Protective Effect of Nafamostat Mesilate on Cellular and Lysosomal Fragility of Acinar Cells in Rat Cerulein Pancreatitis

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Summary

This in vivo and in vitro study demonstrates the protective effects of a new synthetic protease inhibitor—nafamostat mesilate, FLIT-175—on increased cellular and lysosomal fragility within acinar cells during the early stage of cerulein-induced acute pancreatitis in rats. FLIT-175 prevented hyperamylasemia, pancreatic edema, congestion owing to amylase, and lactic dehydrogenase (LDH) discharge from acini as well as cathepsin-B leakage from lysosomes dose-dependently in doses of 1–10 mg/kg • h. These results suggest that FLIT-175 can protect against pancreatitis at subcellular levels in lysosomes and cellular or organelle membranes. Proteases may well play the important role in the pathogenesis of acute pancreatitis, and such a low molecular protease inhibitor may be useful clinically in the treatment of acute pancreatitis.

Key Words: Cerulein-induced pancreatitis; cathepsin-B; lactic dehydrogenase (LDH); nafamostat mesilate (FUT-175).

Introduction

It has been reported that lysosomal functions are impaired and lysosomal fragility increased in acinar cells during experimental acute pancreatitis (1,2). Since cathepsin-B, as a lysosomal enzyme, can activate trypsinogen and trypsin can activate many other digestive enzymes (3–6), intracellular colocalization of lysosomal enzymes and pancreatic digestive enzymes are thought to play important triggering roles in the autodigestion of acinar cells in acute pancreatitis. Looking at the important roles of proteases in the pathogenesis of acute pancreatitis (7–10), it may be rational to use protease inhibitors clinically to treat acute pancreatitis.

In this article, we report the protective effects of a new synthetic, potent, low molecular protease inhibitor, nafamostat mesilate (6-amidino-2-naphthyl-p-guanidinobenzoate dimethane-sulfonate, FUT-175) on the exocrine pancreas in cerulein-induced acute pancreatitis in rats. We found that the interstitial pancreatic edema and hyperamylasemia that characterize cerulein-induced pancreatitis were protected and that cellular and lysosomal fragility were reduced by this protease inhibitor.

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Materials and Methods

Male Wistar rats (125–150 g; Shizuoka Experimental Animals, Shizuoka, Japan) were used for this experiment on secretagogue-induced pancreatitis. All the animals were kept in light–dark cycle-regulated (5:00–17:00) and temperature-controlled (23 ± 3°C) animal quarters with free access to tap water and diet (Purina Rodent Chow, Purina Mills, Inc., St. Louis, MO).

After a 12-h fast, a PE-50 cannula (Clay Adams, Parsippany, NJ) was placed in the superior vena cava through the right external jugular vein under light ip sodium pentobarbital (15 mg/kg) anesthesia, and its potency was maintained by continuous infusion of heparinized (30 U/mL) 150 mM NaCl solution at 0.21 mL/h with an infusion pump (Harvard Apparatus, South Natick, MA). The animals were housed in shoe-box cages and given 12 h to recover from the effects of surgery and anesthesia; they had free access to tap water and diet before the next cerulein infusion.

Animals were subsequently divided into the following three groups: 15 control rats (CONT) infused only with heparinized saline at 0.58 mL/h for 3.5 h; 18 cerulein rats (CER) infused with heparinized saline as above (0.58 mL/h for 3.5 h) but with cerulein (Sigma Chemical, St. Louis, MO) added to the infusate so that each animal received 5 mg/kg 9 h; and 90 FUT-175 plus cerulein rats identical to the cerulein group, but FUT-175 was infused in five different concentrations (F1, 0.5; F2, 1; F3, 2; F4, 5; and F5, 10 mg/kg • h for 2 h before and throughout the 3.5 h of cerulein infusion). The infusion experiments were begun at 8 AM to rule out the effect of circadian rhythm on the rat exocrine pancreas. During the infusion, tap water and food were removed from their cages. FUT-175 was kindly provided by the Torii Pharmaceutical Co., Ltd. (Tokyo, Japan).

Serum Amylase, Pancreatic Water, Pancreatic Amylase, and Cathepsin-B Content

At selected times for each group, rats were killed by a large dose of iv pentobarbital after blood had been drawn for determinations of serum amylase levels, and portions of the pancreas were quickly removed and trimmed of fat. One portion of each pancreas was used for determination of pancreatic water content by comparing its wt immediately after sacrifice (wet wt) with that of the same sample after incubation at 150°C for 48 h in a desiccator (Isotemp Oven®, Fisher Scientific, Fair Lawn, NJ) (dry wt).

Another portion of the pancreas was homogenized in cold phosphate-buffered saline (PBS) (pH 7.4) containing 0.5% Triton X-100 (Fisher Scientific) in a Brinkman Polytron (Brinkman Instruments, Inc., Westbury, NY) and the activity of amylase and cathepsin-B, as a lysosomal enzyme, as well as the deoxyribonucleic acid (DNA) content were measured in the resulting supernatant after low-speed centrifugation (150g, 15 min, 4°C). Both pancreatic amylase and cathepsin-B content were expressed as U/mg DNA.

LDH Discharge from Dispersed Pancreatic Acini

In another rat in each group, dispersed pancreatic acini were prepared by collagenase (Cooper Diagnostics, Freehold, NJ) digestion and gentle shearing as previously described (11). Acini were suspended in HEPES-Ringer buffer (pH 7.4) containing (mM): NaCl, 115; KCl, 5; MgSO4, 1; Na2HPO4, 1; HEPES, 10; CaCl2, 1.26; glucose, 15; Eagle’s basal amino acids; bovine serum albumin (BSA), 0.1% (Sigma); and soybean trypsin inhibitor, 0.01% (Cooper Diagnostics), which was preoxygenated by bubbling 100% O2 through it. The acini were incubated in this buffer under an atmosphere of O2 in a shaking water bath maintained at 37°C. At 30-min intervals for up to 120 min, aliquots were removed. The LDH activity in the suspending medium and the pelleted acini was measured. LDH discharge from acini was expressed as the percent of total LDH activity present in the acini at the onset of in vitro incubation.

Lysosomal Enzyme Discharge from Lysosomes

In other rats from each group, portions of the pancreas were homogenized with a Dounce® homogenizer in ice-chilled 5 mM MOPS buffer. Unbroken cells and debris were removed by low-speed cen-