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INDIRECT STRONG INHIBITION BY ACID-STABLE TRYPSIN-PLASMIN INHIBITOR (ASTPI) OF THE ACTIVATIONS OF ELASTASE AND PLASMA FIBRINOLYSIS IN A DOG MODEL OF ACUTE PANCREATITIS

HIROYUKI SUMI* and YASUSHI ETO

Department of Physiology, Miyazaki Medical College, Kiyotake-cho, Miyazaki-gun Miyazaki 889–16, Japan

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Acid-stable trypsin-plasmin inhibitor (ASTPI) had almost no direct effect on the increased levels of the plasma enzymes elastase, plasmin and plasminogen activators in an acute pancreatitis model in the dog, whereas it strongly inhibited the activation of such enzymes by intravenous administration. *In vivo* experiments also showed strong inhibition by ASTPI of pancreas tissue elastase and plasma fibrinolysis. Anti-shock and anti-pancreatitis effects of ASTPI observed in clinical therapy may be related to this "indirect inhibition".

KEY WORDS: Acid-stable trypsin-plasmin inhibitor; elastase; plasmin; plasminogen activator; acute pancreatitis.

INTRODUCTION

Many reports have shown an increase of urinary ASTPI in various clinical conditions such as rheumatic fever, inflammation, cancer,[1–4] etc. ASTPI is a very acidic and acid stable glycoprotein (Mr 67,000, gel filtration) excreted by the kidney and has increased levels in the plasma of dialyzed patients.[5–7] Recently, it has been demonstrated that ASTPI has the same N-terminal amino acid sequence to the human endothelial cell growth factor ECGF-2b.[8]

ASTPI has a broad inhibition spectrum and reacts with extracellular proteases such as trypsin, chymotrypsin and plasmin, and with intracellular proteases such as acrosin, elastase, cathespin G and earthworm proteases.[9–12] However, Gustavsson *et al.*[13] recently showed that the anti-trypsin activity of ASTPI disappears in the presence of α_2-macroglobulin and α_1 antitrypsin, as well as decreases in anti-plasmin and anti-plasminogen activator activities using fibrin substrate.[7,14,15]

In the present paper, we studied the effects of highly purified ASTPI on enzyme levels in plasma and tissues in a dog model of acute pancreatitis. The "indirect inhibition" by ASTPI of the activation of elastase and plasma fibrinolysis is shown for the first time.

* Correspondence.

MATERIALS AND METHODS

Highly purified urinary ASTPI, pyrogen-free with Mr 67,000, and a specific activity of 2,700 U/mg (Lot 007E), prepared as described previously,[7] was kindly supplied by JCR Pharmaceutical Co. Ltd. (Kobe).

Adult mongrel dogs (20 males) weighing about 10 kg and previously confirmed to be free from abnormality in plasma enzyme system, were fasted for 24 h before use. Experimental pancreatitis was induced with the closed duodenal loop method of Pfeffer *et al.*[16] For adminitration experiments, 1 group consisting of 5 animals had 10,000 or 30,000 U/kg of ASTPI injected intravenously for 4 to 5 successive hours starting immediately after preparation of the blind loop. Furthermore, additional ASTPI (2,500 or 7,500 U/kg) was injected i.v. immediately before and after preparation of the blind loop, and at 6 and 12 hours postoperatively, respectively (total doses: 20,000 and 60,000 U/kg). All these animal experiments were performed under appropriate conditions in the Experimental Animal Center, Miyazaki Medical College.

Plasma elastase activity was determined with the endo-point method of Bang *et al.*[17] using synthetic amido substrates; Suc-Ala-Ala-Ala-pNA, Pyro- Glu-Pro-Val-pNA and MeOSuc-Ala-Ala-Pro-Val-pNA. H-D-Val-Leu-Lys-pNA was used for plasmin and both pyro-Glu-Gly-Arg-pNA and H-D-Ile-Pro-Arg-pNA were used for plasminogen activator (urokinase and tissue plasminogen activator) substrates. The Congo red-elastin hydrolyzing activity of elastase was measured by the method of Shotton;[18] the activity was expressed as absorbance per minute at 405 nm. Activated elastase and total elastase activities of the pancreas tissue were determined by the method of Geokas *et al.*,[19] (i.e., Pro-elastase activity = Total elastase activity-Activated elastase activity). Thromboelastography (TEG) was performed with a TEG apparatus (Hellige) according to the routine procedure. The instrument used advances the film with a speed of 1 mm per min.

RESULTS

The time course changes of plasma elastase, plasmin, urokinase, and tissue plasminogen activator activities were studied with each specific amido substrate (Table 1). A significant increase of elastase activity was observed from 6 h to 18 h after preparation of the duodenal blind loop compared to the preoperative values; after 18 h the activities were more than 6 times higher with each at the 3 substrates. All these increases were statistically significant ($p < 0.01$). The activities of plasmin, tissue plasminogen activator and urokinase were elevated 1.2, 0.9, and 2.8 times, respectively.

In *in vitro* experiments, the effect of ASTPI for these activated enzymes in plasma was very weak. Less than 9.0% of any of these enzymes was inhibited even when the high concentration of 1,000 U/ml of ASTPI was used (Figure 1, upper columns). In contrast, when administered to the dogs *in vivo* (Figure 1, lower columns) ASTPI had powerful effects with more than 86% inhibition on the activation of plasma proelastase, and to a lesser extent on pro-urokinase.

The strong anti-elastase activity of ASTPI *in vivo* was also confirmed with the natural substrate elastin. As shown in Figure 2a, the elastase activity (4.22 ± 0.98 U/g) of the pancreatitis group 18 h after operation was about 5 times higher than that of the control group (0.94 ± 0.88 U/g). Elastase activation was strongly inhibited (ca. 99%, $p < 0.01$; $n = 5$) by ASTPI administration; the activity of the administered

Table 1 Activation of plasma enzymes in acute pancreatitis

	After induction of acute pancreatitis			
	Before	6 h	12 h	18 h
Elastase				
Suc-Ala-Ala-Ala-pNA	0.07 ±0.01	0.20* ±0.09	0.40** ±0.12	0.46** ±0.09
Pyro-Glu-Pro-Val-pNA	0.03 ±0.01	0.14* ±0.10	0.34*** ±0.05	0.35*** ±0.07
MeOSuc-Ala-Ala-Pro-Val-pNA	0.02 ±0.01	0.07** ±0.02	0.14*** ±0.02	0.16*** ±0.01
Plasmin				
H-D-Val-Leu-Lys-pNA	0.29 ±0.06	0.24 ±0.05	0.29 ±0.08	0.34 ±0.05
TPA				
H-D-Ile-Pro-Arg-pNA	0.56 ±0.17	0.34 ±0.12	0.53 ±0.15	0.49 ±0.12
UK				
Pyro-Glu-Gly-Arg-pNA	0.11 ±0.05	0.14 ±0.11	0.27 ±0.02	0.31* ±0.14

After induction of acute pancreatitis, the time course change of several plasma enzymes were measured. Elastase: Incubation of 0.1 ml of plasma at 37 °C for 2 h in the presence of substrates, Ala-Ala-Ala-pNA, Pyro-Glu-Pro-Val-pNA or MeOSuc-Ala-Ala-Pro-Val-pNA- Plasmin: Incubation of 0.025 ml of plasma at 37 °C for 1 h in the presence of substrate H-D-Val-Leu-Arg-pNA. Urokinase (UK): Incubation of 0.025 ml of plasma at 37 °C for 1 h with substrate Pyro-Glu-Gly-Arg-pNA. Tissue plasminogen activator (TPA): Incubation of 0.025 ml of plasma at 37 °C for 1 h in the presence of substrate, H-D-Ile-Pro-Arg-pNA. In all reaction systems, 1 ml of substrate at concentration of 5×10^{-4} M in 0.1 M phosphate buffer pH 7.4 was used. The reaction was stopped by adding 0.2 ml of 50% acetic acid, and the absorbance of *p*-nitroanilide (pNA) formed was measured at 405 nm. The enzyme activities of the sham operated (control) dogs were also similar ($p > 0.5$) (not shown). Each value represents the mean ± S.D. ($n = 5$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

group (0.97 ± 0.91 U/g) was almost the same as that of the sham operated control group. The calculated pro-elastase activity in the pancreatitis group (10.70 ± 1.64 U/g) was less than that of the control group (13.92 ± 4.63 U/g). As shown in Figure 2b, an inhibitory effect of ASTPI on the decrease of pro-elastase activity was also observed.

The TEG pattern of each dog 18 h after the induction of pancreatitis is shown in Figure 3. Inhibition of the decrease of the Ma value (fibrinolytic parameter) was observed in the ASTPI administered group; the average value for the control group was 20.2 ± 18.5 mm, whereas the administered group was 41.6 ± 10.4 mm ($p < 0.01$; $n = 5$). On the other hand, the blood clotting parameters of *r* and *k* values were not much changed ($p > 0.5$).

DISCUSSION

An appreciable decrease in the anti-protease activities of ASTPI *in vitro* systems has been reported in the presence of several plasma proteins.[7,14,15] Using RIA,[20] ELISA[21] and fluorometric methods,[22] the same antigenicity of many body fluids or organs has been shown. The molecular similarity of ASTPI to the endothelial cell growth factor[8] has been recently shown. The physiological significances of ASTPI, its action as "inhibitor" or"growth factor" are problems requiring clarification.

In 1971, Kwaan *et al.*[23] reported an activation of plasma plasminogen activator

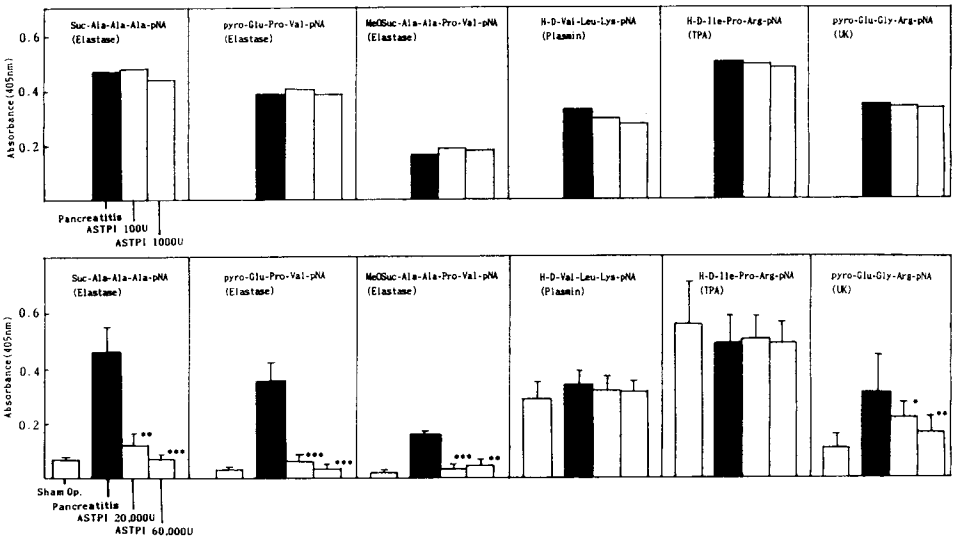

Figure 1 Effect of ASTPI on plasma enzymes 18 h after induction of acute pancreatitis *in-vitro* and *in-vivo*. The upper columns show the additional effect of ASTPI on acute pancreatitis plasma. ASTPI (100 and 1,000 units/ml) was added to dog plasma (0.05 ml) 18 h after induction of acute pancreatitis, and pre-incubated at 37 °C for 5 min prior to measuring the residual activities. The assay conditions were the same as those for Table 1. Lower columns show the effect of ASTPI injection on plasma enzymes 18 h after induction of acute pancreatitis. From the left to the right of each panel, mean ± S.D. values for the sham operation (control) group ($n = 5$), pancreatitis control group ($n = 5$), ASTPI 20,000 U/kg group ($n = 5$) and ASTPI 60,000 U/kg group ($n = 5$) are shown respectively. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

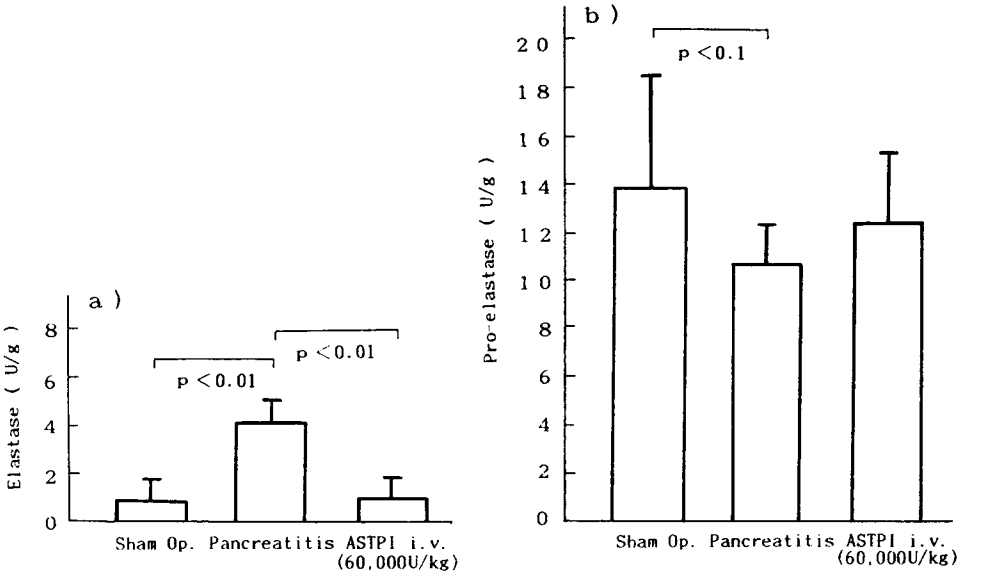

Figure 2 Effect of ASTPI administration on elastase and pro-elastase activities in pancreas tissue. The elastase (a), and pro-elastase activity (b), which is activated by trypsin treatment, present in extracts (0.1 M phosphate buffer, pH 7.4) of pancreas tissue, were measured with Congo red-elastin as substrate. Each value (elastase U/g tissue) represents the mean ± S.D. ($n = 5$).

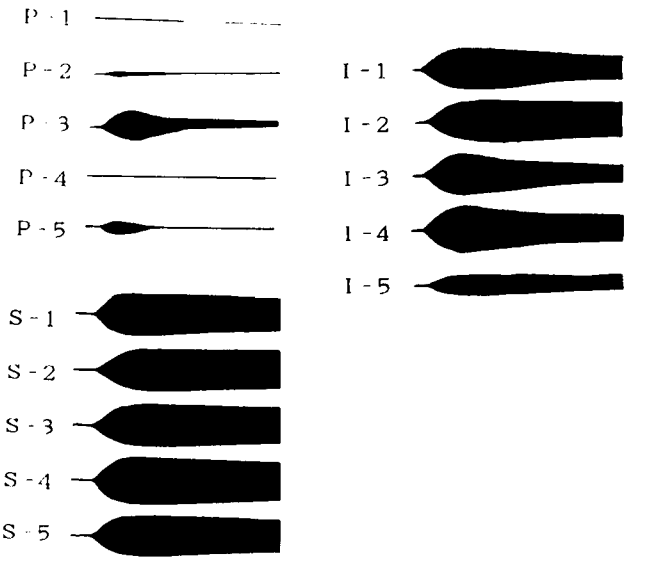

Figure 3 Thromboelastographic patterns of acute pancreatitis, ASTPI administered and sham operated (control) dogs. All blood was tested 18 h after induction of acute pancreatitis: P 1–5, acute pancreatitis; I 1–5, ASTPI administered (60,000 U/kg); and S 1–5, sham operated (control), respectively.

in experimental acute pancreatitis. The same phenomenon was observed in the present study; although it had no effect *in vitro*, ASTPI *in vivo* showed strong inhibition of the activation of plasma and pancreas tissue enzymes.

The anti-pancreatic properties of urinary ASTPI were first described in 1978 by ourselves.[24] In experimental acute pancreatitis induced by the Elliett's method,[25] the survival rate of dog could be increased by ASTPI administration, with partial inhibition of the activation of serum lipase and amylase. Recently, strong inhibition by ASTPI of the activation of glucuronidase activity has also been reported in the experimental model for shock in the dog.[26,27] Oda *et al.*[26] found the inhibition of lung swelling in acute pancreatitis in ASTPI administration.

The cell wall stabilizing effect of ASTPI similar to that of steroid hormone may induce the strong inhibition of the activation of the tissue type enzymes elastase and plasma fibrinolysis observed.

Acknowledgements

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