Effects of Urinary Trypsin Inhibitor on Pancreatic Enzymes and Experimental Acute Pancreatitis

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Therapeutic effect and the mechanism of the action of human urinary trypsin inhibitor (MTI) on experimental acute pancreatitis were studied. MTI significantly increased survival rate of animals with experimental acute pancreatitis induced by the infusion of trypsin or phospholipase A₂ into pancreas or by a closed duodenal loop. The efficacy of MTI on these types of pancreatitis were higher than those of aprotinin. Pancreatic enzymes were released from pancreatic slice by trypsin or phospholipase A₂, and this release was inhibited by MTI. Further, these pancreatic enzymes caused a secondary release of enzymes from other pancreatic slice, suggesting that these enzymes injured pancreatic tissue and that a chain reaction of pancreatic enzyme activation may play an important role in the pathogenesis of acute pancreatitis. MTI suppressed the secondary enzyme-induced pancreatic injury more strongly than aprotinin. These results suggest that MTI may suppress pathogenesis and development of pancreatitis by inhibiting the chain reaction of pancreatic enzyme activation.

Acute pancreatitis is generally accepted as a chemical autolysis of the pancreas triggered by the activated pancreatic enzyme, trypsin. Based on this viewpoint, aprotinin has been used for the treatment of acute pancreatitis (1). On the other hand, it has been found that the infusion of trypsin alone into the pancreatic duct could not induce a severe acute pancreatitis (2). Therefore, pathogenesis and development of acute pancreatitis cannot be attributed only to the activation of trypsin, and it was suggested that inhibition of various pancreatic enzymes may be necessary for the effective treatment of acute pancreatitis.

The authors have found that a trypsin inhibitor in human urine inhibits pancreatic enzymes such as trypsin, α-chymotrypsin, lipase, amylase, elastase, and carboxypeptidase (3). This trypsin inhibitor in human urine has been reported to be an acid glycoprotein with molecular weight of 67,000-70,000 and contains 5-12% of neutral sugar (3-5). However, its physiological role has not been elucidated. In the present study, the effects of this urinary trypsin inhibitor, hereafter referred to as MTI, on experimental acute pancreatitis were studied, and the role of pancreatic enzymes other than trypsin in the development and progress of acute pancreatitis was investigated.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 180–230 g and mongrel dogs weighing 8–15 kg were used.

Trypsin Inhibitors. Pyrogen-free MTI was prepared from fresh urine of healthy human by the method of Proksh et al (5). The inhibitory action of MTI on trypsin was determined by the method of Kassell (6), by which 1
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Unit of MTI inhibits 2 μg of trypsin by 50%. The specific activity of the MTI preparation used in this study was 2613 units/mg protein. The MTI preparation showed a single band in polyacrylamide gel electrophoresis, and the molecular weight was measured as 67,000 by gel filtration with Sephadex G-100. Aprotinin (Mochida) was used.

Assay of Proteases. Protease activity was measured by the method of Bruhn et al (7), using casein as a substrate. Elastase-like activity was measured by the method of Bieth et al (8), using succinyl-L-alanyl-L-alanine p-nitroanilide as a substrate.

Various protease activities released from pancreatic tissue were classified using several enzyme inhibitors. Total protease activity was defined as caseinolytic activity in the absence of inhibitors. Trypsin-like activity was calculated as total protease activity minus protease activity in the presence of 0.7 mM N-p-tosyl-L-lysine chloromethyl ketone (9). Alpha-chymotrypsin-like activity was calculated as total protease activity minus protease activity in the presence of 0.7 mM L-1-p-tosylamide-2-phenylethyl chloromethyl ketone (9). Carboxypeptidase-like activity was calculated as total protease activity minus protease activity in the presence of 0.5 mM P-chloromercuribenzoate (11) and cathepsin G-like activity as total protease activity minus the sum of elastase-like activity and protease activity in the presence of 14 mM phenylmethylsulfonyl fluoride (12). Total protease activity minus the sum of activities of elastase-like, trypsin-like, α-chymotrypsin-like, carboxypeptidase-like, cathepsin A, B-like, and cathepsin G-like proteases is referred as the other type of protease.

Effect of MTI on Trypsin-Induced Canine Pancreatitis. Dogs were infused with 15 ml of saline containing 15,000 national formulary units (NFU) (13) of trypsin (Canada Packers) and 0.6 g of sodium taurocholate (taurocholate) into the main pancreatic duct under laparotomy at the rate of 0.5 ml/min, according to the method of Elliott et al (14). One minute after the beginning of the infusion of trypsin and taurocholate, the dogs received MTI or aprotinin, dissolved in 250 ml of lactated Ringer's solution supplemented with 5 w/v% sorbitol, by drip infusion in 4 hr intravenously. The doses were 3000, 10,000 or 30,000 units/kg for MTI and 100,000 units/kg for aprotinin. In addition, 750, 2500 or 7500 units/kg of MTI or 25,000 units/kg of aprotinin were dissolved in 3 ml of physiological saline and intravenously injected to the animals before and immediately after the infusion of phospholipase A2 and taurocholate. The total doses were 4500, 15,000, or 45,000 units/kg for MTI and 150,000 units/kg for aprotinin.

As a control, a group of animals which was infused with trypsin and taurocholate, was treated with vehicle by the same method. Survival of the animals was observed daily for 10 days.

Effect of MTI on Protease Release from Canine Pancreatic Slices. Canine pancreatic slices, weighing 0.5 g, was incubated in 2.5 ml of 10 mM Tris HCl buffer (pH 8.0) containing 5 mg of taurocholate, 150 mM of NaCl and 2 mM of CaCl₂ with 100 NFU of trypsin and 1000 or 3000 units/ml of MTI or 3000 units/ml of aprotinin at 37°C for 1 hr, or 200 units of phospholipase A₂ and 1000 or 3000 units/ml of MTI or 3000 units/ml of aprotinin at 37°C for 3 hr. The total protease activity in the supernatant of the reaction mixture was determined.

Effect of MTI on Pancreatic Enzymes Released by Trypsin or Phospholipase A₂. Canine pancreatic slice, weighing 0.5 g, was incubated in 2.5 ml of 10 mM Tris HCl buffer (pH 8.0) containing 5 mg of taurocholate, 150 mM of NaCl, and 2 mM of CaCl₂ with 100 NFU of trypsin at 37°C for 1 hr or with 200 units of phospholipase A₂ at

Effect of MTI on Trypsin-Induced Murine Pancreatitis. Rats were injected with 0.1 ml of saline containing 500 NFU of trypsin and 10 mg of taurocholate into the common bile duct under laparotomy by the method of Lankish et al (15). MTI, at Doses of 30,000 or 100,000 units/kg, or 300,000 units/kg of aprotinin was dissolved in 12.5 ml/kg of physiological saline and infused intravenously to the animals over a 45-min period beginning 5 min before the infusion of trypsin. As a control, a group of animals which received trypsin and taurocholate was treated with vehicle by the same method. Survival of the animals was observed daily for 4 days.

Effect of MTI on Murine Pancreatitis Caused by Closed Duodenal Loop. According to the method of Ferrie et al (16), rats were laparotomized and a 3-cm-long duodenal loop was made, placing the opening of common bile duct at the center of the loop. Three hours after preparing the duodenal loop, the bile duct was ligated 3 cm from its opening. MTI, dissolved in 0.66 ml/kg of physiological saline, was injected into the loop at a dose of 13,000 or 40,000 units/kg. As a control, a group of animals was treated with vehicle after the ligation. The ligature was maintained for 5 hr. Survival of the animals was observed daily for 4 days.

Effect of MTI on Phospholipase A₂-Induced Canine Pancreatitis. Dogs were infused with 15 ml of saline containing 80 units (17) of phospholipase A₂ (Boehringer-Mannheim) and 0.6 g of taurocholate into the main pancreatic duct at the rate of 0.5 ml/min under laparotomy by the method of Elliott et al (14). One minute after the beginning of the infusion of phospholipase A₂ and taurocholate, the dogs received MTI or aprotinin, dissolved in 250 ml of lactated Ringer's solution supplemented with 5 w/v% sorbitol, by drip infusion in 4 hr intravenously. The doses were 3000, 10,000 or 30,000 units/kg for MTI and 100,000 units/kg for aprotinin. In addition, 750, 2500 or 7500 units/kg of MTI or 25,000 units/kg of aprotinin were dissolved in 3 ml of physiological saline and intravenously injected to the animals before and immediately after the infusion of phospholipase A₂ and taurocholate. The total doses were 4500, 15,000, or 45,000 units/kg for MTI and 150,000 units/kg for aprotinin.

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