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Oral Administration of Sepimostat Mesilate Prevents Acute Alcohol Pancreatic Injury in Rats

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Abstract

The preventive effect of a novel synthetic serine protease inhibitor, sepimostat mesilate (sepimostat), on acute alcohol pancreatic injury, induced by exocrine hyperstimulation and ethanol administration, was assessed and compared with that of a similar protease inhibitor, camostat mesilate (camostat).

Conscious rats were infused with 1 μg mL^{-1} h^{-1} caerulein intravenously for 6 h and with 0·1 g mL^{-1} h^{-1} ethanol for 9 h, with the latter infusion beginning 3 h after the start of the caerulein infusion. Sepimostat or camostat was administered orally 1 h before the caerulein infusion.

Rats infused with caerulein plus ethanol showed increased plasma amylase and lipase activities, and aggravated pancreatic interstitial oedema when compared with rats given caerulein alone. Sepimostat at 10 and 30 mg kg^{-1} prevented the increase in plasma amylase and lipase activities caused by caerulein plus ethanol infusion. Sepimostat at 30 mg kg^{-1} suppressed the histological change. Camostat did not show any preventive effects at the equivalent dose. When conscious rats were infused with 1 μg mL^{-1} h^{-1} caerulein alone intravenously for 6 h, plasma amylase and lipase activities were increased compared with rats given saline. Neither drug prevented the increase in these activities at 30 mg kg^{-1}.

Our results suggest that sepimostat has superior preventive effects on alcohol-induced acute pancreatic injury compared with camostat. Sepimostat may thus be a useful drug in the therapy of alcohol-induced pancreatitis.

Alcohol consumption is a major cause of acute and chronic pancreatitis (Singh & Simsek 1990; Haber et al 1995), and alcohol-related pancreatitis (alcohol pancreatitis) is a severe and intractable disease (Gullo et al 1988). However, the mechanism of the pathogenesis of alcohol pancreatitis has remained unclear, and there is no beneficial therapy for this disease.

Sepimostat mesilate (sepimostat; 6-amidino-2-naphthyl-4-[(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethanesulphonate; Figure 1) is a novel oral synthetic serine protease inhibitor that was developed in Japan. This drug has been reported to be a strong inhibitor of serine proteases (Nakamura et al 1995) and of complement in-vivo (Oda et al 1990). Sepimostat is reported also to have protective effects against acute pancreatitis induced by trypsin, phospholipase A_2, caerulein and a choline-deficient ethionine supplement diet in-vivo (Murakami et al 1990; Wang et al 1995). Furthermore, in a clinical study it was confirmed to be a useful drug for chronic pancreatitis caused by intracellular activation of pancreatic enzymes (Takeuchi et al 1996). Camostat mesilate (camostat) is a protease inhibitor similar to sepimostat (Tamura et al 1977;

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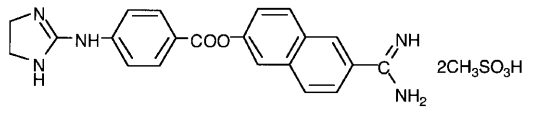

Figure 1. Chemical structure of sepimostat.

Kisfalvi et al 1995), and has been used as a therapeutic drug for chronic pancreatitis in Japan (Ishii et al 1980). However, the efficacy of these protease inhibitors toward alcohol pancreatitis has not yet been investigated.

Clinically, it is well known that alcohol consumption aggravates the recovery from acute and chronic pancreatitis, which is the representative pathogenesis of alcohol pancreatitis. Recently, by combining ethanol administration and exocrine hyperstimulation induced by a supramaximal dose of caerulein, a cholecystokinin (CCK) analogue, we have developed a new experimental model in which ethanol administration aggravates the recovery from caerulein-induced pancreatic injury, such as hyperamylasaemia and histological changes (Yuasa et al 1998).

In this study, we have assessed the preventive effect of sepimostat on alcohol pancreatitis and compared it with that of camostat in rats.

Materials and Methods

Drugs and chemical reagents

Sepimostat was a generous gift from Torii Pharmaceutical Co. Ltd (Tokyo, Japan). Sepimostat is water soluble, and has a molecular weight of 565·63. Camostat was purchased from Ono Pharmaceutical Co. Ltd (Osaka, Japan). Caerulein was purchased from Sigma Chemical Co. (St Louis, MO). Ethanol, L-Type Wako Amylase kit, and Lipase Color Auto Test Wako kit were purchased from Wako Pure Chemical Co. (Osaka, Japan). All other chemicals were of the best quality commercially available.

Experimental protocol

Six-week-old male Wistar rats (Shizuoka Animal Facility Center, Shizuoka, Japan) were housed in a controlled environment, exposed to a 12-h light/dark cycle, with laboratory rat food pellets and water freely available for a minimum of five days. Following an overnight fast, the animals (160–190 g) were anaesthetized with intraperitoneal pentobarbital sodium (45 mg kg^{-1}). A silicone elastomer catheter was then inserted into the right jugular vein and tunnelled subcutaneously to the neck. The catheter was protected at the exit point by being passed through a stainless steel spring sewn onto the skin. The catheter and spring were then connected to a follow-through swivel that permitted continuous infusion of solution into the animal while it was in a metabolic unit. The animals were infused with saline at a rate of 0·5 mL h^{-1} via the catheter to avoid thrombus formation, and allowed to recover overnight before initiation of the experimental procedures.

Drug effect on caerulein and ethanol-induced alcohol pancreatic injury. The animals were randomly allocated to ten experimental groups before the operation. On the day of the experiment, either sepimostat, camostat (both diluted with distilled water) or distilled water was orally administered (5·0 mL kg^{-1}) by use of a gastric needle 1 h before the caerulein infusion was started; then saline was infused (0·5 mL h^{-1}). The animals in each group (n = 12) received caerulein and ethanol as follows: group 1, normal group, saline was infused at a rate of 1·0 mL h^{-1} for 12 h. Group 2, caerulein group, caerulein was infused at a rate of 1·0 μg mL^{-1} h^{-1} for 6 h, and then saline was subsequently infused at a rate of 1·0 mL h^{-1} for 6 h. Group 3, ethanol group, saline was infused at a rate of 1·0 mL h^{-1} for 3 h, and thereafter ethanol was infused at a rate of 0·1 g mL^{-1} h^{-1} for 9 h. Group 4, caerulein plus ethanol group (pancreatitis model), caerulein was infused at a rate of 1·0 μg mL^{-1} h^{-1} for 6 h, with a 9-h ethanol infusion at a rate of 0·1 g mL^{-1} h^{-1} beginning after the first 3-h of the caerulein infusion. Groups 5, 6, and 7, sepimostat groups, 1 h after sepimostat (3, 10, or 30 mg kg^{-1}) had been given, caerulein and ethanol were infused as in the pancreatitis model. Groups 8, 9 and 10, camostat groups, 1 h after camostat (3, 10, or 30 mg kg^{-1}) had been given, caerulein and ethanol were infused as in the pancreatitis model. After the total 12-h infusion period, the animals were anaesthetized with ether, and blood from the inferior vena cava was collected in chilled heparinized tubes for the determination of amylase and lipase activities. Immediately after blood collection, the pancreas was removed and fixed in 20% formaldehyde for histological examination.

Drug effect on caerulein-induced pancreatic injury. The animals were randomly allocated into four experimental groups, and treated as follows: drug administration and then saline infusion for 1 h were conducted according to the procedures described in the first experiment. The animals in each group (n = 10) received caerulein or saline. Group 11, normal group, saline was infused at a rate of 1·0 mL h^{-1} for 6 h. Group 12, caerulein group, caerulein was infused at a rate of 1·0 μg mL^{-1} h^{-1} for 6 h. Group 13, sepimostat group, 1 h after sepimostat (30 mg kg^{-1}) had been given, caerulein was infused as in the caerulein group. Group 14, camostat group, 1 h after camostat (30 mg kg^{-1})

had been given, caerulein was infused as in the caerulein group. After the 6-h infusion period, the animals were anaesthetized with ether, and blood from the inferior vena cava was collected in chilled heparinized tubes for the determination of amylase and lipase activities.

Assays

Plasma was obtained by centrifugation of blood samples and was then used for the assays. Plasma amylase activity was measured by an enzymatic assay (Satomura et al 1988) using a L-Type Wako Amylase kit. Plasma lipase activity was measured by an enzymatic assay (Yuasa et al 1998) using a Lipase Color Auto Test Wako kit.

Histological examination

The pancreas, fixed in 20% formaldehyde, was sectioned and stained with haematoxylin eosin. The sections were then evaluated by the light microscopy method (Yuasa et al 1998).

Statistical analysis

Data were expressed as the mean ± s.e.m. Comparisons between the pancreatitis model group and other groups were made by Dunnett's method (JMP Software, SAS Institute Inc.). $P < 0{\cdot}05$ was considered to be statistically significant.

Results

Drug effect on caerulein and ethanol-induced alcohol pancreatic injury

Plasma amylase activity in the caerulein plus ethanol group increased 1·6-fold compared with that in the caerulein group (8250 ± 618 vs 5170 ± 301 int. units L^{-1}, $P < 0{\cdot}01$) (Figure 2A). This aggrandizement was significantly suppressed by the oral administration of 30 mg kg^{-1} sepimostat given before the caerulein infusion. Plasma lipase activity in the caerulein plus ethanol group also increased, 3·1-fold compared with that in the caerulein group (778 ± 109 vs 254 ± 25 int. units L^{-1}, $P < 0{\cdot}01$) (Figure 2B). This increase was significantly suppressed by the oral administration of 10 or 30 mg kg^{-1} sepimostat. However, camostat (3–30 mg kg^{-1}, p.o.) did not significantly affect either activity in the caerulein plus ethanol group.

Histological changes in the pancreas in each group were investigated by light microscopy. Rats in the caerulein group had interstitial oedema,

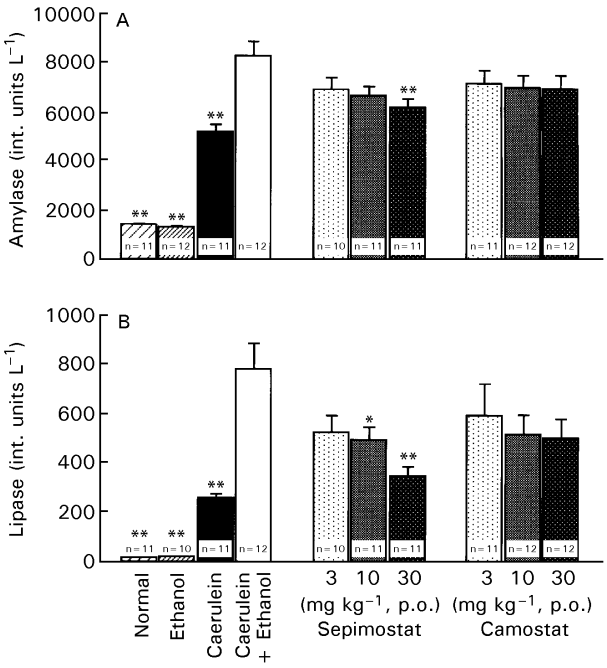

Figure 2. Effects of sepimostat and camostat on plasma amylase (A) and lipase (B) activities increased by caerulein plus ethanol infusion at 12 h after the start of caerulein infusion. Conscious rats were infused with 1·0 μg mL^{-1} h^{-1} caerulein intravenously for up to 6 h (time: 0–6 h) with 0·1 g mL^{-1} h^{-1} ethanol infusion for 9 h (time: 3–12 h). Drugs were administered orally 1 h before the start of the caerulein infusion. Data are expressed as the mean ± s.e.m. of 10–12 animals. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ compared with the caerulein plus ethanol group by Dunnett's test.

acinar cell vacuolization, and inflammatory cell infiltration (Figure 3A). Intense interstitial oedema was found in the caerulein plus ethanol group (Figure 3B). The interstitial oedema induced by caerulein and ethanol was reduced by the oral

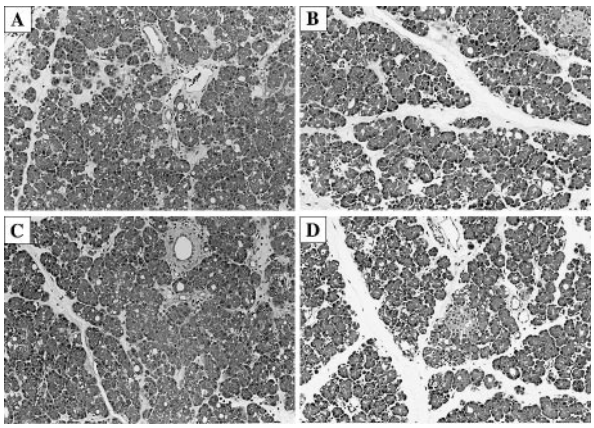

Figure 3. Effects of sepimostat and camostat on caerulein plus ethanol-induced histological changes in the rat pancreas at 12 h after the start of caerulein infusion. A. Caerulein group, B. caerulein plus ethanol group, C. sepimostat (30 mg kg^{-1}) group, D. camostat (30 mg kg^{-1}) group. Sections were stained with haematoxylin and eosin, and evaluated by light microscopy. Original magnification ×50.

Table 1. Effects of sepimostat and camostat on plasma amylase and lipase activities increased by caerulein infusion. Conscious rats were infused with 1·0 μg mL^{-1} h^{-1} caerulein intravenously for 6 h. Drugs were administered orally 1 h before the start of the caerulein infusion.

	Amylase (int. units L^{-1})	Lipase (int. units L^{-1})
Normal	1290 ± 50	19 ± 1**
Caerulein	13570 ± 1120	4580 ± 450
Sepimostat (30 mg kg^{-1})	13500 ± 1010	3960 ± 520
Camostat (30 mg kg^{-1})	12500 ± 900	3540 ± 330

Values are the mean ± s.e.m. of 10 animals. **$P < 0·01$ compared with the caerulein group by Dunnett's test.

administration of 30 mg kg^{-1} sepimostat, but not by 30 mg kg^{-1} camostat (Figure 3C, D).

Drug effect on caerulein-induced pancreatic injury

Plasma amylase activity in the caerulein group increased 11-fold compared with that in the normal group (13570 ± 1120 vs 1290 ± 50 int. units L^{-1}, $P < 0·01$; Table 1). A supramaximal dose of caerulein also elevated the plasma lipase activity 241-fold over the normal group (4580 ± 450 vs 19 ± 1 int. units L^{-1}, $P < 0·01$; Table 1). Neither sepimostat nor camostat (30 mg kg^{-1}, p.o.) significantly affected these aggrandizements in the caerulein group (Table 1).

Discussion

Sepimostat and camostat, both oral synthetic serine protease inhibitors, exhibit potent inhibitory effects against trypsin (Tamura et al 1977; Oda et al 1990). Therefore, it is not surprising that they have superior protective effects on various types of experimental acute pancreatitis induced by activated trypsin (Murakami et al 1990; Kisfalvi et al 1995; Wang et al 1995). In clinical studies, both drugs were found to have a therapeutic potential in chronic pancreatitis caused by activation of trypsin in the pancreas (Ishii et al 1980; Takeuchi et al 1996). However, the majority of chronic pancreatitis cases are those of alcohol pancreatitis caused by alcohol consumption (Pitchumoni 1998), and there is no beneficial therapy for this disease. To investigate whether sepimostat might be helpful as a therapeutic drug for alcohol pancreatitis, we established acute alcohol-induced pancreatic injury in rats, of which some had been pretreated with sepimostat, to determine whether the drug could prevent this alcoholic injury. We compared the effect with that of camostat.

In this study, we produced an experimental model in which ethanol infusion aggravated the recovery from caerulein-induced pancreatic injury. In this model, increases in plasma amylase and lipase activities, and aggravated pancreatic interstitial oedema were observed and compared with the activities and oedema found for caerulein-induced pancreatic injury. The aggravation of these parameters by ethanol administration has been reported by others (Letko et al 1991; Foitzik et al 1994). Sepimostat (10 and 30 mg kg^{-1}) reduced the values of these parameters aggravated by ethanol infusion, whereas camostat (10 and 30 mg kg^{-1}) did not. Wang et al (1995) reported that the oral administration of greater than 30 mg kg^{-1} sepimostat inhibited intracellular activation of pancreatic enzymes such as trypsin, and prevented caerulein-induced acute pancreatitis. Therefore, to examine whether sepimostat prevented the alcohol-related acute pancreatitis by suppressing the caerulein-induced pancreatic injury, we administered sepimostat or camostat (30 mg kg^{-1}, p.o.) to rats, and then administered caerulein to produce pancreatitis. As a result, neither drug could suppress the increase in plasma amylase and lipase activities at this dose, thus caerulein-induced acute pancreatitis could not be prevented. Our results suggest that sepimostat (10 to 30 mg kg^{-1}, approx. clinical dose) lessened the alcohol-related acute pancreatitis by suppressing not the pancreatic injury induced by caerulein, but the caerulein-induced pancreatic injury aggravated by ethanol infusion in the recovery phase.

Based on the results of a controlled clinical trial, it was reported that sepimostat had a similar therapeutic effect as camostat on chronic pancreatitis, but that sepimostat had a stronger therapeutic effect on alcohol pancreatitis than camostat (Takeuchi et al 1996). We do not know how sepimostat suppressed the effects of alcohol or what effects were suppressed in our study. However, our results appear to support the results of the controlled clinical trial. Further study of the therapeutic effects of sepimostat on alcohol pancreatitis is necessary.

In this study, it is evident that sepimostat has superior preventive effects on the alcohol-related experimental pancreatitis compared with camostat. Sepimostat may thus be a useful drug in the therapy of alcohol-induced pancreatitis.

References

Foitzik, T., Lewandrowski, K. B., Castillo, C. F., Rattner, D. W., Klar, E., Warshaw, A. L. (1994) Exocrine hyperstimulation but not pancreatic duct obstruction increases the susceptibility to alcohol-related pancreatic injury. Arch. Surg. 129: 1081–1085

Gullo, L., Barbara, L., Labo, G. (1988) Effect of cessation of alcohol use on the course of pancreatic dysfunction in alcoholic pancreatitis. Gastroenterology 95: 1063–1068

Haber, P., Wilson, J., Apte, M., Korsten, M., Pirola, R. (1995) Individual susceptibility to alcoholic pancreatitis: still an enigma. J. Lab. Clin. Med. 125: 305–312

Ishii, K., Takebe, T., Hirayama, A., Uehara, S., Murashima, Y., Suga, T., Kishino, H., Sato, T., Noto, T., Saito, Y., Hatta, Y., Naito, S., Nakajima, S., Takayama, T., Hayakawa, T., Nakano, S., Suzuki, T., Murata, I., Hirono, T., Miyazaki, I., Kinami, Y., Kubota, Y., Maeda, S., Hosoda, S., Bamba, T., Kasima, K., Ibayashi, H., Abe, M., Tsuchiya, R. (1980) Suien ni taisuru FOY-305 no yakko hyoka. Tashisetu nijyumokenho. Gendai Iryo 12: 261–278

Kisfalvi, K., Papp, M., Friess, H., Buchler, M., Goracz, Ulm, G. (1995) Beneficial effects of preventive oral administration of camostate on cerulein-induced pancreatitis in rats. Dig. Dis. Sci. 40: 546–547

Letko, G., Nosofsky, T., Lessel, W., Siech, M. (1991) Transition of rat pancreatic juice edema into acute pancreatitis by single ethanol administration. Path. Res. Pract. 187: 247–250

Murakami, H., Togawa, M., Takahashi, S., Kasahara, N., Yamamoto, J., Matsuura, N., Koshiyama, Y., Ino, Y., Oda, M. (1990) Pharmacological studies of 6-amidino-2-naphthyl 4-[(4,5-dihydro-1H-imidazol-2-yl)amino]benzoate dimethanesulfonate. Effects on experimental pancreatitis. Arzneim. Forsch. 40: 1352–1358

Nakamura, K., Johmura, A., Oda, M., Ino, Y., Uchiyama, H., Ohtani, H., Miyazaki, H., Kurumi, M., Akizawa, Y., Oka, T. (1995) Inhibitory effects of sepimostat mesilate (FUT-187) on the activities of trypsin-like serine proteases in vitro. Yakugaku Zasshi 115: 201–212

Oda, M., Ino, Y., Nakamura, K., Kuramoto, S., Shimamura, K., Iwaki, M., Fujii, S. (1990) Pharmacological studies on 6-amidino-2-naphthyl[4-(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethane sulfonate (FUT-187). I: Inhibitory activities on various kinds of enzymes in vitro and anticomplement activity in vivo. Jpn. J. Pharmacol. 52: 23–34

Pitchumoni, C. S. (1998) Chronic pancreatitis: a historical and clinical sketch of the pancreas and pancreatitis. Gastroenterologist 6: 24–33

Satomura, S., Iwata, T., Sakata, Y. (1988) Synthesis of *p*-nitrophenyl 6^5- *o*-benzyl-α-maltopentaoside, a substrate for alpha amylases. Carbohydr. Res. 176: 107–115

Singh, M., Simsek, H. (1990) Ethanol and the pancreas. Current status. Gastroenterology 98: 1051–1062

Takeuchi, T., Watanabe, S., Shiratori, K., Matsuno, S., Mitamura, K., Homma, T., Nakano, S., Saito, Y., Iwasaki, Y. (1996) Mesirusan sepimosutatto (FUT-187) no manseisuien ni taisuru rinsyohyoka. Mesirusan kamosutatto wo taisyoyaku to sita nijyumokenhikakushiken. J. Clin. Exp. Med. (Igakunoayumi) 178: 953–968

Tamura, Y., Hirado, M., Okamura, K., Minato, Y., Fujii, S. (1977) Synthetic inhibitors of trypsin, plasmin, kallikrein, thrombin, C_1r, and C_1 esterase. Biochim. Biophys. Acta 484: 417–422

Wang, Z. H., Manabe, T., Ohshio, G., Hirano, T., Okada, N., Imamura, T., Kawaguchi, Y., Yotsumoto, F., Yamaki, K., Imamura, M. (1995) Effect of the oral administration of sepimostat mesilate on cerulein induced acute pancreatitis in rats. Arzneim. Forsch. 45: 1082–1086

Yuasa, C., Irimura, K., Oka, T. (1998) Ethanol administration delays recovery from acute pancreatitis induced by exocrine hyperstimulation. J. Pharmacol. Toxicol. 39: 221–228