Cytoprotective Effects of Ulinastatin against Hypoxic Injury to LLC-PK1 Cells

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Dear Sir,

Ulinastatin (UT) is a glycoprotein with a molecular weight of 67,000. UT is a Kunitz-type proteinase inhibitor that inhibits the enzymatic activities of trypsin, α-chymotrypsin, leukocyte elastase, hyaluronidase, etc. [1,2]. UT has been shown to exert a protective effect against nephrotoxic renal injury caused by gentamicin, mercuric chloride [3], or cis-platin [4] and against ischemic renal injury [5]. In the present study, we investigated the effect of UT against hypoxic injury to a cultured renal cell line: LLC-PK1. As the proximal tubule is the most susceptible cell type in ischemic renal injury, LLC-PK1 cells that originate from porcine kidney proximal tubules represent an appropriate model for studying hypoxic injury.

LLC-PK1 cells obtained from the American Type Culture Collection (ATCC CRL-1392) were cultured as previously reported [6]. The effect of hypoxia was studied 7 days after seeding, when the cells reached a confluent monolayer. Each was washed twice with 2 ml of Dulbecco’s phosphate-buffered saline. After removing the buffer, the reaction was initiated by addition of 1 ml of medium 199 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal calf serum and an appropriate amount of sodium bicarbonate to adjust the pH to 7.4. UT (Mochida Pharmaceutical Company, Tokyo; 3,000 U/ml) was added to one group of cells. The cells were incubated at 37°C in a hypoxic atmosphere of 5% CO2-90% N2-5% O2 for 12, 24, and 48 h. At the end of the incubation period, the medium was removed immediately by suction. The medium samples were then analyzed for enzyme activities. γ-Glutamyl transpeptidase (γ-GTP) and lactate dehydrogenase (LDH) were assayed using an autoanalyzer (Hitachi 736-60). N-acetyl-β-D-glucosaminidase (NAG) was assayed by employing the m-creosulfonphthaleinyl-N-β-glucosaminidase-method (NAG assay kit; Shionogi, Osaka, Japan). The changes in the contents of these enzymes were used as the markers of cell injury. The enzyme contents in the experimental medium incubated free of cells in an identical manner were subtracted from the original values. The remaining cells were solubilized with 1 ml of 0.1 N NaOH to determine the cellular protein content using bovine γ-globulin as the protein standard (Bio-Rad Laboratories, Richmond, Calif, USA) and to normalize the enzyme levels. Data are expressed as mean values ± SE (n = 6) and were analyzed using Student’s t test.

Figure 1 shows a detailed time course of the release of enzymes during hypoxia. A constant release of γ-GTP and NAG was observed. The addition of UT (3,000 U/ml) significantly reduced
the γ-GTP release after 24 and 48 h and the NAG release after 48 h. Only a moderate increase of LDH occurred during the first 24 h of hypoxia, but a dramatic increase was seen between 24 and 48 h. UT remarkably suppressed the drastic LDH release.

The protective effect of UT against renal tubular injury has been attributed to its pharmacological properties that include membrane stabilization, maintenance of hemodynamic stability, and the increase of solute ex-