Protective Effect of a Pancreatic Elastase Inhibitor Against a Variety of Acute Pancreatitis in Rats

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ABSTRACT—Protective effect of trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride (compound 1), a pancreatic elastase inhibitor, on three types of acute pancreatitis models was examined in rats. Mild, moderate and severe acute pancreatitis were induced by cerulein, the closed duodenal loop method and retrograde injection of a taurocholate plus trypsin solution into the pancreatic duct, respectively. Intravenous infusion of compound 1 at a dose of 30 mg/kg/hr resulted in lower increases in serum amylase, lipase, blood urea nitrogen (BUN) and creatinine levels in rats with mild cerulein-induced edematous pancreatitis. Compound 1 had no beneficial effect on pancreatitis in rats with moderate pancreatitis. In rats with severe pancreatitis, prophylactic treatment of compound 1 (30 mg/kg/hr) reduced both elevated serum BUN level and ascitic volume, and it histologically inhibited the extent of pancreatic edema and hemorrhage. These results suggest that pancreatic elastase is partially responsible for pancreatic edema and hemorrhage exhibited by rats with severe acute pancreatitis.

Keywords: Acute pancreatitis, Pancreatic elastase, Closed duodenal loop, Cerulein, Taurocholate

The pathogenesis of acute pancreatitis in humans is complicated and is not fully understood. Trypsin has long been considered a key enzyme in the initiation of this autodigestive disease. However, protease inhibitors such as nafamostat mesilate and gabexate mesilate exhibited few effects in patients with severe acute pancreatitis in spite of their potent inhibitory activities on trypsin, chymotrypsin, kallikrein, plasmin, thrombin, phospholipase and complement (1–4).

Pancreatic elastase is also a pancreatic enzyme, similar to trypsin, chymotrypsin, kallikrein and phospholipase. It is known to hydrolyze elastin, which is a primary component of vascular tissue (5, 6). The elevation of serum pancreatic elastase was observed in patients with acute pancreatitis (7–10) as well as in animals with acute pancreatitis (11, 12). Therefore, the inhibitory effect of trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride (compound 1), a pancreatic elastase inhibitor (13), was examined in rats with acute pancreatitis induced by a variety of methods to investigate the participation of pancreatic elastase in the pathogenesis of this disorder.

Three types of rat acute pancreatitis models, mild, moderate and severe, were used to examine the effects of compound 1 on the progression of pancreatitis. Mild, moderate and severe acute pancreatitis were induced by cerulein, the closed duodenal loop (CDL) method and retrograde injection of a taurocholate plus trypsin solution into the pancreatic duct in rats, respectively. The severity of pancreatitis of each model corresponded to human patients presenting with mild, moderate and severe acute pancreatitis.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 220–300 g (SLC, Hamamatsu, Japan) were used. The animals were maintained on ordinary laboratory food and tap water ad libitum under a 12-hr light-dark cycle. For intravenous infusion of test compounds, under ether anesthesia, a polyethylene catheter was inserted into the left external jugular vein and exteriorized through a subcutaneous tunnel in the retroscapular area. Three or four days after the operation, the experiment was performed as described below.

All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

Pancreatic and neutrophil elastases activity

Porcine pancreatic elastase activity was determined by succinyl-Ala-Ala-Ala-*p*-nitroanilide hydrolysis. Succinyl-Ala-Ala-Ala-*p*-nitroanilide (2 mM) and compound 1 were incubated with the enzyme (0.02 μg protein) in Tris-HCl buffer (0.2 M, pH 8.0) in a final volume of 202 μl. After incubation at 37℃ for 1 hr, the absorbance at 405 nm of *p*-nitroanilide generated from the substrate was measured. The inhibitory effect on human neutrophil elastase was determined according to the method of Kawabata et al. (14) with minor modifications. Succinyl-Ala-Pro-Ala-*p*-nitroanilide (0.2 mM) and compound 1 were incubated with the enzyme (1.1 μM) in Tris-HCl buffer (0.1 M, pH 8.0) containing 0.2 M sodium chloride in a final volume of 202 μl. After incubation at 37℃ for 1 hr, the absorbance at 405 nm of *p*-nitroanilide generated from the substrate was measured.

Cerulein-induced mild acute pancreatitis

Mild edematous acute pancreatitis was induced by intravenous infusion of cerulein in rats according to the method of Hirano and Takeuchi (15) with minor modifications. Rats were anesthetized with urethane (1.25 g/kg, i.p.). As the pancreatic blood flow markedly decreased in rats with acute pancreatitis, higher doses of compound 1 was required to act on the pancreatic tissue. Because intravenous infusion of compound 1 at 100 mg/kg/hr for 12 hr was toxic to rats (n=10), the dose of compound 1 was set at 10–30 mg/kg/hr. A solution of cerulein (5 μg/kg/hr) and compound 1 (10–30 mg/kg/hr) was intravenously infused for 5 hr by an infusion pump (KDS Type 220; Muromachi Kikai Co., Tokyo) at a rate of 0.6 ml/hr via the left femoral vein. After the cessation of infusion, arterial blood was taken from the left common carotid artery to measure arterial oxygen pressure. Venous blood was also taken from the inferior vena cava, and serum was then separated by centrifugation at 1500×*g* for 10 min at 4℃. The pancreas was quickly removed, weighed, dried in an oven at 100℃ for 24 hr, and then reweighed to measure pancreatic water content. Pancreatic function was assessed by pancreatic weight and serum amylase and lipase levels; hepatic function, by serum GOT and GPT levels; renal function, by serum blood urea nitrogen (BUN) and creatinine levels; and pulmonary function, by arterial oxygen pressure.

CDL-induced moderate acute pancreatitis

Moderate acute pancreatitis was induced by the CDL method in rats according to the method of Nevalainen and Seppä (16) with minor modification. The rats were fasted for 24 hr before the operation, but were allowed free access to water. The rats were laparotomized along the midline under ether anesthesia. A Teflon AWG tube (spaghetti tube), 2.5 cm in length, 3.0 mm in the outer diameter, and 2.4 mm in bore diameter, was inserted from the forestomach into the duodenum. A closed loop was created using the intraduodenal teflon tube by ligating the duodenum with surgical sutures at two points on both sides of the common bile duct. The blood supply to the duodenal closed loop was carefully preserved. The intraduodenal tube restored a free passage for the intestinal contents. The length of the closed loop was approximately 2.0 cm. After the forestomach was closed, the abdomen was sutured. Sham-operated rats also had a teflon tube inserted. The duodenum was ligated at a point proximal to the common bile duct.

Time-course changes in pancreatic, hepatic, renal and pulmonary functions were evaluated 1, 2 and 3 days after the induction of pancreatitis. Pancreatic function was assessed by pancreatic weight and serum amylase and lipase levels; hepatic function, by serum GOT and GPT levels; renal function, by serum BUN level; and pulmonary function, by arterial oxygen pressure and pulmonary vascular permeability.

The rats were intravenously infused by an infusion pump with compound 1 at a dose of 30 mg/kg/hr (0.6 ml/hr) for 24 hr beginning with the formation of the CDL. During the intravenous infusion of compound 1, the rats were placed in Bollman's cages. Pancreatic and hepatic functions were evaluated 24 hr after the formation of the CDL, because the extent of pancreatitis reached peak values at that time (see "Results").

Rats were anesthetized with urethane (1.25 g/kg, i.p.), and arterial blood was taken from the left common carotid artery to measure arterial oxygen pressure. Venous blood was also taken from the inferior vena cava, and serum was then separated by centrifugation at 1500×*g* for 10 min at 4℃. The pancreas was quickly removed, weighed, dried in oven at 100℃ for 24 hr and reweighed to measure pancreatic water content. To examine pulmonary vascular permeability, rats were injected with 0.5% Evans blue (1 ml/rat, i.v.) 30 min before the anesthesia. After anesthesia, the lungs were perfused through the right cardiac ventricle with physiological saline and quickly removed. The lungs were then minced and soaked in formamide (3 ml) for 2 days at 4℃.

Taurocholate plus trypsin-induced severe acute pancreatitis

Severe acute pancreatitis was induced by retrograde injection (0.5 ml/kg) of a 5% sodium taurocholate plus 1% trypsin solution into the pancreatic duct via the common bile duct in rats. Intraductal injection of the mixture of taurocholate at a concentration of approximately 5% and trypsin at a concentration of approximately 1% was fre-

quently used to induce severe acute pancreatitis in rats (17, 18). Taurocholate plus trypsin solution was injected for 1 min by using an infusion pump. The rats were intravenously infused with compound 1 at a dose of 30 mg/kg/hr (0.6 ml/hr) for 6.5 or 12.5 hr from 30 min before the induction of pancreatitis by an infusion pump. Infusion of compound 1 was started 30 min before the induction of pancreatitis to examine its prophylactic effect because pancreatic hemorrhage in rats with taurocholate plus trypsin-induced pancreatitis was observed within a few minutes after the intraductal injection. During the intravenous infusion of compound 1, the rats were placed in Bollman's cages. Pancreatic, hepatic, renal and pulmonary functions were evaluated in rats with severe acute pancreatitis 6 or 12 hr after the induction of pancreatitis. Pancreatic function was assessed by serum amylase and lipase levels and ascitic volume; hepatic function, by serum GOT and GPT levels; renal function, by serum BUN level; and pulmonary function, by arterial oxygen pressure.

Rats were anesthetized with urethane (1.25 g/kg, i.p.), and arterial blood was taken from the left common carotid artery to measure arterial oxygen pressure. Venous blood was also taken from the inferior vena cava, and serum was then separated by centrifugation at $1500 \times g$ for 10 min at 4℃. The pancreas was quickly removed to perform histologic evaluation.

In another series of experiments, the inhibitory effect of a pancreatic elastase inhibitor on mortality was evaluated in rats with severe acute pancreatitis. The rats were intravenously infused with compound 1 at a dose of 30 mg/kg/hr (0.6 ml/hr) by an infusion pump for 6.5 hr from 30 min before the induction of pancreatitis. During the intravenous infusion of compound 1, the rats were placed in Bollman's cages. The survival rate was measured for 24 and 48 hr after the induction of pancreatitis.

Biochemical determinations

Serum amylase, lipase, GOT, GPT, BUN and creatinine were measured with an automatic analyzer (7250 type; Hitachi, Tokyo). Aliquots (500 μl) of 1:1 to 1:50 dilutions of the serum were used in the assay. Arterial oxygen pressure was measured with a Ciba Corning 280 Blood Gas System (Ciba-Corning, Tokyo).

Measurement of pulmonary vascular permeability

To obtain the supernatant of formamide solution, minced lungs were centrifuged at $1500 \times g$ for 10 min at 4℃. Absorbance at 620 nm was measured with a spectrophotometer (V-520; Nihon Kohden, Tokyo). Results are expressed as μg of Evans blue per ml.

Histology

Pancreata were fixed in 10% buffered formalin and embedded in paraffin. Three-micron sections were cut and stained with hematoxylin and eosin. Pancreatic interstitial edema was graded on a scale ranging from 0 to 2 (0 = none, 1 = local, 2 = extensive). Hemorrhage and necrosis were graded on a scale ranging from 0 to 3 (0 = absence of lesions, 1 = one or a few spots of slight lesions, 2 = local lesions, 3 = extensive lesions).

Statistics

All values are expressed as the mean±S.E.M. The inhibition constant (K_i) for elastases was determined by the method of Graf (19). The differences between treatment and control groups were determined by the Tukey-Kramer test or Dunnett's multiple range test. Probabilities of $<5\%$ ($P<0.05$) were considered significant.

Drugs

Trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride (compound 1) was prepared by the Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki). Cerulein sulfate, sodium taurocholate and porcine trypsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Porcine pancreatic elastase and human neutrophil elastase were purchased from Elastin Products Company, Inc. (Owensville, MO, USA) and Athens Research and Technology, Inc. (Athens, GA, USA), respectively. Succinyl-Ala-Ala-Ala-*p*-nitroanilide and succinyl-Ala-Pro-Ala-*p*-nitroanilide were obtained from Peptide Institute, Inc. (Osaka). All compounds were dissolved in physiological saline. All compound doses are given as the free base.

RESULTS

Inhibitory effect on pancreatic elastase

Trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride (compound 1) strongly inhibited porcine pancreatic elastase, with a K_i value of 5×10^{-8} M. However, it had no inhibitory effect on human neutrophil elastase at a concentration of 3×10^{-5} M.

Inhibitory effect in cerulein-induced mild acute pancreatitis

In rats with cerulein-induced pancreatitis, pancreatic water content, and serum amylase, lipase, BUN and creatinine levels were significantly elevated (Fig. 1). Serum GOT and GPT levels and arterial oxygen pressure were not significantly different between rats with pancreatitis and control rats (data not shown, $n=7-8$). Cerulein-treated rats showed gross pancreatic edema without pancreatic hemorrhage. Elevated pancreatic water content and serum parameter levels were not affect-

ed by intravenously infused compound 1 at 10 mg/kg/hr for 5 hr in rats with cerulein-induced pancreatitis (data not shown, n=8). Intravenous infusion of compound 1 (30 mg/kg/hr) for 5 hr from the start of cerulein infusion showed only a tendency to reduce the increase in serum amylase, BUN and creatinine levels in rats with cerulein-induced pancreatitis, but significantly inhibited increases in the serum lipase level (Fig. 1).

Time-course changes in pancreatic, hepatic, renal and pulmonary functions in CDL-induced moderate acute pancreatitis

Rats with moderate acute pancreatitis induced by CDL showed marked pancreatic edema and mild pancreatic hemorrhage. Rats with CDL-induced pancreatitis did not die for at least 3 days after the creation of the loop (n=10). Pancreatic water content, and serum amylase

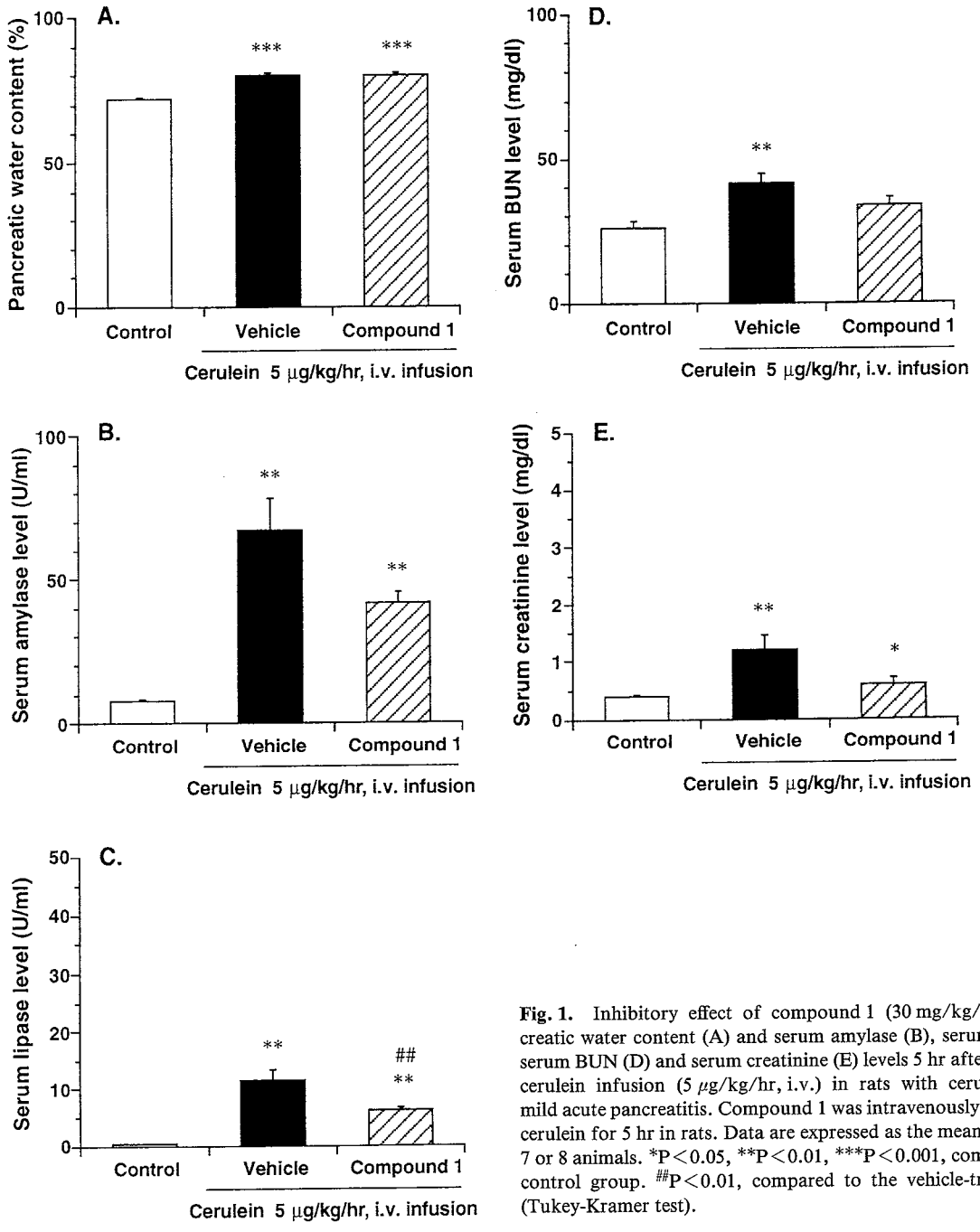


Fig. 1. Inhibitory effect of compound 1 (30 mg/kg/hr) on pancreatic water content (A) and serum amylase (B), serum lipase (C), serum BUN (D) and serum creatinine (E) levels 5 hr after the start of cerulein infusion (5 μg/kg/hr, i.v.) in rats with cerulein-induced mild acute pancreatitis. Compound 1 was intravenously infused with cerulein for 5 hr in rats. Data are expressed as the mean±S.E.M. of 7 or 8 animals. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to the control group. $^{\#\#}P<0.01$, compared to the vehicle-treated group (Tukey-Kramer test).

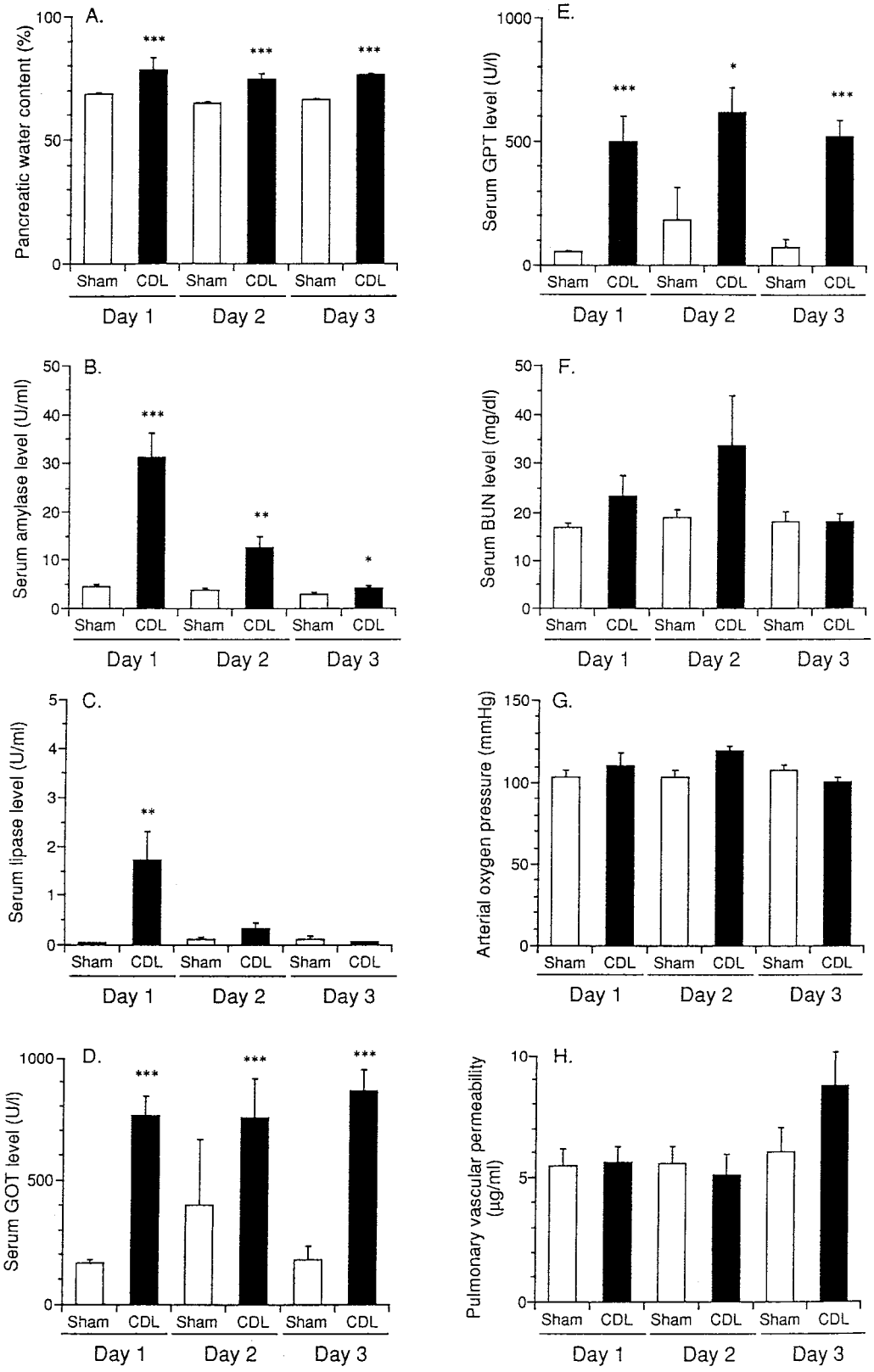


Fig. 2. Time-course of pancreatic water content (A); serum amylase (B), lipase (C), GOT (D), GPT (E) and BUN (F) levels; arterial oxygen pressure (G); and pulmonary vascular permeability (H) 1, 2 and 3 days after closed duodenal loop (CDL) creation in rats with CDL-induced moderate acute pancreatitis. Data are expressed as the mean ± S.E.M. of 10 or 11 animals. *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared to the sham-operated group (Dunnett's multiple range test).

and lipase levels significantly increased in CDL rats (Fig. 2: A–C). The peak of this elevation was observed 1 day after the creation of CDL, and the elevation of pancreatic water content was sustained for at least 3 days (Fig. 2A). Ascitic volume was not markedly different between sham-operated (0.4±0.1 ml) and CDL (1.6±0.6 ml) rats 1 day after the creation of CDL (n=10–11). Serum GOT and GPT levels were also significantly elevated in CDL rats (Fig. 2: D and E). However, a slight increase in serum BUN level was observed 2 days after the creation of CDL (Fig. 2F). Arterial oxygen pressure in CDL rats was not different from that in sham-operated rats, whereas pulmonary vascular permeability in CDL rats was slightly increased 3 days after the creation of CDL (Fig. 2: G and H).

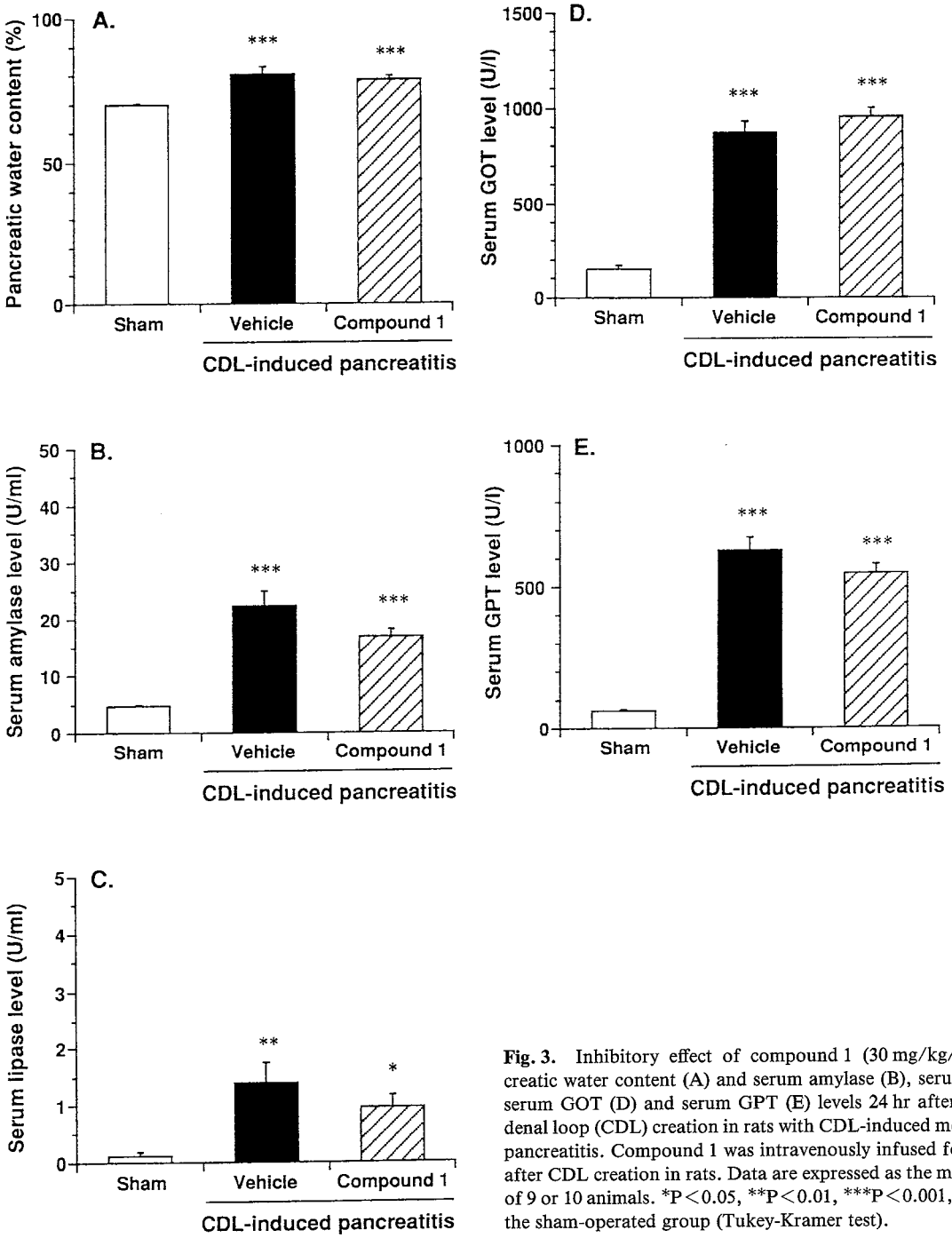
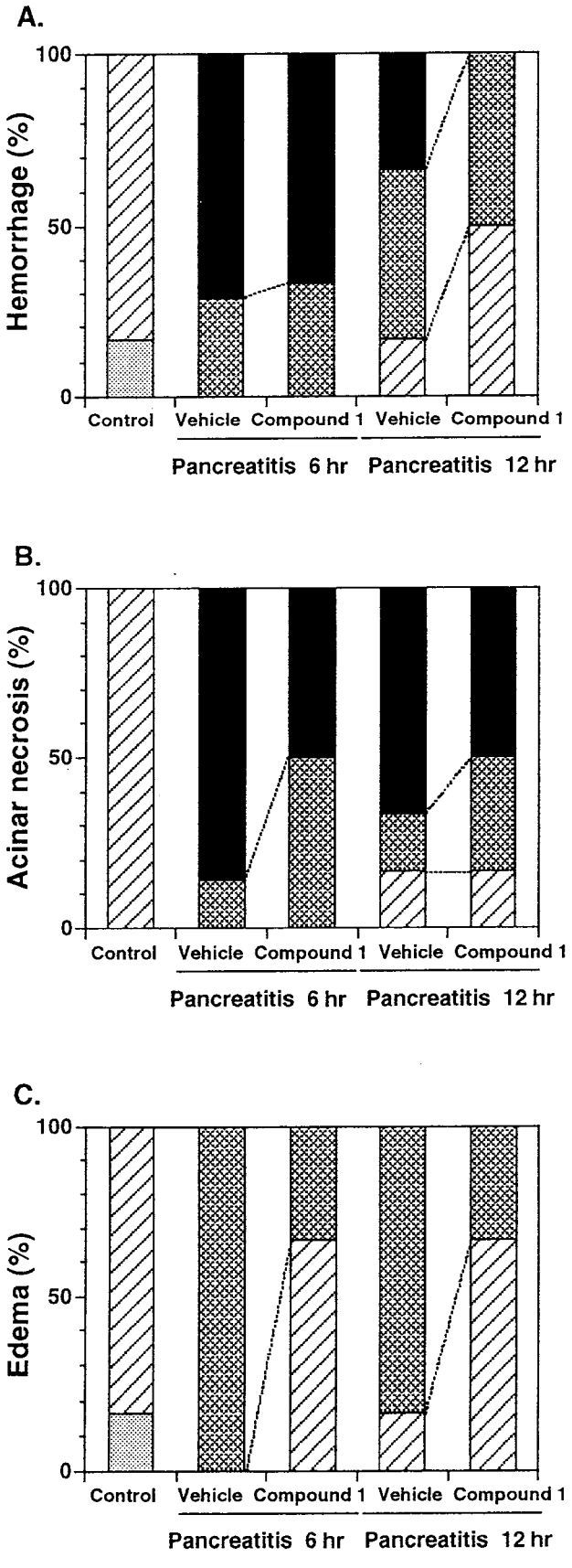


Fig. 3. Inhibitory effect of compound 1 (30 mg/kg/hr) on pancreatic water content (A) and serum amylase (B), serum lipase (C), serum GOT (D) and serum GPT (E) levels 24 hr after closed duodenal loop (CDL) creation in rats with CDL-induced moderate acute pancreatitis. Compound 1 was intravenously infused for 24 hr soon after CDL creation in rats. Data are expressed as the mean±S.E.M. of 9 or 10 animals. *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared to the sham-operated group (Tukey-Kramer test).



Inhibitory effect in CDL-induced moderate acute pancreatitis

In CDL rats, intravenous infusion of compound 1 (30 mg/kg/hr) for 24 hr from the creation of CDL had no effect on the increase in pancreatic water content and serum amylase, lipase, GOT and GPT levels 24 hr after the creation of CDL (Fig. 3).

Inhibitory effect in taurocholate plus trypsin-induced severe acute pancreatitis

Severe acute pancreatitis was induced by retrograde injection of a 5% sodium taurocholate plus 1% trypsin solution into the pancreatic duct in rats, and the histologic findings are summarized in Fig. 4. Pancreatic edema, hemorrhage and necrosis were observed in rats with severe acute pancreatitis 6 or 12 hr after the induction of pancreatitis (Figs. 4 and 5). Although 10 mg/kg/hr of intravenously infused compound 1 for 6.5 hr showed only a tendency to reduce the elevated ascitic volume in rats with severe pancreatitis, it had no effect on pancreatic edema, hemorrhage, and necrosis and serum parameter levels (data not shown, n=7). Prophylactic treatment of intravenous infusion of compound 1 (30 mg/kg/hr) for 6.5 or 12.5 hr inhibited the extent of the pancreatic hemorrhage 12 hr after the induction of pancreatitis, whereas it had no effect 6 hr after the induction of pancreatitis (Fig. 4A). Compound 1 showed no beneficial effect on the extent of the pancreatic necrosis (Fig. 4B). On the other hand, compound 1 inhibited the extent of the pancreatic edema 6 and 12 hr after the induction of pancreatitis (Fig. 4C). Serum amylase and lipase levels showed no significant difference between compound 1- and vehicle-treated rats (Fig. 6: A and B). However, compound 1 showed lower increase in serum BUN level and ascitic volume 6 or 12 hr after the induction of pancreatitis (Fig. 6: C and D). Serum GOT and GPT levels, and arterial oxygen pressure in rats with taurocholate plus trypsin-induced pancreatitis were not different from those in the control rats (data not shown, n=6–9). The survival rate of compound 1- and vehicle-treated groups (prophylactic treatment of intravenous infusion for 6.5 hr) was almost the same 24 hr (91% vs 82%) and 48 hr (36% vs 36%) after the induction of pancreatitis (n=11).

Fig. 4. Inhibitory effect of compound 1 (30 mg/kg/hr) on pancreatic histology [hemorrhage (A), acinar necrosis (B) and edema (C)] at 6 and 12 hr after the induction of pancreatitis in rats with taurocholate plus trypsin-induced severe acute pancreatitis. Compound 1 was intravenously infused for 6.5 or 12.5 hr from 30 min before the induction of pancreatitis in rats. Histologic grading of pancreatic hemorrhage and acinar necrosis was made using a scale ranging from 0 to 3 (0 = absence of lesions ▒, 1 = one or a few spots of slight lesions ▨, 2 = local lesions ▩, 3 = extensive lesions ■). Histologic grading of pancreatic edema was made using a scale ranging from 0 to 2 (0 = none ▒, 1 = local ▨, 2 = extensive ▩).

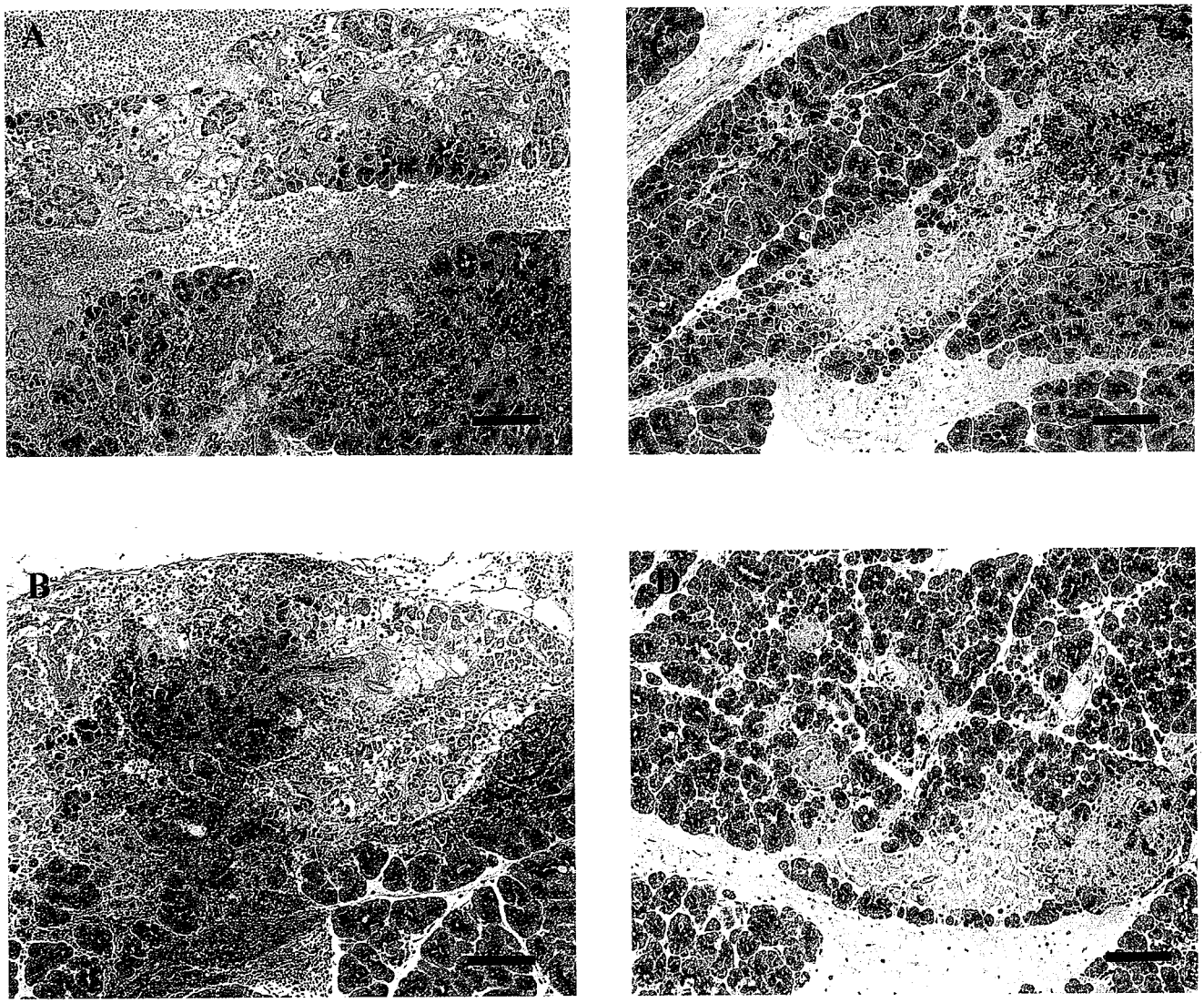


Fig. 5. Typical histologic features of severe taurocholate plus trypsin-induced acute pancreatitis in rats. Pancreatic tissue was obtained from rats with pancreatitis at 6 (A) or 12 hr (B) after the induction of pancreatitis or rats with pancreatitis infused with compound 1 (30 mg/kg/hr) at 6 (C) or 12 hr (D) after the induction of pancreatitis. Compound 1 was intravenously infused for 6.5 or 12.5 hr from 30 min before the induction of pancreatitis. Magnification: ×100; bar represents 100 μm.

DISCUSSION

The effects of trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride (compound 1), a pancreatic elastase inhibitor, on acute pancreatitis were examined in rats using a variety of experimental conditions to investigate the participation of pancreatic elastase in the pathogenesis of acute pancreatitis. Three types of rat acute pancreatitis models, mild, moderate and severe, were used.

Mild acute pancreatitis was induced by cerulein in rats. Intravenous infusion of compound 1 showed a tendency to reduce the increases in serum amylase, lipase, BUN and creatinine levels, but had no effect on elevated pancreatic water content. Active pancreatic elastase content in the pancreatic tissue was previously reported to be elevated (20–22). Taken together, pancreatic elastase may be partly involved in the induction of mild edematous pancreatitis in rats.

Moderate acute pancreatitis was induced by the CDL method in rats. The process of the induction of acute pancreatitis in CDL-induced pancreatitis has been reported to be similar to that in patients with gallstone-induced acute pancreatitis. Since long-term investigation of the development and progression of acute pancreatitis induced by CDL has not been performed, time-course changes in the extent of pancreatitis and organ dysfunc-

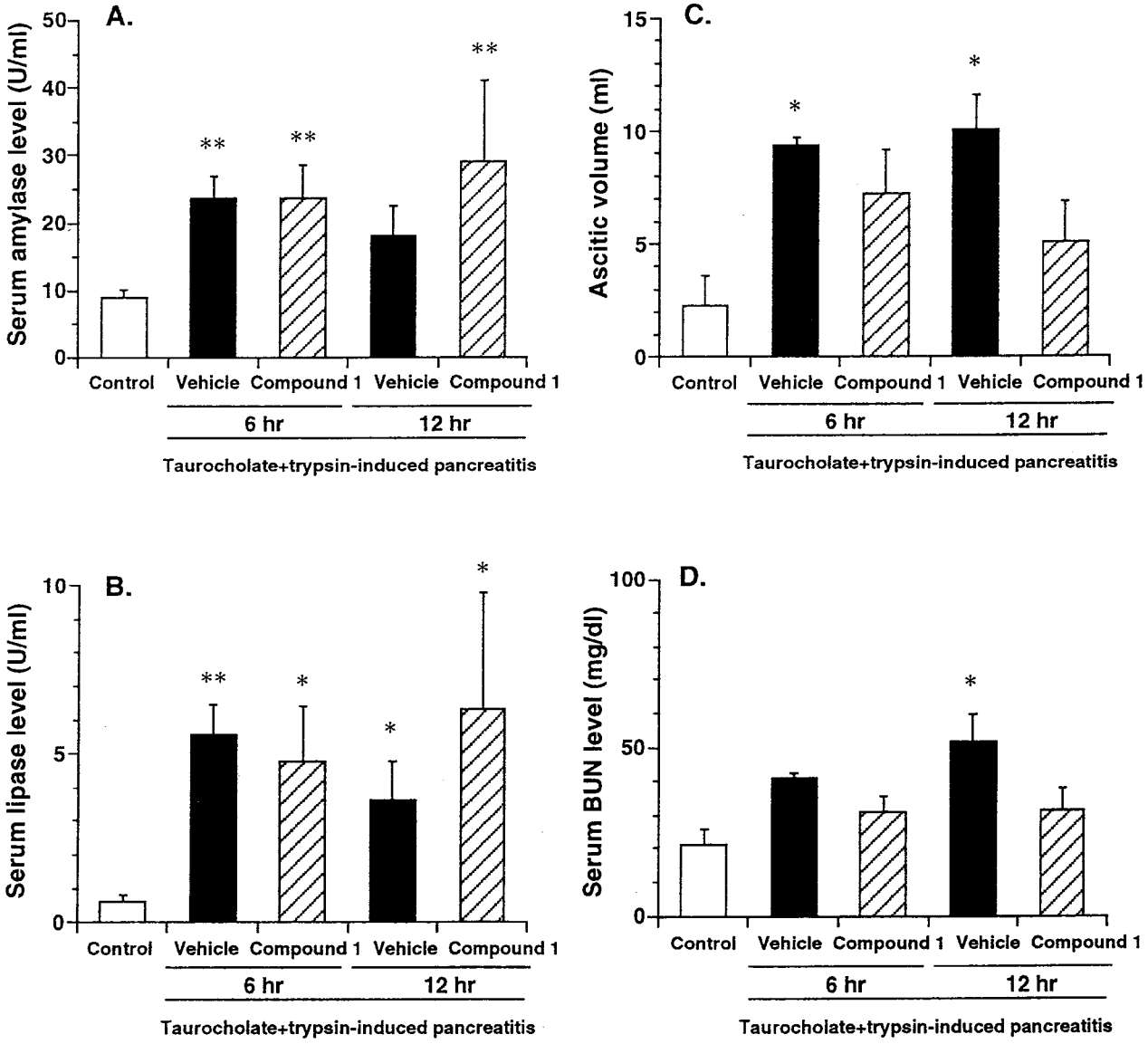


Fig. 6. Inhibitory effect of compound 1 (30 mg/kg/hr) on serum amylase (A) and serum lipase (B) levels, volume of ascites (C) and serum BUN level (D) 6 and 12 hr after the induction of severe taurocholate plus trypsin-induced acute pancreatitis in rats. Compound 1 was intravenously infused for 6.5 or 12.5 hr from 30 min before the induction of pancreatitis in rats. Data are expressed as the mean±S.E.M. of 6 to 9 animals. *$P<0.05$, **$P<0.01$, compared to the control group (Tukey-Kramer test).

tions were also examined in this study. In rats with CDL-induced pancreatitis, marked pancreatic edema and mild pancreatic hemorrhage were produced, and the peak pancreatitis was observed 1 day after the creation of the loop. Elevated serum amylase and lipase levels gradually recovered within 3 days, whereas sustained elevation of pancreatic water content was observed, suggesting that pancreatic acinar cells were exhausted due to long-term pancreatitis in CDL rats. Slight renal and pulmonary dysfunctions were elicited after the induction of pancreatitis in CDL rats, but CDL-induced pancreatitis did not cause death. Hepatic dysfunction was also observed in CDL rats, probably due to pancreatic juice regurgitated into the bile duct or extravasated into the vascular system attacking liver tissue. In patients with moderate and severe acute pancreatitis, multiple organ failure including hepatic, renal and pulmonary dysfunction is common and highly fatal. In this report, CDL-induced pancreatitis did not produce marked organ failure, whereas CDL produced moderate acute pancreatitis in rats, indicating that the CDL-induced pancreatitis model was not directly comparable to patients with acute pancreatitis. In this model, intravenous infusion of compound 1 had no effect on the elevated pancreatic water content, or serum amyl-

ase, lipase, GOT and GPT levels 1 day after the creation of CDL. The serine protease inhibitors nafamostat mesilate and FUT-187, which inhibit trypsin, chymotrypsin, kallikrein, plasmin, thrombin, phospholipase and complement, were reported to have beneficial effects on CDL-induced acute pancreatitis in rats (23, 24). These findings suggest that pancreatic enzymes other than pancreatic elastase might be involved in the pathogenesis of CDL-induced pancreatitis in rats.

Severe acute pancreatitis was induced by retrograde injection of sodium taurocholate plus trypsin solution into the pancreatic duct in rats. Prophylactic intravenous infusion of compound 1 inhibited the extent of pancreatic edema and hemorrhage, but it had no beneficial effect on the extent of pancreatic necrosis in rats with severe acute pancreatitis. Animals treated with compound 1 also showed a lower increase in serum BUN level and ascitic volume, although serum amylase and lipase levels showed no significant difference between compound 1- and vehicle-treated rats. These results suggest that pancreatic elastase may play a role in the induction of pancreatic edema and hemorrhage in rats with severe acute pancreatitis. However, compound 1 partly inhibited the pancreatitis only at the high dose (30 mg/kg/hr) in rats with severe acute pancreatitis as well as rats with mild acute pancreatitis. A high dose of intravenously infused compound 1 might be required to act on the pancreatic tissue because the pancreatic blood flow markedly decreases in rats with pancreatitis. Serum pancreatic elastase level has been found to increase in rats and pigs with bile-induced hemorrhagic pancreatitis (11, 12). Pancreatic elastase is known to hydrolyze elastin, which is a primary component of vascular tissue (5, 6), and is thought to participate in the pancreatic hemorrhage seen in severe acute pancreatitis. Fric et al. (25, 26) reported that intraperitoneal injection or peritoneal lavage with glutaryl-trialanine-ethylamide, a pancreatic elastase inhibitor, reduced mortality and pancreatic hemorrhage in rats with taurocholate-induced severe acute pancreatitis. The results of this study are partly consistent with their findings. On the other hand, pancreatic necrosis in rats with severe acute pancreatitis is known to be induced by the activation of lipase and phospholipase or the release of proteases from inflammatory cells in the pancreatic tissue. Therefore, compound 1, a pancreatic elastase inhibitor, was not thought to inhibit the pancreatic necrosis in severe acute pancreatitis model.

Levy et al. (27) reported that hypovolemia caused renal dysfunction in dogs with acute, bile-induced pancreatitis. This renal dysfunction was replicated by the intravenous infusion of trypsin, chymotrypsin, pancreatic elastase and phospholipase A_2, but not by lipase or amylase. Thus, the inhibitory effect of pancreatic elastase inhibitor on renal dysfunction in rats with severe pancreatitis in this study may be due to a decrease in the volume of ascites (hypovolemia) or prevention of direct cytotoxicity of pancreatic elastase extravasated into the vascular system against the renal tissue.

In conclusion, pancreatic elastase partially participates in the pancreatic edema and hemorrhage seen in mild and severe acute pancreatitis in rats. It is further suggested that CDL-induced pancreatitis does not produce vital organ failure, unlike that seen in patients with moderate or severe acute pancreatitis.

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