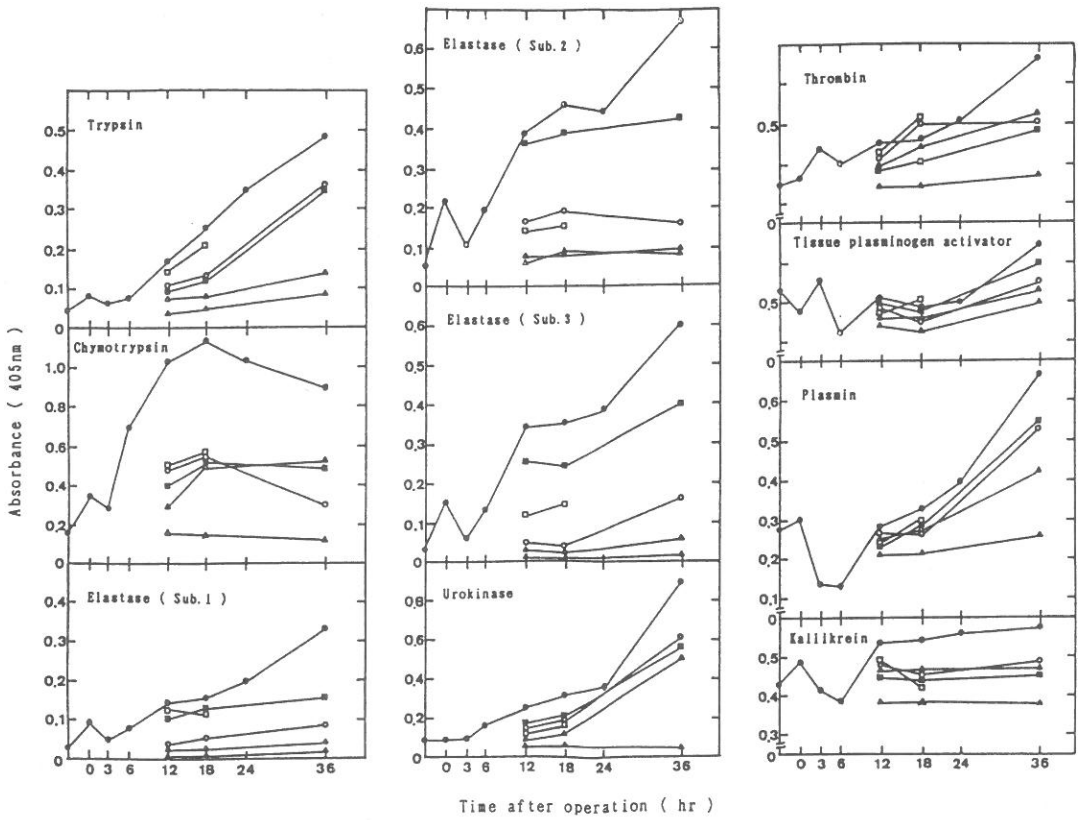

Fig. 3. Effect of UTI injection on acute pancreatitis
Plasma enzyme activities were measured under the same measuring conditions as in Fig. 1 in the presence of 10 kinds of synthetic amide substrates(5×10^{-4}M).
●——● Acute pancreatitis (Control group)
△——△ UTI 60,000 U/kg group
○——○ UTI 20,000 U/kg group
□——□ UTI 6,000 U/kg group
■——■ Aprotinin 120,000 KIU/kg group
▲——▲ Sahm operation group

On the other hand, as shown in Figs. 2a and 2b the elastase activity was increased most significantly in all enzymes upon the onset of acute pancreatitis compared with the activity before preparation of acute pancreatitis or that in the sham operation group, e.g., the plasma elastase activity measured by substrates, pyro-Glu-Pro-Val-pNA and

MeOSuc-Ala-Ala-Pro-Val-pNA, 12hr after preparation of acute pancreatitis was over 5 times ($p < 0.001$) as high as the pre-operative value. The activities of chymotrypsin ($p < 0.001$), urokinase ($p < 0.001$), trypsin ($p < 0.01$), thrombin, kallikrein and tissue plasminogen-activator also tended to increase at the onset of acute pancreatitis.

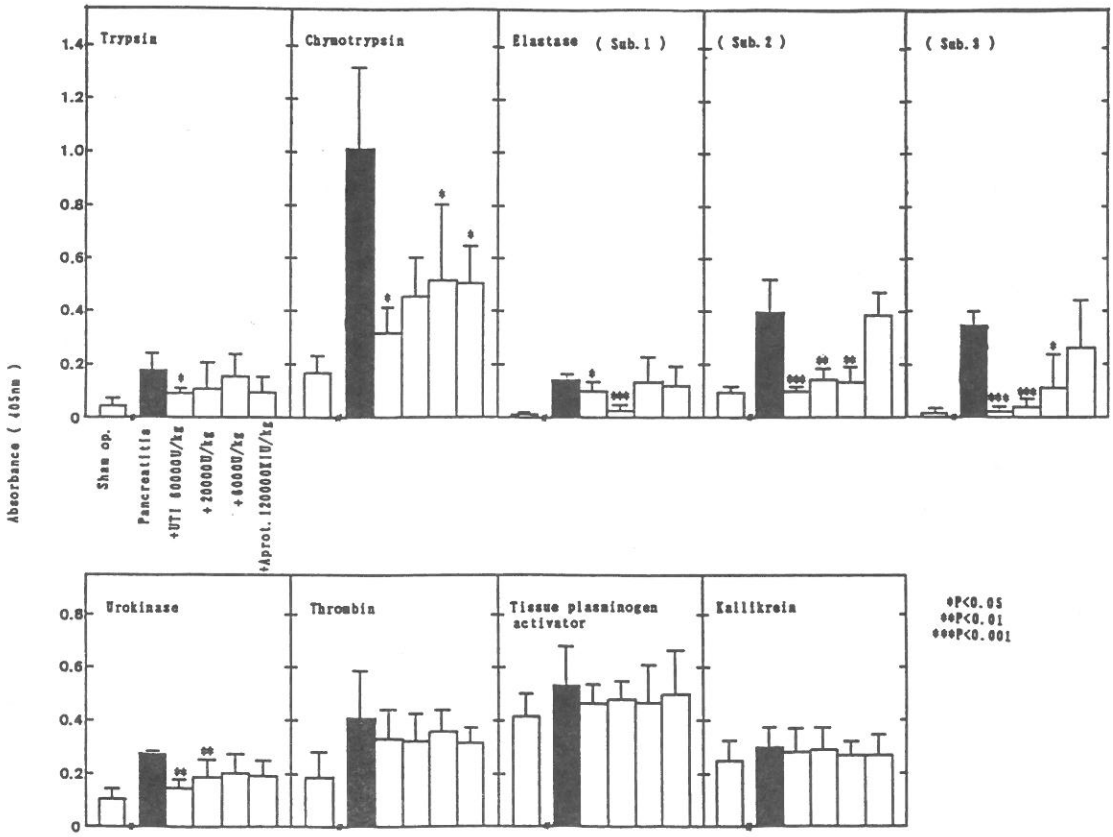

Fig. 4. Effect of UTI injection on plasma enzymes 12hr after preparation of acute pancreatitis
With the same measuring conditions as used in Fig.1, plasma enzyme activities were measured 12hr after preparation of acute pancreatitis. From the left to the right of each panel, mean±S.D. values for the sham operation (control) group (N= 5), apancreatitis control group(N= 5), UTI, 6,000 U/kg group(N= 5) and aprotinin 120,000 KIU/kg group (N= 4) are shown, respectively.

2. *Effect of UTI on plasma enzyme activities*

Fig. 3 shows the effect of UTI on activities of various plasma enzymes (12,18 and 36hr after preparation of acute pancreatitis). The effect was somewhat different, but UTI exerted an inhibitory effect on activities of all enzymes examined. Fig. 4 summarizes the results observed 12hr after preparation of acute pancreatitis. UTI (20,000 and 60,000 U/kg) inhibited the activation of trypsin, chymotrypsin or urokinase ($p < 0.01$). Especially, the activity of plasma elastase was inhibited strongly by UTI when measured by MeOSuc-Ala-Ala-Pro-Val-pNA, Suc-Ala-Ala-

Ala-pNA or pyro-Glu-Pro-Val-pNA ($p < 0.001$). For example, the plasma elastase activity was inhibited by over 65% immediately after the i.v. injection of UTI (20,000 U/kg) compared with that of the control group ($p < 0.005$) (although the inhibition was not significant because of the increase in mortality of the pancreatitis groups at and after preparation of the inflamed condition, the plasma elastase activity 18hr after preparation of acute pancreatitis in the UTI 60,000 U/kg group was inhibited by over 78.1%). On the contrary, UTI could not almost inhibit the plasma thrombin, tissue plasminogen activator or kallikrein activity. Inhibition of the elastase activity by the control drug, aprotinin (120,000 KIU/kg) 12 and 18 hr after preparation of acute pancreatitis was less than 28.7% ($p < 0.5$) even in any substrates, unlike UTI, the inhibitory effect of aprotinin was weak.

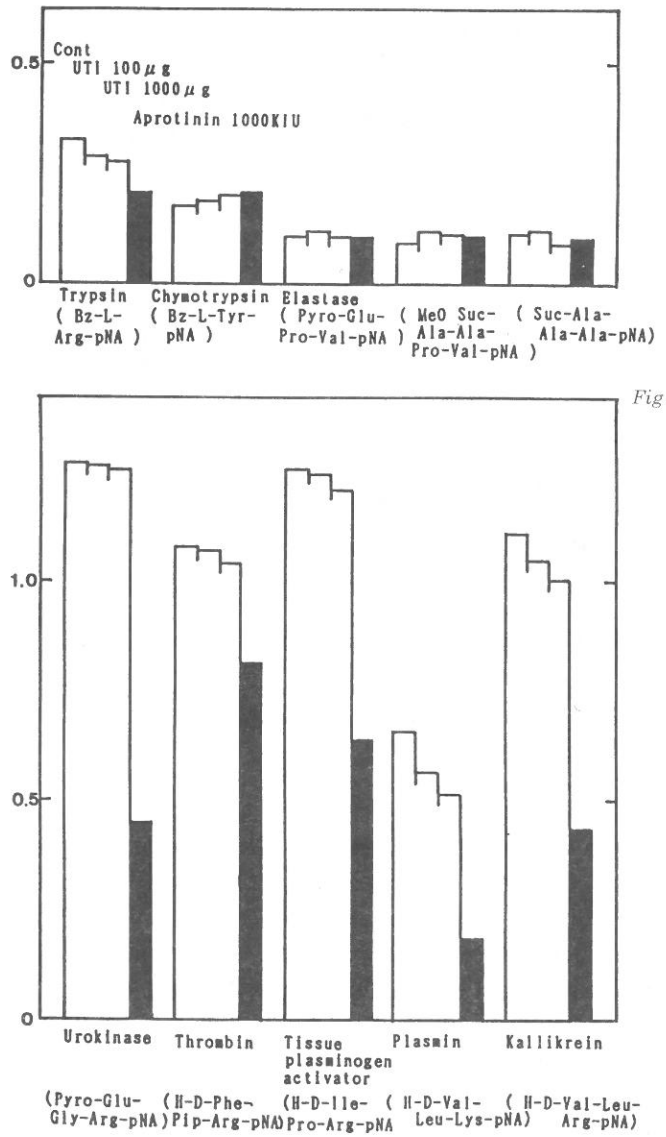

Fig. 5. Effect of UTI addition to acute pancreatitic plasma
UTI (100 and 1,000 μg/0.1ml)or aprotinin (1,000KIU/0.1ml)was added to dog plasma (0.05ml) 36hr after preparation of acute pancreatitis, and pre-incubated at 37℃ for 5 min to measure the residual activities. The concentration of each substrate was 5×10^{-4}M. ncubation was performed at 37℃ for 2 hr by using 0.1M phosphate buffer of pH 7.4.

3. *Effect of UTI on activity of pancreas tissue elastase*

An in-vitro adding effect of UTI on activities of plasma enzymes previously activated by acute pancreatitis was assessed and compared with its potent i.v. effect. Fig. 5 shows the result. Even though UTI was added in concentrations from 100 to as high as 1,000 μg /0.1ml plasma, UTI exerted almost no direct inhibitory effect or exhibited only a slight inhibitory effect on the activities of plasma plasmin, kallikrein and trypsin. In contrast, aprotinin (1,000 KIU/kg) under the same conditions inhibited kallikrein, plasmin, urokinase, tissue plasminogen activator, thrombin and trypsin.

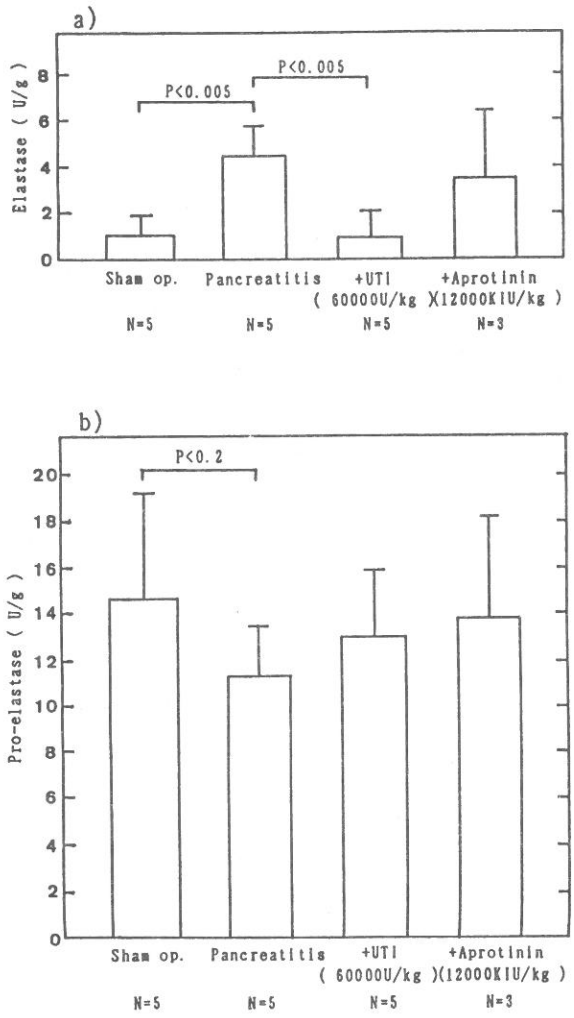

Fig. 6. Effect of UTI injection on elastase and pro-elastase activities in pancreas tissue. The elastase activity(a) and pro-elastase activity(b) which is activated by trypsin treatment, both being present in extracted solution(0.1M phosphate buffer of pH 7.4) of pancreas tissue, were measured by using Congored-elastin as substrate.(Each activity was expressed by elastase U/g tissue.)

The inhibitory effect of UTI on elastase activity was also evaluated by using a natural substrate, Congored-elastin. Owing to the presence of a large amount of plasma inhibitors, the Congored-elastin method was unable to measure plasma enzyme activities. However, a potent elastolytic activity could be detected in the extracted solution of pancreas tissue.

The elastase activity in acute pancreatitis (4. 43 ± 1. 35 U/g pancreas tissue) measured by this method was significantly higher (about 5-fold, $p < 0.005$) than that of the control (sham operation) group (0. 92 ± 0. 90 U/g tissue) as shown in Fig. 6 a. Furthermore, the elastase activity in the UTI 60,000 U/kg group was 0. 95 ± 1. 19 U/g tissue, indicating over 99% inhibition ($p < 0.001$) of the elastase activation in the pancreas tissue by UTI. The simultaneously measured pro-elastase activity in the pancreas tissue was 14. 84 ± 4. 55 and 11. 58 ± 2. 14 U/g tissue in the control (sham operation)and pancreatitis group, respectively; inversely, the tissue pro-elastase activity tended to decrease in pancreatitic state ($p < 0.2$, Fig. 6 b).

DISCUSSION

The time course changes in plasma enzymes of dogs with experimental acute pancreatitis were measured by 10 kinds of synthetic amide (chromozyme) substrates. As the result, the activities of all enzymes, viz., trypsin measured by Bz-L-Arg-pNA, chymotrypsin by Bz-L-Tyr-pNA, elastase by MeOSuC-Ala-Pro-Val-pNA, Suc-Ala-Ala-Ala-pNA and pyro-Glu-Pro-Val-pNA, urokinase by pyro-Glu-Gly-Arg-pNA, thrombin by H-D-Phe-Pip-Arg-pNA, tissue plasminogen activator by H-D-Ile- Pro-Arg-pNA, plasmin by H-D-Val-Leu-Lys-pNA and kallikrein by H-D-Val-Leu-Arg-pNA were found to be increased although the increased degrees were different as seen in Fig. 1 . Particularly, the plasma elastase activity was verified to reach over 10 times as high as that before preparation of acute pancreatitis or that in the control (sham operatiion) group (Fig. 2).

Trypsin has been regarded as most important plasma enzyme(protease) in acute pancreatitis because the enzyme is well correlated with the pathology of pancreatitis[14] . In addition, it has been considered that trypsin which has once been activated in the pancreas acts a trigger for sequential activation of phospholipase, elastase and other enzymes to not only result in aggravation of the pathologic conditions but also implicate with formation of toxin playing an important role in its prognostic course[15,16] . The increase in plasma kinin level, reduction of plasma kininogen level and increase in plasma kininase activity during experimental acute pancreatitis in dogs have been reported by Tharl et al.[17], Ryan et al.[18] and Nugent et al.[19] , respectively, suggesting a great change in the kallikrein-kinin system. It is known that in human acute pancreatitis, activated kallikrein combines with plasma α_2- macroglobulin and the formed complex is able to hydrolyze synthetic amides such as H-D-Pro-Phe-Arg-pNA[20] . Using synthetic substrates, Rund et al.[21] recently demonstrated that the kallikrein activity in peritoneal exudate was more capable of activation than that in plasma during experimental

acute pancreatitis in pigs. These activated trypsin and kallikrein have been also confirmed experimentally to cause local vasodilation, hyper-permeability, stagnation of blood flow and thrombogenic hemorrhage eighter directly or through the tissue kinin and histamin systems[17,22,23]. In addition, the complement system has been believed to relate with damage of acinous cell membrane in the incipient stage of development of acute pancreatitis[24]. Thus, activation of various enzyme system during acute pancreatitis has been reported by many investigators. Measurement of amount of the antigen against the plasma elastase has become possible since the recent development or radioimmunoassay (RIA)[25,26], but there has been almost no extensive study of the change in the elastase activity to date.

Plasma elastase activity may be detected in not only pancreas[27] but also leukocyte[28], platelet[29], spleen[30] and aortic wall[31]. Splenic and leukocytic elastases are known to have substrate specificities[32] in experiments using various synthetic amides and also have different reactivities to aprotinin[33]. Even though elastase is originated from a same pancreas, two kinds of elastase (elastase 1 and elastase 2) differing in their physicochemical and immunological properties are isolated[34]. Pancreas-derived elastase detectable in plasma[35] is generally accepted as elastase 2 . Recently we could isolate another type of elastase providing different properties from those of conventional elastase, which was dissociable from α_2- macroglobulin fraction of plasma of patients with acute pancreatitis by the gel filtration method in the presence of SDS[36].

Elastase may digest vascular wall elastin which is not affected by trypsin, resulting in development of hemorrhagic pancreatitis from edematous pancreatitis[1] . In fact, the injection of elastase into the dog pancreatic duct in vivo could induce hemorrhagic pancreatitis[37]. Stokke et al.[38] also described that the i.v. injection of homologous pancreatic elastase to a miniature pig could produce blood hypercoagulation and an ARDS-like change accompanied with leukocyte infiltration and edema in pancreas. Clinically, activated elastase has been proved frequently from the pancreatic tissue of patients who died of hemorrhagic pancreatitis[13]. If elastase plays an important role in etiology or pathogenesis of acute pancreatitis, any antielastase agent may be used in the treatment of pancreatitis. A recent study by Fric et al.[39] is of great interest to substantiate this view. They developed oligopeptides including Glu-Ala-Ala-Ala-NH-Et and others with a high specivicity as anti-elastase agents, and verified that the agents could prolong life span and inhibit strongly hemorrhage into peritoneal exudate in rats with experimental acute pancreatitis.

The result of this study also suggested that from a substrate specificity point of view, pancreas-derived elastase[9,10] may act in MeOSuc-Ala-Ala-Pro-Val-pNA (Sub. 1) and Suc-Ala-Ala-Ala-pNA (Sub. 2) and

leukocyte derived elastase[11] in pyro-Glu-Pro-Val-pNA (Sub. 3). As shown in Figs. 3 and 4, in use of any substrates it was found that the elastolytic activity was most strongly inhibited by UTI as compared with inhibition of other enzymes [over 65.1% ($p < 0.005$) of the elastolytic activity was inhibited by UTI (20,000 U/kg) 12 hr after preparation of acute pancreatitis and over 78.1% ($p < 0.001$) by the agent (60,000 U/kg) 18 hr laterl.

To ascertain an inhibitory effect of UTI on elastase activation during acute pancreatitis, we measured the elastase activity in pancreas tissue by using a natural substrate (Congored-elastin). Because, the presence of 'elastase-like enzyme' in plasma, which can decompose synthetic amide substrates of elastase but cannot the natural elastin substrate, has recently been detected[40]. Ogawa et al.[41] reported that the biliary hydrolyzing activity on Suc-Ala-Ala-Ala-pNA was a different enzyme from elastase. The plasma elastase activity was not detected by Congored-elastin because the sensitivity of this substrate to the enzyme was low and the activity was influenced by plasma inhibitors. However,the elastase activity in the extracted solution of pancreas tissue was detected by this natural substrate. Figs. 6a and 6b show the results of measurement by this method: The activity of active-form elastase in pancreas tissue was also increased significantly during acute pancreatitis same as that in plasma, while an elastase precursor (pro-elastase) which is activated by addition of trypsin to the extracted solution of pancreas tissue was found to be decreased inversely during acute pancreatitis[42]. The change like this resembled well the result observed by Geokas et al.[43] in the dog with experimental acute pancreatitis induced by the injection of bile or trypsin into the pancreatic duct[43]. This indicates that activation from pro-elastase to elastase occurs in acute pancreatitic tissue. To the contrary, almost no change in the elastase and pro-elastase activities was seen in the UTI (60,000 U/kg) group, proving that UTI strongly inhibited activation of elastase in pancreas tissue. Aprotinin (120,000 KIU/kg) had no such inhibitory effect. It is generally accepted that although UTI had a potent inhibitory activity on active-form trypsin, chymotrypsin, plasmin, acrosin, blood coagulation system in-vitro[44,45] or leukocyte elastase, UTI has almost no direct inhibitory effect on pancreatic elastase, especially in use of natural substrate[44,46]. Fig. 5 presents the result obtained by examination of a directly adding effect of UTI to already activated enzymes of dog plasma in an in-vitro system; UTI was confirmed to have almost no adding effect on various enzyme activities. This result appears to be not conflicting with the result of a recent study by Jonsson and Ohlsson[47] reporting that UTI strongly inhibits leukocytic elastase and trypsin in a pure substrate system, but it can not inhibit the enzymes in the coexistence of plasma α_1-antitrypsin or α_2-macroglobulin which affects the effect of UTI.

The result of the present study suggests that UTI exerts an anti-

pancreatitis effect by inhibiting more strongly the activation of pancreatic pro-enzymes or escape of pancreatic pro-enzymes into blood than already activated enzymes.

The presence of inhibitors, which are generically named as acid stable trypsin inhibtors (ASTIs) having the same antigenicity as UTI, in various human body fluids or organs including normal tissues, has recently been recognized by RIA[48] using antibody of UTI, enzyme-linked immunosorbent assay[49] (ELISA) or tissue staining method[50]. Furthermore, based on the fact that ASTIs are increased after the onset of various diseases including acute pancreatitis[51-53], it has been gradually elucidarted that ASTI may possibly act as a short of defense reaction and also it may be produced by enzymatic molecular breakage from a kind of precursor protein(pro-inhibitor)present in plasma as the mechanism of formation[54]. Differing from aprotinin, UTI is only one human-derived natural inhibitor with a broad spectrum available at present, and it is a physiologically rational drug for the treatment of acute pancreatitis in the above-mentioned sense.

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