Inhibitory Effect of Human Urinary Trypsin Inhibitor (Urinastatin) on Lysosomal Thiol Proteinases

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Abstract—Effects of human urinary trypsin inhibitor, urinastatin, a compound clinically prescribed for treatment of acute pancreatitis, on lysosomal thiol proteinases were studied. Urinastatin had inhibitory effect on the activities of cathepsins B and H *in vitro*. In the experimental acute pancreatitis induced by a closed duodenal loop, urinastatin prevented the enhancement of esterolytic activity and the activities of cathepsins B and H. Urinastatin also improved the activities of inhibitors of cathepsins B and H in the case of pancreatitis.

Human urinary trypsin inhibitor, urinastatin, is an acid glycoprotein with a molecular weight of about 67,000 and has a potent inhibitory effect on the activities of trypsin and α -chymotrypsin (1). This compound is at present clinically prescribed for treatment of acute pancreatitis. Recent evidence obtained experimentally indicates that digestive enzyme activation during the early stages of acute pancreatitis may result from an admixture of zymogens with lysosomal hydrolases capable of activating digestive enzymes (2–4). Among the lysosomal enzymes, a thiol proteinase such as cathepsin B is regarded as an important enzyme which is responsible for triggering the activation of zymogens (5). In the present work, the inhibitory effect of urinastatin on the activities of lysosomal thiol proteinases such as cathepsins B and H was clearly shown *in vitro* and in experimental acute pancreatitis.

Urinastatin, which shows a single band in polyacrylamide gel electrophoresis, was kindly provided by Mochida Pharmaceutical Co., Japan. The specific activity of urinastatin was 2,600 unit/mg protein. One unit of urinastatin inhibits 1 μ g of trypsin. A commercial sample of Trasylol (10,000 kallikrein inhibitor units (KIU) per ml, Bayer A.G., West Germany) served as aprotinin. The activity of one KIU corresponds to 0.14 μ g of crystalline aprotinin. Gabexate mesilate was obtained

from Ono Pharmaceutical Co., Japan. Acute pancreatitis was induced in male Wistar rats (about 250 g in weight) by a closed duodenal loop, according to Takasugi et al. (6). Eighteen hours later, the survivors were anesthetized with pentobarbital sodium, perfused through the aorta with 0.9% NaCl, and then the pancreas was removed. The tissue was homogenized with 4 volumes of distilled water containing 0.1 mM EDTA. The homogenate was centrifuged at 12,000 $\times$ g for 20 min at 4°C, and the supernatant was used as an enzyme sample for cathepsin assay. Extract for the inhibitor assay was prepared with the supernatant fraction, according to Lenney et al. (7). Cathepsins B and H activities were determined with carbobenzoxy-L-arginyl-L-arginine 4-methylcoumaryl-7-amide and L-arginine 4-methylcoumaryl-7-amide, respectively, according to Barrett (8). The activity of papain was assayed with α -N-benzoyl-DL-arginine- β -2-naphthylamide as a substrate, as described by Barrett (9). Esterolytic activity was measured with tosyl-L-arginine methyl ester hydrochloride (TAME) as a substrate by the method of Roberts (10). Cathepsins B and H used in the inhibitor assay were purified from rat liver according to Lenney et al. (7). The inhibitor was assayed under the conditions used for the assay of cathepsins B and H, replacing some of the buffer with inhibitor

solution.

Figure 1 shows the effects of urinastatin, aprotinin, and gabexate mesilate on the activities of lysosomal thiol proteinases, cathepsins B and H, and a plant thiol proteinase, papain, *in vitro*. Among these thiol proteinases, a striking homology of amino acid sequences has been demonstrated (11). The 4,000 unit/ml of urinastatin and 4,000 KIU/ml of aprotinin corresponded to 0.02 $\mu\text{mol/ml}$ and 0.09 $\mu\text{mol/ml}$, respectively, as calculated from their molecular weight and specific activity. The activities of thiol proteinases such as cathepsins B and H and papain were clearly inhibited by 4,000 unit/ml of urinastatin, whereas a 4.5 times higher concentration of aprotinin and a 25 times higher concentration of gabexate mesilate, on a molar basis, had no inhibitory effect on the activities of the enzymes. Aprotinin and gabexate mesilate also have a potent inhibitory effect on the activity of trypsin; however, their inhibition spectra against pancreatic and other enzymes have been reported to be narrower than urinastatin (1).

In the experimental acute pancreatitis induced by a closed duodenal loop, 2 of the 7 rats died in the pancreatitis group, whereas

none of the rats died in both the sham-operated and urinastatin-treated groups. The mortality of the pancreatitis group was lower than that reported by Takasugi et al. (6); however, signs of acute pancreatitis such as edema, fat necrosis and hemorrhage were evident. In addition, esterolytic activity which is considered to be the most prominent index of experimental acute pancreatitis was markedly increased in the pancreatitis group as shown in Fig. 2. The activities of cathepsins B and H and their inhibitors in the pancreas are also shown in Fig. 2. The activities of cathepsins B and H were increased in the pancreatitis group, compared to the findings in the sham-operated group. The infusion of urinastatin prevented the elevation of the esterolytic and catheptic activities in the case of pancreatitis. Macroscopic examination of the pancreas from the urinastatin-treated group showed a remarkable ameliorating effect on edema, fat necrosis and hemorrhage. The activities of inhibitors of cathepsins B and H were inhibited in the pancreatitis group compared with those of the sham-operated group, and the recovery of activities of inhibitors was observed in the urinastatin-treated group. Regulation of the function of

Fig. 1. Effect of urinastatin, aprotinin and gabexate mesilate on thiol proteinases. The enzymatic activity (cathepsin B, 0.9 mU/ml; cathepsin H, 1.0 mU/ml; papain 0.7 $\mu\text{g/ml}$) without urinastatin (or aprotinin or gabexate mesilate) was taken as 100%, and inhibition % shown on the ordinate was calculated from enzymatic activity with urinastatin (or aprotinin or gabexate mesilate). One mU of the cathepsin activity was defined as that quantity releasing one nmol of 7-amino 4-methyl-coumarin per min. Each point shows the mean of triplicate assays. UT: Urinastatin, Ap: Aprotinin, GXT: Gabexate mesilate.

Fig. 2. Esterolytic activity and activities of cathepsins B and H and the activities of their inhibitors in the pancreas. Acute pancreatitis was induced by the closed duodenal loop method. Urinastatin, the dose of which was adjusted with distilled water, was injected into the lumen of the closed duodenal loop. Eighteen hours after the operation, the pancreas was removed and enzymatic and inhibitor activities were measured. One unit of the esterolytic activity was defined as the amount of enzyme hydrolyzing one μ mol of TAME per hr at 37°C. One mU of the cathepsin activity was defined as that quantity releasing one nmol of 7-amino 4-methyl-coumarin per min. One unit of inhibitor was defined as the amount that decreased cathepsin activity by one unit. S: Sham-operated group, P: Pancreatitis group, U5: Urinastatin (50,000 unit/kg)-treated group, U10: Urinastatin (100,000 unit/kg)-treated group. Results show the mean from 5 rats $\pm$ S.E.M., and the data were analyzed by Student's *t*-test. **P*<0.05, ***P*<0.01, ****P*<0.001.

cathepsins B and H by their inhibitors may play an important role in the development of pancreatitis.

The inhibitory effect of urinastatin on the activities of lysosomal thiol proteinases such as cathepsins B and H observed in the present experiments may throw new light on the mechanism of action of urinastatin in acute pancreatitis.

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