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The therapeutic effect of a new synthetic protease inhibitor (E-3123) on hemodynamic changes during experimental acute pancreatitis in dogs

Katsusuke SATAKE, Sin-Soo HA, Akihito HIURA, and Hideki NISHIWAKI
First Department of Surgery, Osaka City University Medical School, Osaka, Japan

Summary: The therapeutic effect of a new synthetic protease inhibitor on hemodynamic changes was studied in experimental acute pancreatitis. Pancreatitis was induced by the injection of autologous bile mixed with trypsin into the main pancreatic duct after ligating the accessory duct. Plasma beta-endorphin concentrations and cardiovascular function were measured. Seventeen dogs (control group) were given 10 ml/kg/hr of lactate Ringer's solution intravenously 1 hr before the induction of pancreatitis and throughout the experiment. Seven dogs (the low protease inhibitor group) were given an intravenous bolus injection of 0.4 mg/kg of a new synthetic protease inhibitor, E-3123 (4-(2-succiminido-ethylthio)4-geranidinobenzoate methanesulfate) 30 min after the induction of pancreatitis and then a continuous intravenous infusion at 3 μg/kg/min throughout the experiment. Seven dogs (the high protease inhibitor group) received an intravenous bolus injection of 3 mg/kg and a continuous intravenous infusion at 50 μg/kg/min of E-3123 according to the same method as in the low protease inhibitor group. The mortality rate during the experiment was 41% (7/17) in the control group, 28.5% (2/7) in the high protease inhibitor group and 0% in the low protease inhibitor group. The increase in the plasma beta-endorphin levels in the control group was statistically significant. When E-3123 was given 30 min after the induction of pancreatitis, the increase in the plasma beta-endorphin levels in the high protease inhibitor group was also found to be increased statistically significant, compared with preinduction levels, but the increase was statistically significantly lower than that in the control group. Plasma beta-endorphin levels in the low protease inhibitor group, however, did not increase. The protease inhibitor infusion as used in this experiment can bring about improvement in hypotension and myocardial depression to an extent by inhibiting the release of beta-endorphin, suggesting that the inhibitory effect of the protease inhibitor on beta-endorphin release contributes to the improvement in hemodynamic changes during pancreatitis. There may also be an optimal therapeutic dose of this drug for the treatment of hypotension and myocardial depression secondary to beta-endorphin release. *Gastroenterol Jpn 1993;28:64-71.*

Key words: *acute pancreatitis cardiovascular function; beta-endorphin; synthetic protease inhibitor (E-3123).*

Introduction

Acute pancreatitis is a disease of variable intensity, ranging a mild, self-limiting disease to a life-threatening condition causing multiple organ failure. Survival has improved during the past decade, mainly to the use of vigorous fluid replacement and intensive care management. The pathophysiology is, however, still obscure, and there is no specific treatment available.

Since publication of the theory of "tryptic autodigestion" of the pancreas as a cause of pancreatitis, several pancreatic enzymes have been implicated in the triggering of acute pancreatitis.

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Address for correspondence: Katsusuke Satake, M.D., First Department of Surgery, Osaka City University Medical School, Asahi-Machi, Abeno-ku, Osaka 545, Japan.
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Activated protease, such as trypsin[1] and elastase[2], have been found in complexes with protease inhibitors. These findings indicate there is liberation of activated proteases during acute pancreatitis. In addition, some studies have shown activation of the various cascade systems in the body, such as the complement, kinin, coagulation and fibrinolytic cascades, which may lead to the induction of multiple organ failure during acute pancreatitis. The systemic sequelae of acute pancreatitis lead to significant mortality and morbidity. Generally, the mild form of acute pancreatitis responds to conservative treatment with mortality and morbidity remaining low. However, severe acute pancreatitis is characterized morphologically by parenchymal necrosis and is difficult to diagnose precisely and has a doubtful prognosis.

Initially, promising therapeutic approaches to acute pancreatitis derived from experience with animal models involved either resting the pancreas (e.g., using cimetidine, anticholinergic drugs, glucagon, calcitonin) or the use of inhibitors of activated pancreatic proteases. Despite the use of these principles, the mortality and morbidity of severe acute pancreatitis remains a significant problem.

Recently, new synthetic protease inhibitors have been developed and considerable attention has been paid to these agents that inhibit activated proteases. Like trasylol, new synthetic protease inhibitors[3,4,5] can inhibit trypsin and kallikrein but they also possess a broader spectrum of protease inhibition than trasylol by their additional capacity to inhibit plasmin, thrombin $C_1\gamma$ and C_1 esterase and phospholipase A.

In a limited number of animal studies, synthetic protease inhibitors have been reported to exert a beneficial effect on the course of acute pancreatitis. However, in all of these studies the effect of synthetic protease inhibitors before or soon after the induction of acute pancreatitis was investigated.

The present study was undertaken to investigate the therapeutic effect of a new synthetic protease inhibitor (E-3123) on hemodynamic changes in dogs with experimentally induced acute pancreatitis.

Materials and Methods

Adult mongrel dogs of either sex, weighing between 15 and 20 kg, were anesthetized by an intravenous injection of 15 mg/kg of pentobarbital sodium and ventilated mechanically using room air. The iliac vein was cannulated through the saphenous vein and 3.8% sodium citrate solution was added to the blood samples taken. Control blood sample were taken before the procedure and succeeding samples were taken 30 min and one hour later and at hourly intervals for a period of five hours.

Plasma amylase levels were measured using a blue-starch method (Shionogi, Co. Japan). Beta-endorphin was measured with an immunological kit for beta-endorphin (Special Reference Laboratory, Japan).

A catheter was introduced into the femoral artery and connected to a pressure transducer Ap-610G (Nihon Kohden, Japan) to record systemic blood pressure. An ultrasonic transit flow meter (T101) was connected to the ascending aorta to measure cardiac output. A cut probe FBT (Nihon Kohden, Japan) was placed in the left renal artery and connected to an electromagnetic flow meter MFV 1200 (Nihon Kohden, Japan) to measure renal blood flow. After stabilization of blood pressure, pancreatitis was induced by the injection of 0.5 ml/kg of autologous bile mixed with 10,000 u/kg of trypsin (Sigma Co. St. Louis, MO, U.S.A.) into the main pancreatic duct after ligating the accessory duct.

Various parameters were then measured in the three groups of dogs. Seventeen dogs were used for the control group. Each had an intravenous injection of 10 ml/kg/hr of lactate Ringer's solution one hour before the induction of acute pancreatitis and continuous infusion throughout the experiment. The low protease inhibitor group (7 dogs) received an intravenous bolus injection of 0.4 mg/kg of a new synthetic protease inhibitor, E-3123 (4-(2-succiminidoethylthio) 4-geranidinobenzoate methanesulfonate) 30 min after the induction of pancreatitis and then a continuous intravenous infusion at 3 μg/kg/min throughout the experiment. The high protease inhibitor

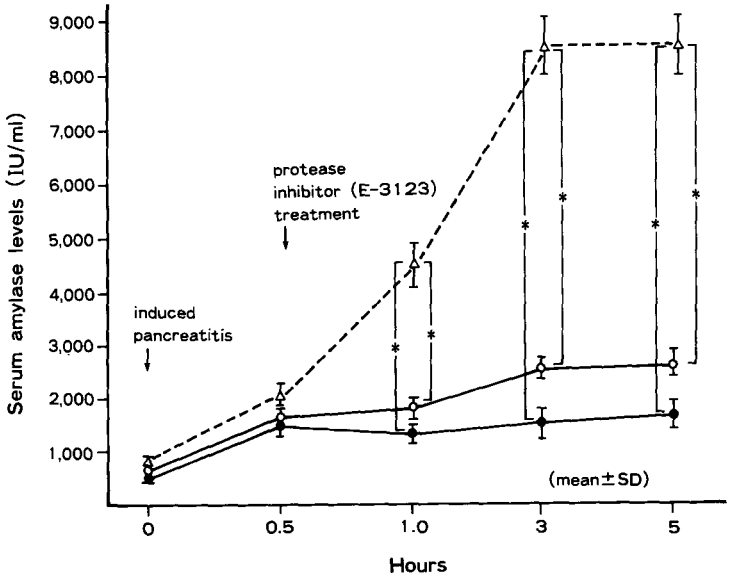

Figure 1. Plasma amylase levels in the control and protease inhibitor groups. The increase in amylase levels in the control group was satistically significant compared with pre-induction levels ($P<0.005$). There is no remarkable increase in plasma amylase levels in either of the protease inhibitor groups. The increase in amylase levels in the control group was statistically significantly compared with both the protease inhibitor group ($P<0.05$). △---△: control group. ○---○: low protease inhibitor group. ●---●: high protease inhibitor group. *: $P<0.05$ comparison of contral group.

group (7 dogs) received an intravenous bolus injection of 3 mg/kg and continuous intravenous infusion at 50 μg/kg/min of E-3123 according the same methods as in the lower protease inhibitor group.

The results were expressed as mean and standard deviation. Statistical analysis included an analysis of variance and an unpaired Student's test in the same group and Scheffe's multiple comparison test in different groups. Results were considered to be significant if $P<0.05$.

Results

Seven of the 17 dogs in the control group died during the experiment and two of the 7 dogs in the high protease inhibitor group also died, but no deaths occurred in the low protease inhibitor group.

Plasma amylase levels in the control group and protease inhibitor groups are shown in **Figure 1**. Serum amylase levels in the control group increased markedly but no remarkable increase in serum amylase levels was observed in both protease inhibitor groups.

Plasma beta-endorphin levels in the control group and protease inhibitor groups are shown in **Figure 2**. The mean plasma beta-endorphin level before the induction of pancreatitis in the control group was 30.1 pg/ml (±6.8 SD). It increased gradually to 88.1 pg/ml (±24.3 SD) 1 hr later and reached 206.1 pg/ml (±13.9 SD) 5 hr after the induction of pancreatitis. This increase was statistically significant ($P<0.05$). The mean plasma beta-endorphin levels before the induction of pancreatitis in the high and low protease inhibitor groups were 28.9 pg/ml (±7.8 SD) and 31.7 pg/ml (±15.9 SD) respectively, which were similar to the level found in the control group before the induction of pancreatitis. An increase in beta-endorphin levels was also seen in the high protease inhibitor group, with levels increasing to 79.5 pg/ml (±14.9 SD) 1 hr later and reaching 65.2 pg/ml (±18.2 SD) 5 hr after induction. This increase was also statistically significant ($P<0.05$). The levels were, however, lower than those of the control group ($P<0.05$). On the contrary, there was no significant increase in the level of plasma beta-endorphin detected in the low protease inhibitor group. The levels were lower than those of the high protease inhibitor group.

Arterial blood pressure changes in the control and protease inhibitor groups are shown in **Figure 3** and **Table 1**. Soon after the induction of pancreatitis arterial blood pressure began to decrease steadily. However, it increased 30 min after the

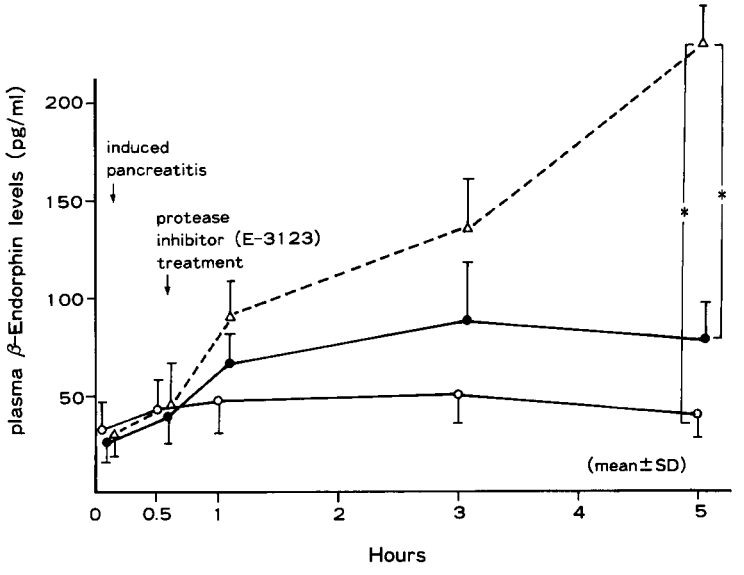

Figure 2. Plasma beta-endorphin levels in the control and protease inhibitor groups. The increase in the control group was statistically significant compared with preinduction levels ($P<0.01$). In the high protease inhibitor group, it also increased statistically significant compared with preinduction levels ($P<0.05$). No increase in beta-endorphin levels was seen in the low protease inhibitor group. Comparison of three groups shows statistically significant difference.

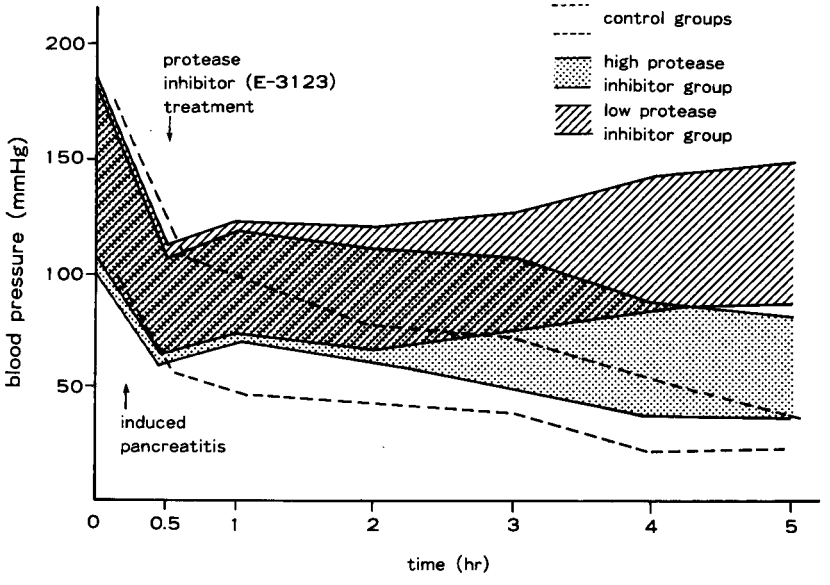

Figure 3. Typical cases of arterial blood pressure changes after the induction of acute pancreatitis. In the control group, the arterial blood pressure decreased and pulse pressure narrowed gradually after the induction of acute pancreatitis (while line). The arterial blood pressure in the low protease inhibitor group increased after the protease inhibitor treatment instituted and tended to return to preinduction levels following this. Pulse pressure was well maintained (oblique line). In the high protease inhibitor group, the arterial blood pressure also increased 30 min after the protease inhibitor treatment was instituted, but it decreased gradually thereafter (dotted area).

bolus injection and continuous intravenous infusion was commenced in both the low and high protease inhitor groups. In the high protease inhibitor group, arterial blood pressure decreased gradually after three hours. However, in the low protease inhibitor group it was well maintained.

Cardiac output in the control group and protease inhibitor groups are shown in **Figure 4**. The mean cardiac output before the induction of pancreatitis in the control group was 149.5 ml/kg/min (±17.2 SD), which decreased gradually to 22.5 ml/kg/min (±7.6 SD). This decrease was statistically significant ($P<0.01$). Mean cardiac outputs in the low and high protease inhibitor groups were 148.6 ml/kg min (±11.7 SD) and 142.1 ml/kg min (±18.4 SD) respectively, which were similar to results for the control group. They decreased to 93.3 ml/kg min (±8.1 SD) and 96.4 ml/kg min

Table 1. Change of mean arterial pressure (mmHg)

Time	Control group n=10	Protease inhibitor group	
		Low n=7	High n=5
Before induction of acute pancreatitis	124.4 (±21.1)	131.6 (±20.0)	128.0 (±14.7)
30 min[a]	70.9 (±16.4)[b]	76.4 (±27.8)[b]	70.7 (±14.7)[b]
1 h[a]	64.7 (±13.8)[b]	88.5 (±20.0)[b]	86.0 (±16.6)[b]
2 h[a]	57.7 (± 9.6)[b]	83.4 (±13.4)[b]	77.5 (±19.6)[b]
3 h[a]	55.3 (±10.0)[b]	96.8 (±14.8)[b]	69.0 (±10.2)[b]
4 h[a]	35.5 (± 8.3)[b]	106.92 (±13.0)[bcd]	56.1 (± 7.1)[bc]
5 h[a]	33.9 (± 3.6)[b]	109.5 (±15.8)[cd]	54.6 (± 5.4)[bc]

a: After induction of acute pancreatitis.
b: $P<0.05$ compared with the preinduction level.
c: $P<0.05$ compared with the control group.
d: $P<0.05$ compared with the high protease inhibitor group.

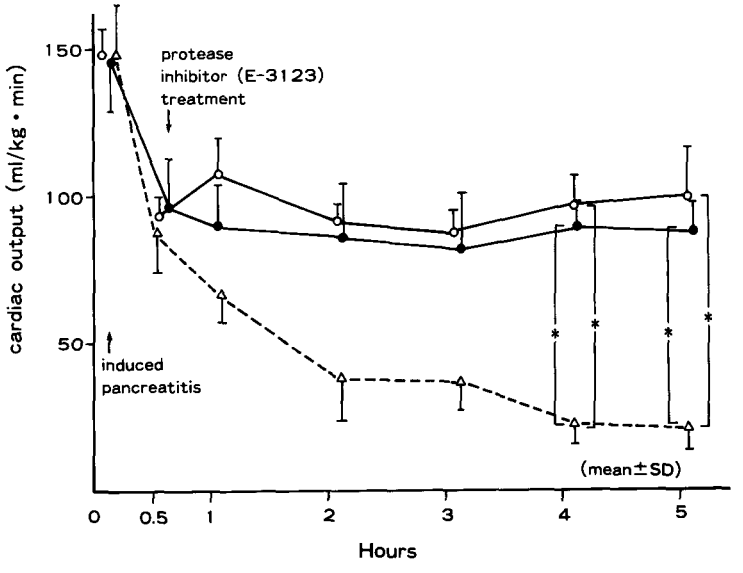

Figure 4. Cardiac output changes in the control and protease inhibitor groups. For the control group, cardiac output decreased markedly compared with pre-induction levels ($P<0.01$). For both protease inhibitor groups, it also decreased statistically significant compared with pre-induction levels ($P<0.05$). Comparison of the control group and both the protease inhibitor groups shows statistically significant difference ($P<0.05$). But no statistically difference was observed between the low and high protease inhibitor groups. △---△: control group. ○---○: low protease inbitor group. ●---●: high protease inhibitor group. *: $P<0.05$ comparison of control group.

(±20.3 SD) respectively 30 min later. After treatment with protease inhibitor these was no further decrease in cardiac output. Although the decrease in cardiac output in both protease inhibitor groups compared with pre-induction levels was statistically significant ($P<0.05$), significantly higher levels were maintained than in the control group ($P<0.05$), particularly in the low protease inhibitor group.

Decrease in renal arterial blood flow as shonw in **Figure 5** was statistically significant in the control ($P<0.005$) and high protease inhibitor group ($P<0.005$) and in the low protease inhibitor group ($P<0.01$) compared with levels prior to induction of pancreatitis. Flow rates were maintained well in the low protease inhibitor group.

Discussion

The concept that the severity of acute pancreatitis and its complications may be reduced by inhibitors of pancreatic enzymes has received much attention over the past 30 years. The most extensively investigated inhibitor is trasylol, which

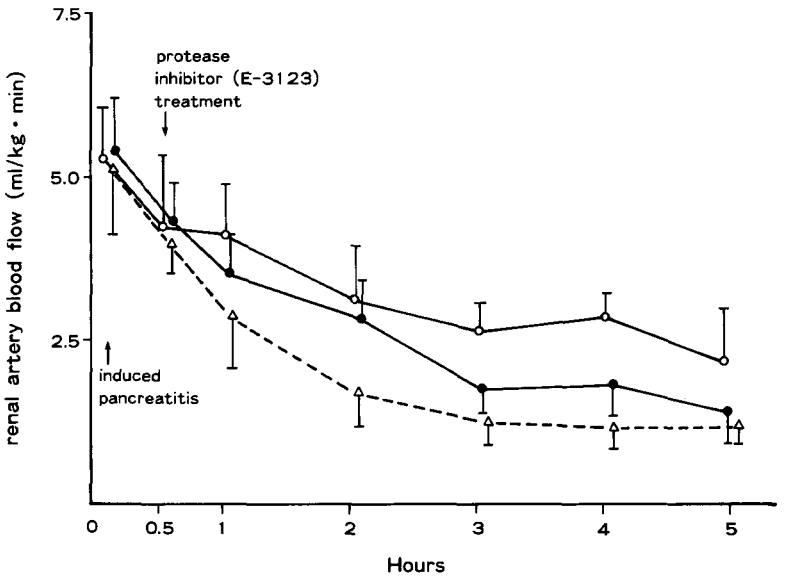

Figure 5. Renal arterial blood flow in the control group and protease inhibitor groups. For the control group and the high protease inhibitor group, it decreased statistically significant compared with pre-induction levels ($P<0.005$). For the low protease inhibitor group, it also decreased statistically significant compared with the pre-induction level ($P<0.01$). △---△: control group. ○---○: low protease inbitor group. ●---●: high protease inhibitor group.

inhibits trypsin and kallikrein. Experimental studies have produced conflicting results, with a controlled clinical trial conducted in 1965 showing it to be of no benefit. Interest in trasylol was aroused because of a controlled clinical trial in 1974, which indicated a decrease in mortality from 25% to 7.5%[6]. However, there have also been randomized double-blind clinical studied showing this agent to be of no benefit.

Recently, new synthetic protease inhibitors have been developed and considerable attention has been paid to these new agents. Like trasylol, the protease inhibitors gabexate mesilate (FOY), nafamostate mesilate (FUT) and the new synthetic protease inhibitors, E-3123, inhibit trypsin and kallikrein. These agents also posses a broader spectrum of protease inhibition than aprotinin by their additional capacity to inhibit plasmin, thrombin, $C_1\gamma$ and C_1 esterase and phospholipase A.

In a limited number of animal studies, synthetic protease inhibitors have been reported to exert beneficial effects on the course of acute pancreatitis. All of these reports, however, have looked at the effect of synthetic protease inhibitors on acute pancreatitis upon administration of the agent before or soon after the induction of acute pancreatitis. There have been no studies to investigate the therapeautic effect of these agents on hemodynamic changes during acute pancreatitis where these agents have been given some time after the induction of acute pancreatitis.

Shock and cardiovascular dysfunction are complications of acute pancreatitis and are important causes of mortality and morbidity. It has been suggested that shock and cardiovascular dysfunction in acute pancreatitis may be caused by hypovolemia after fluid sequestration around the pancreas, which may release a myocardial depressant factor.

Recently, we[7] demonstrated that beta-endorphin may play an important role in the development of hypotension and myocardial depression in acute pancreatitis and that the opiate antagonist naloxones improves hypotension and myocardial depression. Naloxone, however, does not inhibit beta-endorphin release during acute pancreatitis. Recent reports[8-10] indicated that trypsin-like enzymes and bradykinin may induce the production and the liberation of beta-endorphin from tissue. Serum trypsin-like activity and brady kin levels increase during acute pancreatitis, We[11] have also demonstration that an infusion of protease inhibitor (E-3123) commenced soon after the induction of acute pancreatitis inhibits beta-endorphin release during acute pancreatitis and improves hypotension and myocardial through an

inhibitory effect on trypsin-like enzyme activity and bradykinin release.

In this study, following the induction of acute pancreatitis, there was a statistically significant increase in plasma beta-endorphin levels seen in the control group as mentioned previously. When the new synthetic protease inhibitotr E-3123 was given 30 min after the induction of acute pancreatitis, the increase in plasma beta-endorphin levels in the high protease inhibitor group was statistically significant compared with pre-induction levels but the increase was statistically significantly lower than that of the control group. Plasma beta-endorphin levels in the low protease inhibitor group, however, did not increase and the levels in this group were statistically significantly lower than the levels for the control group.

Soon after the induction of acute pancreatitis, arterial blood pressure in the control group began to decrease progressively, and regardless of the dose of protease inhibitor used, it began to increase after 30 min.

A bolus injection followed by continuous intravenous infusion of E-3123 was given. Although arterial blood pressure in the high protease inhibitor group decreased gradually thereafter, it was well maintained in the low protease inhibitor group.

Cardiac output in the control group decreased steadily after the induction of acute pancreatitis. Cardiac output in both the low and high protease inhibitor groups decreased to the same level as in the control group 30 min after the induction of acute pancreatitis. However, following the bolus injection and a continuous infusion of E-3123, it was well maintained, especially in the low protease inhibitor group. The same tendencies were observed in renal arterial blood flow.

E-3123[12] is the strongest trypsin inhibitor known. Previous results revealed that trypsin-like enzyme activity in the control group increased, but that in the protease inhibitor infusion group commenced soon after the induction of acute pancreatitis remained unchanged. In this study serum amylase levels showed the same pattern. Although trypsin-like enzyme activity was not measured, E-3123 seems to have some inhibitory effect on pancreatic enzyme release.

This is the first time that a protease inhibitor, given after the induction of acute pancreatitis, has been shown to inhibit beta-endorphin release during acute pancreatitis. This occurred in the low protease inhibitor group. In the high protease inhibitor group, beta-endorphin release was also inhibited, but less effectively than in the low protease inhibitor group. Beta-endorphin release secondary to various stimuli can contribute to hypotension and myocardial depression. A protease inhibitor infusion as used in this experiment may bring about improvement in hypotension and myocardial depression to an extent by inhibiting the release of beta-endorphin. Such a result suggests that it is the inhibitory effect of the protease inhibitor on beta-endorphin release that contributes to improvement.

Recently, Mulfertheiner[13] reported that FOY is of proven usefulness in acute pancreatitis by conducting several open clinical trials and two multicentor placebo trials using different doses of FOY in Germany. The first study with FOY 900 mg/day showed positive results in so far as the incidence of surgery for acute pancreatitis was statistically significantly less in FOY treated patients. However, the second study using FOY 4000 mg/day involved 218 patients from 27 centers and did not find any statistical difference in mortality and major complications between the two groups. Also the positive effect associated with the reduction in pancreatic surgery required was not confirmed.

Theoretically, larger doses of protease inhibitor should inhibit protease enzyme activity more and improve the complications of acute pancreatitis mediated by protease enzymes compared with low doses.

However, German studies and our study suggest that there may be an optimal dose of these agents to bring about improvement in complications.

In conclusion, an infusion of an optimal dose of a protease inhibitor (E-3123), given following the induction of acute pancreatitis was found to inhibit beta-endorphin release and improve the hypotension and myocardial depression that arises dur-

ing acute pancreatitis. There may be an optimal therapeutic dose of this drug for the treatment of hypotension and myocardial depression and myocardial depression secondary to beta-endorphin release.

The synthetic protease inhibitor E-3123 (4-(2-succiminidoethylthio) 4-geranidinobenzoate methanesulfonate was a kind gift from the Eisai company in Japan.

References

1. Elias E, Redshaw M, Wood T. Diagnostic importance of changes in circulations of immunoreactive trypsin. Lancet 1977;9:66-68.
2. Wellborn JC, Alston JD, Cannon DJ, Read RC: Serum proteolytic and antiproteolylic activity in acute pancreatitis. Am J Surg 1983;646:834-847.
3. Muramatsu M, Fujii S. Inhibitory effects of w-guanidine esters on trypsin plasmin, plasma kallikrein and thrombin. Biochem Biophys Acta 1972;268:221-224.
4. Tamura Y, Hirado M, Okamura K, et al. Synthetic inhibitors of trypsin, Plasmin, kallikrein, thrombin, C_1 and $C_1\gamma$ esterase. Biochem Biophys Acta 1977;484:417-422.
5. Fujii S, Hitomi Y. New synthetic inhibitors of C_1 and $C_1\gamma$ esterase, thrombin, plasmin, kallikrein and trypsin. Biochem Biophys Acta 1981;661:342-345.
6. Trapnell JE, Rigby CC, Talbot CH, Duncan EHL. A control clinical trial of trasylol in the treatment of acute pancreatitis. Br J Surg 1974;61:177-182.
7. Satake K, Hiura A, Nishiwaki H, et al. Plasma beta-endorphin and the effect of naloxone on hemodynamic changes during experimental acute pancreatitis in dogs. Surg Gynecol & Obstet 1989;168:402-406.
8. Kudo T, Maeda S, Nakamae J, et al. Changes of the Met-Enkepholin-like peptide content induced by noxious stimuli in the rat incisor pulp. Life Sci 1983;33:677-680.
9. idem. A possible relationship from precursor proteins to opioid peptides and noxious stimulation in the rat incisor pulp. Life Sci 1983;33:691-684.
10. Kudo T, Keeroi M, Inoki M. In vitro production and release of opioid peptides in the tooth pulp induced by bradykinin. Neuropeptide 1986;7:391-392.
11. Satake K, Hiura A, Ha S-S, et al. Effect of a new synthetic protease inhibitor on beta-endorphin release during acute pancreatitis in dogs. Pancreas 1991;6:441-447.
12. Miyamoto K, Hishinuma I, Nagakawa J, et al. Effect of E-3123, a new protease inhibitor, on several protease acute and on experimental acute pancreatitis. Folia Pharmacul Japan 1988;91:285-293.
13. Malfertheinor P. Somatostatin and protease inhibitors: Two Principle in treatment of acute pancreatitis. Proc. 4th meeting of International Association of Pancreatology 1990;13. (Abstr)