Effect of Urinary Trypsin Inhibitor on Pancreatic Cellular and Lysosomal Fragility in Cerulein-Induced Acute Pancreatitis in Rats

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We evaluated the protective effect and the mechanism of action of the trypsin inhibitor, urinastatin, extracted from human urine, in experimental acute pancreatitis induced by a supramaximal dose of cerulein (5 μg/kg/hr for 3.5 hr). Urinastatin in a dose of 10,000 units/kg/hr was given by three different methods of continuous infusion: (1) 2 hr before and during cerulein infusion, (2) only during cerulein infusion, and (3) starting 1 hr after the beginning of cerulein infusion and continued for 3.5 hr. In protocol 1 and 2 urinastatin was significantly more protective than in 3. In protocol 1 urinastatin was very protective in all parameters tested (serum amylase level, pancreatic water and amylase content, distribution of lysosomal enzymes, cellular and lysosomal fragility). These results suggest that the administration of urinastatin before and during cerulein infusion may suppress the pathogenesis and evolution of pancreatitis by inhibiting the chain reaction of pancreatic enzyme activation closely related to redistribution of lysosomal enzyme and lysosomal fragility.

KEY WORDS: cerulein; acute pancreatitis; urinastatin; lysosomal enzyme.

Recent studies have suggested that a blockade of exocytosis of digestive enzymes and intracellular activation of trypsinogen by lysosomal hydrolases are likely causes of the experimental acute pancreatitis induced by a supramaximal dose of cerulein or by a choline-deficient ethionine-supplemented (CDE) diet (1-4). Moreover, lysosomal fragility has been reported to precede activation of digestive enzymes in the acinar cells (1, 5-7).

Thus, the greatest prospect of improving acute pancreatitis is now focused on the intrapancreatic inhibition of activators of digestive enzymes, particularly trypsin, which seems to be a key enzyme in the initiation of the proteolytic cascade. In this study, we evaluated the protective effect of the protease inhibitor urinastatin (UTI), an acid glycoprotein extracted from human urine (8, 9), which, like gabexate mesilate (FOY) and other synthetic protease inhibitors, inhibits trypsin, elastase, carboxypeptidase, and α-chymotrypsin (10, 11).

MATERIALS AND METHODS

Male Wistar rats weighing 225–250 g (Shizuoka Experimental Animals, Shizuoka, Japan) were used. The ani-
mals were given free access to tap water and feed (Purina Rodent Chow, Purina Mills, Inc., St. Louis, Missouri). After a 16-hr fast, rats were anesthetized with intraperitoneal sodium pentobarbital (15 mg/kg), and the superior vena cava was catheterized via the right external jugular vein. After about 12 hr, the rats were divided into the following five groups: (1) 15 control rats (CONT)—infused with heparinized saline only at a speed of 0.58 ml/hr for 5 hr; (2) 18 cerulein rats (CER)—infused with cerulein (Sigma Chemical, St. Louis, Missouri) 5 μg/kg/hr in saline for 3.5 hr. (3) 54 urinastatin rats (UTI)—urinastatin (UTI) (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) infused for 2 hr before and throughout the 3.5 hr of cerulein infusion in a dose of 10,000 units/kg/hr (UTI1), or (4) only throughout the 3.5 hr of cerulein infusion (UTI2), or (5) from 1 hr after the beginning of cerulein infusion for 3.5 hr (UTI3).

**Serum Amylase Levels, Pancreatic Water, Amylase, and Cathepsin B Content.** One hour after the end of cerulein infusion, rats were killed by a large dose of intravenous pentobarbital after blood sampling, and portions of the pancreas were quickly removed. One small portion of the pancreas was used for the determination of pancreatic edema by comparing the wet weight to dry weight (150 °C for 48 hr). Other small portions of the pancreas were homogenized (150 g at 4 °C for 15 min) in 5 ml of cold phosphate-buffered saline (pH 7.4) containing 0.5% Triton X-100 (Fisher Scientific) in a Brinkmann Polytron (Brinkmann Instruments, Inc., Westburg, New York). Pancreatic amylase (12) and cathepsin B (13) activity and deoxyribonucleic acid (DNA) concentration (14) were measured in the resulting supernatant. Enzyme activities were expressed as units per milligram of DNA.

**Histological Examinations.** Samples of rat pancreatic tissue from each group were used for light microscopical examination. Histological change, interstitial edema, acinar cell vacuolization, and inflammatory cell infiltration were graded on a 0–4 scale.

**Distribution of Cathepsin B in Subcellular Fractions.** Other portions of the pancreas were homogenized in 6 ml of ice-chilled 5 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 6.5) (Sigma Chemical) containing 1 mM MgSO4 and 250 mM sucrose and centrifuged (150 g at 4 °C for 15 min). Subcellular fractionation was performed by differential centrifugation, as described by Saluja et al (6). Briefly, the supernatant was centrifuged (1500 g at 4 °C for 15 min) to yield a pellet (zymogen fraction, 1.3 kilo g pellet; 1.3 KP), and a second pellet (lysosomal fraction, 12 kilo g × g pellet; 12 KP) and a supernatant (microsomal and soluble fraction, 12 kilo g × g supernatant; 12 KS).

The cathepsin B activity in each fraction was measured and expressed as the percentage of the total activity.

**Lactic Dehydrogenase (LDH) Discharge from Dispersed Pancreatic Acini.** From the pancreas of another rat from each group, dispersed acini were prepared as described previously (15). Acini were suspended in HEPES (Sigma Chemical) -Ringer buffer (pH 7.4) containing Eagles’ basal amino acids, bovine serum albumin (0.1%) (Sigma Chemical), and soybean trypsin inhibitor (0.01%) (Cooper Diagnostics). The acini were incubated in this buffer under O2 in a shaking water bath maintained at 37 °C. At 30-min intervals (up to 120 min), aliquots were removed and LDH activity was measured (16) in both the suspending medium and the pelleted acini. LDH discharge from the acini was expressed as a percentage of the total LDH activity.

**Cathepsin B Leakage from Lysosomes.** Other rats from each group were sacrificed, and portions of the pancreas were homogenized in MOPS. After centrifugation (150 g at 4 °C for 15 min), the resulting supernatant was centrifuged (12,000 g at 4 °C for 12 min) to obtain a combined zymogen–lysosome pellet. This pellet was resuspended in 5 mM MOPS buffer and incubated for varying intervals (up to 120 min) at 25 °C in a shaking water bath. The samples were then recentrifuged (12,000 g at 4 °C for 12 min) to separate particulate and soluble lysosomal activity. Soluble cathepsin B activity was expressed as the percent of the total cathepsin B activity.

**Analysis of Data.** The results reported in this communication represent the mean ± SEM for N determinations. Differences between groups were evaluated by Student’s t test, With significant differences defined as those associated with a probability value (P) of less than 0.005.

**RESULTS**

**Serum Amylase Levels and Pancreatic Water, Amylase, and Cathepsin B Content.** Infusion of cerulein (5 μg/kg/hr) for 3.5 hr was found to cause an increase in serum amylase levels (27 ± 3 units/ml vs control group, 6 ± 1 units/ml). When UTI was infused before and as well as during the cerulein infusion, the cerulein-induced increase in serum amylase levels was markedly attenuated, and the infusion of UTI only during the cerulein infusion also had a significant effect. However, if UTI was started 1 hr after the beginning of cerulein infusion, it had no significant effect (Figure 1). Pancreatic edema (CER; 87 ± 3%) was also markedly inhibited.