Urinary trypsin inhibitor ameliorates renal tissue oxygenation after ischemic reperfusion in rats

Satoshi Taie, Masaaki Ueki, Kosuke Chujo, Takehiko Asaga, Yasuyuki Iwanaga, Junichiro Ono, and Nobuhiro Maekawa

Department of Anesthesiology and Emergency Medicine, Kagawa University, School of Medicine, 1750-1 Ikenobe, Miki-cho, Kagawa 761-0793, Japan

Abstract

Purpose. In order to determine the mechanism of the protective effect of a urinary trypsin inhibitor (UTI) on renal ischemic reperfusion injury, we measured the tissue oxygen partial pressure (p_{O_2}) in both the renal cortex and medulla in rats, using electron paramagnetic resonance (EPR) oximetry.

Methods. We allocated the rats to three groups: normal saline (NS) group, a UTI 50 000 U·kg^{-1} (LD) group, and a UTI 150 000 U·kg^{-1} (HD) group, with the normal saline and UTI being administered 30 min before ischemia. Renal ischemia was achieved by inflating the balloon of a vascular occluder that had been placed around the abdominal aorta just above the bifurcation of the renal artery. Cortical and medullary p_{O_2} were measured every 10 min during ischemia (30 min) and reperfusion (60 min) by EPR oximetry; also, systemic cardiopulmonary parameters were measured.

Results. The p_{O_2} in the cortex and medulla decreased to less than 2 mmHg during ischemia in all groups. At 60 min after reperfusion, the p_{O_2} values in the NS group were not fully restored, whereas those in the LD and HD groups were completely restored to the pre-ischemic values. There were no significant differences between the HD and LD groups. There were no differences between any groups in cardiopulmonary parameters.

Conclusion. Because UTI improved renal oxygenation after reperfusion without changing cardiopulmonary parameters, the pharmacological properties of UTI, such as its renal protection and anti-shock activity, may be explained in part, by this improvement in tissue oxygenation.

Key words: Urinary trypsin inhibitor · Renal ischemia · Tissue p_{O_2} · EPR oximetry

Introduction

Ischemic acute renal failure is a common complication of aortic surgery [1]. Tissue hypoxia with ischemia directly causes cellular damage. After blood flow is re-established, cellular dysfunction mediated via biochemical effectors and oxidative processes leads to acute renal failure [2]. During the course of ischemia and reperfusion, the concentration of oxygen in tissue is a critical variable for cellular function and viability. Nelimarkka [3] has reported that a critical oxygen partial pressure (p_{O_2}) level for oxygen consumption is 15 mmHg for the renal cortex and 13 mmHg for the medulla, and aerobic oxidative metabolism ceases at 6 mmHg in both tissue layers.

Appropriate renal protection could prevent a prerenal syndrome developing into acute tubular necrosis. Ulinastatin a urinary trypsin inhibitor (UTI), is a Kunitz-type protease inhibitor that inhibits the activity or release of lysosomal enzymes such as elastase and cathepsin G [4,5]. It has been reported to help protect against shock [6,7] and to suppress the deterioration of renal function associated with ischemic reperfusion [8,9]. In our previous magnetic resonance spectroscopic study (MRS), after reperfusion, the restoration of depleted ATP was faster and greater when animals were treated with UTI [10]. The effect of UTI on ATP recovery after ischemic reperfusion may be the result of improved oxygenation. In order to determine the mechanism responsible for this effect, we measured renal tissue p_{O_2} in vivo in rats, using electron paramagnetic resonance (EPR) oximetry. EPR oximetry is a less invasive technique than multiple electrode tonometry [11,12]. This technique uses signals from stable, paramagnetic materials implanted into the local tissue and provides rapid, highly accurate, and direct tissue p_{O_2} at the site of interest. We measured tissue p_{O_2} in both the renal cortex and medulla during ischemia and reperfusion, as well as measuring cardiopulmonary parameters.

Materials and methods

The study was approved by our institutional animal investigation committee. We used male Wistar rats weighing 300–350 g. An oxygen-sensitive lithium phthalocyanine (LiPc) crystal (diameter approximately 100 to 200 μm and length 200 to 500 μm) was implanted in the kidney cortex and medulla with a 25-G needle through a peritoneal incision approximately 7 days before the experiments began, in order to reduce possible trauma-related variability in our measurements. The details concerning the characterization and calibration of LiPc for p_{O_2} measurements were reported previously [11–13], and the spectra reflect the average p_{O_2} on the surface area of the crystal. After the 7-day recovery period for the placement of LiPc, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg·kg^{-1}). After a tracheostomy, the rats were mechanically ventilated with 0.7% isoflurane and air. Typical settings for the ventilator were: tidal volume, 10 ml·kg^{-1}; respiratory rate, 60·min^{-1}. Polyethylene catheters (PE-50s) were inserted into the right femoral artery for blood pressure monitoring and the right femoral vein for infusion. The left kidney was exposed through a dorsal incision. A vascular occluder was placed around the abdominal aorta just above the bifurcation of the renal artery. Renal ischemia was achieved by inflating the balloon of the vascular occluder, and was confirmed by a femoral artery blood pressure of almost 0 mmHg. The body temperature was maintained at 37 ± 0.5°C with a heating pad and monitored throughout the experiment.

The animals were allocated to three groups: a normal saline 1-ml (NS) group, low-dose UTI (50 000 U·kg^{-1}; dissolved in 1 ml normal saline: LD) group, and a high-dose UTI (150 000 U·kg^{-1}; dissolved in 1 ml normal saline: HD) group ($n = 6$ per group). The normal saline and UTI were injected 30 min before ischemia. UTI was a gift from Mochida Pharmaceutical (Tokyo, Japan). Normal saline was infused at a rate of 2 ml·h^{-1} during ischemia and reperfusion in all groups. Thirty minutes after the catheter insertion, the measurement of EPR spectra was started.

EPR measurements

The spectra of LiPc were obtained using an EPR spectrometer with a low-frequency (1.2 GHz, L-band) microwave bridge. Rats were placed in the magnetic field and the kidney was positioned so that it was directly under the extended loop resonator, which was adjusted to obtain the signal from the LiPc in the renal cortex or medulla. Typical settings for the spectrometer were: incident microwave power, 10 mW; magnetic field center, 425 gauss; scan range, 1 gauss; modulation

frequency, 27 kHz. Modulation amplitude was set at less than one-third of the EPR line width. Scan time was 30–60 s. Three or four scans were usually averaged to achieve a better signal-to-noise ratio. Each EPR spectrum was measured every 10 min during 30 min of ischemia and 60 min of reperfusion. The line widths of the obtained EPR spectra were converted to p_{O_2}, using a calibration curve determined for the LiPc.

Blood pressure was monitored continuously by a pressure transducer. Arterial blood gases were analyzed before ischemia and at the end of the experiment.

At the end of the experiment, rats were dissected. Gross and microscopic examination of the tissue around the implanted LiPc confirmed that the crystals were implanted into suitable sites in the renal cortex and medulla and there was no significant inflammatory infiltrate or necrosis around the LiPc.

All data values were expressed as means ± SE. Data were analyzed by analysis of variance to test differences between the groups. If the calculated F value exceeded the critical value for the 0.05 probability level, Fisher's protected least significant difference (PLSD) test was used to determined at which point the difference was significant at $P < 0.05$.

Results

Figure 1 and Table 1 show the changes in mean arterial pressure and arterial blood gases, respectively. The changes in mean arterial pressure in the NS and LD groups were almost the same as those in our previous study [10]. Mean arterial pressure did not differ significantly between the groups. The pH and base excess values in the NS group after 60 min of reperfusion tended to be lower than those in the LD group, although the differences between the groups in any of the blood gas parameters were not statistically significant.

Figures 2 and 3 show the changes in cortical and medullary p_{O_2} values, respectively. The changes in cortical and medullary p_{O_2} were similar in each group. The p_{O_2} values of the cortex and medulla decreased from 15.4–21.7 mmHg to less than 2 mmHg during ischemia in all groups. After reperfusion, the recovery of p_{O_2} in the LD and HD groups was faster and greater than that in the NS group. At 60 min after reperfusion, the p_{O_2} values in the NS group (cortex, 12.1 ± 2.7 mmHg; medulla, 14.5 ± 3.2 mmHg) were not fully restored, whereas those in the LD group (cortex, 19.7±2.3 mmHg; medulla, 23.0 ± 2.4 mmHg) and HD group (cortex, 19.9 ± 2.1 mmHg; medulla, 20.8 ± 2.4 mmHg) were completely restored to the pre-ischemic values. There were significant differences in cortical p_{O_2} after 40 and 60 min of reperfusion between the NS and LD groups and after 60 min of reperfusion between the NS and HD groups.

Table 1. Arterial blood gas analysis

	Pre-ischemia			60 min after reperfusion		
	NS	LD	HD	NS	LD	HD
pH	7.45 ± 0.02	7.45 ± 0.01	7.41 ± 0.01	7.29 ± 0.06	7.38 ± 0.04	7.31 ± 0.02
Pa_{CO_2} (mmHg)	37.0 ± 2.1	35.2 ± 1.8	35.7 ± 1.6	33.2 ± 2.2	32.0 ± 2.2	32.1 ± 2.5
Pa_{O_2} (mmHg)	84.2 ± 7.3	80.2 ± 6.2	79.4 ± 4.8	100.6 ± 5.1	96.7 ± 6.7	99.8 ± 4.0
Base excess (mEq·l^{-1})	0.0 ± 0.5	-0.5 ± 1.4	-1.2 ± 0.8	-10.0 ± 3.1	-5.7 ± 2.7	-8.9 ± 0.8

In the normal saline (NS) group, urinary trypsin inhibitor 50 000 U·kg^{-1} (LD) group, and the 150 000 U·kg^{-1} (HD) group, the relevant agents were administered 30 min before ischemia. There were no significant differences in any of the parameters shown between the groups
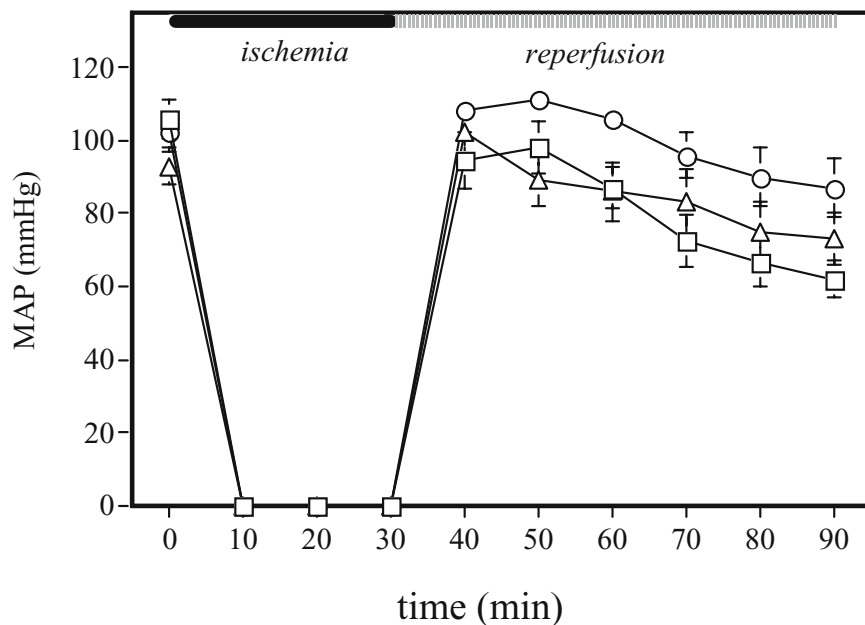

Fig. 1. Time course of changes in mean arterial pressure (*MAP*) during ischemia and reperfusion. In the normal saline (NS) group (*squares*), urinary trypsin inhibitor 50 000 U·kg^{-1} (LD) group (*triangles*), and the 150 000 U·kg^{-1} (HD) group (*circles*) the relevant agents were administered 30 min before ischemia. There were no significant differences between the groups

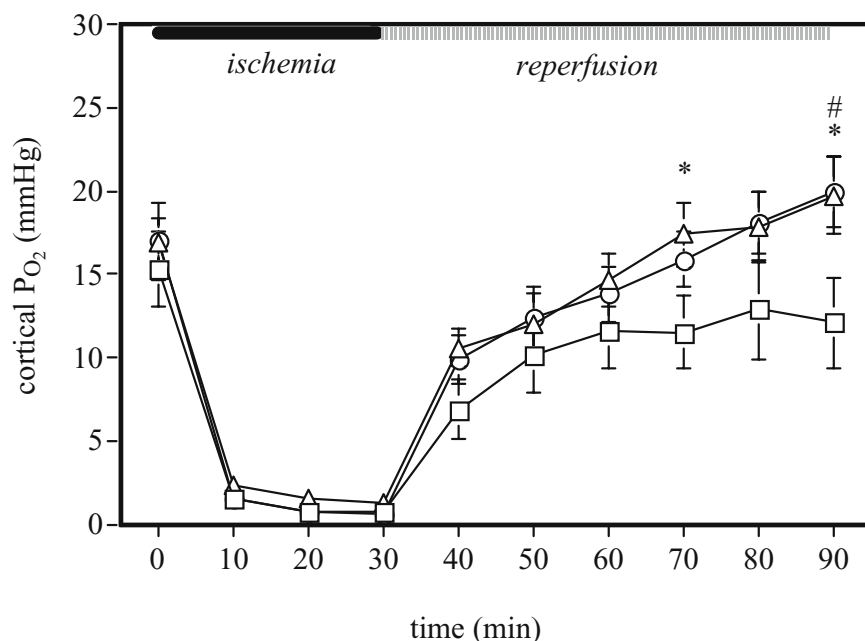**Fig. 2.** Time course of changes in cortical p_{O_2} during ischemia and after reperfusion. $*P < 0.05$ (NS vs LD); $^{\#}P < 0.05$ (NS vs HD). *Symbols*, as in Fig. 1
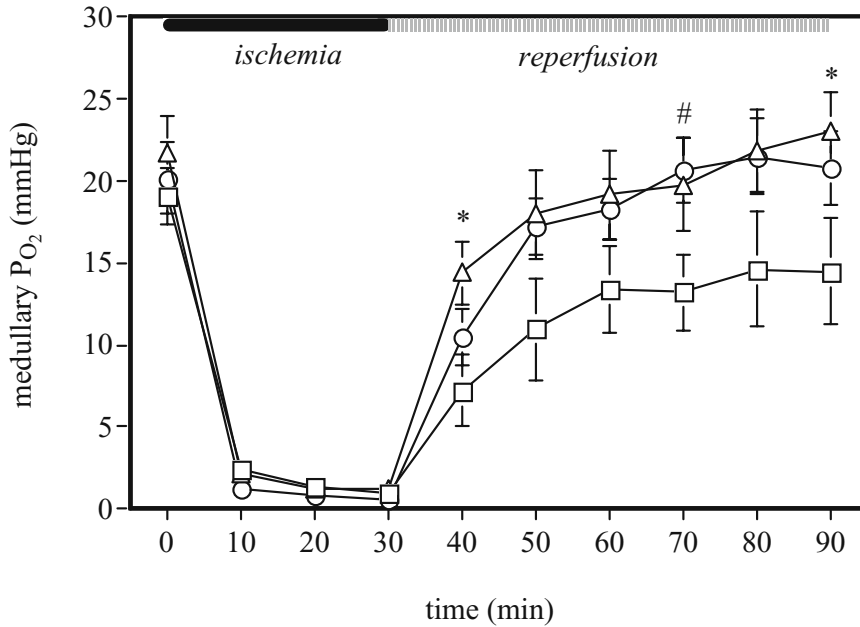
Fig. 3. Time course of changes in medullary p_{O_2} during ischemia and after reperfusion. *$P < 0.05$ (NS vs LD); #$P < 0.05$ (NS vs HD). *Symbols*, as in Fig. 1

There were significant differences in medullary p_{O_2} after 10 and 60 min of reperfusion between the NS and LD groups, and after 40 min of reperfusion between the NS and HD groups. There were no significant differences between the HD and LD groups throughout the experiments.

Discussion

Our findings demonstrate that UTI administered 30 min before aorta occlusion improved renal tissue oxygenation during reperfusion without changing mean arterial pressure and blood gas parameters. During the course of ischemia and reperfusion, the concentration of oxygen in tissue is a critical variable for cellular function and viability. Nelimarkka [3] in a study of renal artery occlusion in dogs, has reported that a critical p_{O_2} level for oxygen consumption is 15 mmHg for the renal cortex and 13 mmHg for the medulla and aerobic oxidative metabolism ceases at 6 mmHg in both tissue layers. In our previous MRS study [10], during ischemia, ATP was rapidly depleted and intracellular Na increased to the same extent in both the NS and UTI groups. After reperfusion, the recovery of ATP in the NS group was incomplete and the recovery of intracellular Na in the UTI group began earlier than that in the NS group, with better recovery of ATP. On electron micrographic analysis, the mitochondria in the NS group were swollen and disorganized with respect to the membrane and internal structure after 60 min of reperfusion, and the morphological changes in the mitochondria in the UTI group were improved in comparison to findings in the NS

group [10]. In the present study, the p_{O_2} values in each group changed around the critical levels during ischemia and reperfusion, and the recovery of p_{O_2} in the UTI groups was faster and more complete than that in the NS group during the reperfusion period. Therefore, it is suggested that the improvement of these cellular functions (i.e., intracellular Na and ATP recovery) by UTI is due to improved tissue oxygenation. UTI has been reported to improve renal functions, as determined by measurements of blood urea nitrogen (BUN) and serum creatinine, and by the histological appearance of the kidneys, during ischemic reperfusion [8,9]. UTI has also been reported to promote the recovery of cardiac function after reperfusion, by reducing the severity of mitochondorial dysfunction in the myocardium during hemorrhagic shock and reperfusion [6]. The effects of UTI on tissue oxygenation found in the present study may contribute to renal and cardiac protection in these conditions of shock.

Tissue oxygenation is affected by oxygen delivery and consumption. Komori et al. [7] have reported that UTI improves the microcirculation in rabbit ears as well as improving urine volume in anaphylactic shock, and they suggested that the action of UTI in maintaining the microcirculation may preserve renal blood flow and retain normal urine volume. Nakajima and Goto [14] have reported that UTI reduces the vasoconstriction in rat mesenterium in endotoxin-induced shock. On the other hand, there are no reports about the effects of UTI on tissue oxygen consumption during ischemia and reperfusion. However, in our previous study [10], UTI promoted the recovery of ATP, intracellular pH, and Na pumps after reperfusion, which requires an

increase in oxygen consumption. Therefore, it is suggested that the improved tissue oxygenation brought about by UTI is mainly due to an improvement in the microcirculation.

The mechanism involved in the improvement of the microcirculation cannot be established from the present study. Proinflammatory cytokines, such as tumor necrosis factor α (TNF α) and interleukin 8, which are induced during reperfusion, activate neutrophils and endothelial cells and lead to increased neutrophil-endothelial adhesion, resulting in microcirculatory disturbance. Recent studies have demonstrated that UTI inhibits the induction of cytokines and adhesion molecules, as well as that of lysosomal enzymes [15–18]. We have also reported that UTI inhibits the increases in renal tissue levels of TNF α, cytokine-induced neutrophil chemoattractant-1, and myeloperoxidase, as well as inhibiting increases in BUN and serum creatinine levels, after lipopolysaccharide stimulation [19]. It is thought that UTI improves the microcirculation by inhibiting interactions among these mediators, neutrophils, and endothelial cells.

Ischemic reperfusion may induce redistribution of blood flow within the kidney. The changes in tissue oxygenation in the renal cortex and medulla during shock and ischemia are controversial. Traditionally, the medulla is considered to have a high energy requirement coupled with a precarious blood flow, which makes this region particularly vulnerable to hypoxic insults [20]. In contrast, a considerable number of studies have indicated that, during shock, the p_{O_2} in the cortex is equal to or lower than that in the medulla [3,21,22]. Whitehouse et al. [22] suggest that the discrepancies in results may be related to methodological differences, such as the use of different species, kidney preparations, and instrumentation, and anesthesia regimens. In the present study, the p_{O_2} values in the renal cortex and medulla changed similarly in each group throughout the experiments. UTI promoted the recovery of both cortical and medullary p_{O_2} after reperfusion; therefore, it is suspected that UTI improves oxygen availability and cellular function in both regions.

Some studies have shown that the protective effects of UTI on shock are dose-dependent [7,23]. In our present study, there was no significant difference in tissue p_{O_2} between low and high doses of UTI. Pre-ischemic adminstration of UTI 50 000 U·kg^{-1} is considered to be sufficient for renal re-oxygenation in rats.

In conclusion, pre-ischemic UTI administration improves renal tissue oxygenation after reperfusion. The pharmacological properties of UTI, such as its renal protection and anti-shock activity, may be explained, in part, by this improvement in tissue oxygenation.

References

1. Cornard MF, Craeford RS, Davison JK, Cambria RPC. Thoracoabdominal aneurysm repair: a 20-year perspective. Ann Thorac Surg. 2007;83:S856–61..

2. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol. 2003;14:2199–210.

3. Nelimarkka O. Renal oxygen and lactate metabolism in hemorrhagic shock: an experimental study. Acta Chir Scand Suppl. 1984;518:1–44.

4. Binns OA, DeLima NF, Buchanan SA, Mauney MC, Cope JT, Thies SD, Shockey KS, Tribble CG, Kron IL. Neutrophil endopeptidase inhibitor improves pulmonary function during reperfusion after 18-hour preservation. J Thorac Cardiovasc Surg. 1996; 112:607–13.

5. Hirose J, Ozawa T, Miura T, Isaji M, Nagao Y, Yamashiro K, Nii A, Kato K, Uemura A. Human neutrophil elastase degrades inter-α-trypsin inhibitor to liberate urinary trypsin inhibitor related proteins. Biol Pharm Bull. 1998;21:651–6.

6. Masuda T, Sato K, Noda C, Ikeda K, Matsunaga A, Ogura M, Shimizu K, Nagasawa H, Matsuyama N, Izumi T. Protective effect of urinary trypsin inhibitor on myocardial mitochondria during hemorrhagic shock and reperfusion. Crit Care Med. 2003;31: 1987–92.

7. Komori M, Takada K, Tomizawa Y, Uezono S, Ozaki M. Urinary trypsin inhibitor improves peripheral microcirculation and bronchospasm associated with systemic anaphylaxis in rabbits in vivo. Shock. 2003;20:189–94.

8. Chen CC, Liu ZM, Wang HH, He W, Wang Y, Wu WD. Effects of ulinastatin on renal ischemia-reperfusion injury in rats. Acta Pharmacol Sin. 2004;25:1334–40.

9. Nakahama H, Obata K, Sugita M. Ulinastatin ameliorates acute ischemic renal injury in rats. Ren Fail. 1996;18:893–8.

10. Taie S, Yokono S, Ueki M, Ogli K. Effects of ulinastatin (urinary trypsin inhibitor) on ATP, intracellular pH, and intracellular sodium transients during ischemia and reperfusion in the rat kidney in vivo. J Anesth. 2001;15:33–8.

11. Swartz HM, Clarkson RB. The measurement of oxygen in vivo using EPR techniques. Phys Med Biol. 1998;43:1957–75.

12. Hou H, Grinberg OY, Williams B, Grinberg S, Yu H, Alvarenga DL, Wallach H, Buckey J, Swartz HM. The effect of oxygen therapy on brain damage and cerebral pO$_2$ in transient focal cerebral ischemia in the rat. Physiol Meas. 2007;28:963–76.

13. Hou H, Grinberg OY, Taie S, Leichtweis S, Miyake M, Grinberg S, Xie H, Csete M, Swartz HM. Electron paramagnetic resonance assessment of brain tissue oxygen tension in anesthetized rats. Anesth Analg. 2003;96:1467–72.

14. Nakajima K, Goto Y. Differentiation of the anti-shock effect of ulinastatin from steroid hormone, by the continuous observation of microcirculation dynamics. Circ Shock. 1992;36:284–9.

15. Kawamura T, Inada K, Akasaka N, Wakusawa R. Ulinastatin reduces elevation of cytocines and soluble adhesion molecules during cardiac surgery. Can J Anaesth. 1996;43:456–60.

16. Aosasa S, Ono S, Seki S, Takayama E, Tadakuma T, Hiraide H, Mochizuki H. Inhibitory effect of protease inhibitor on endothelial cell activation. J Surg Res. 1998;80:182–7.

17. Inoue K, Takano H, Shimada A, Yanagisawa R, Sakurai M, Yoshino S, Sato H, Yoshikawa T. Urinary trypsin inhibitor protects against systemic inflammation induced by lipopolysaccharide. Mol Pharmacol. 2005;67:673–80.

18. Yano T, Anraku S, Nakayama R, Ushijima K. Neuroprotective effect of urinary trypsin inhibitor against focal cerebral ischemia-reperfusion injury in rats. Anesthesiology. 2003;98:465–73.

19. Ueki M, Taie S, Chujo K, Asaga T, Iwanaga Y, Ono J, Maekawa N. Urinary trypsin inhibitor reduces inflammatory response in kidney induced by lipopolysaccharide. J Biosci Bioeng. 2007;104: 315–20.

20. Brezis M, Rosen S. Hypoxia of the renal medulla—its implications for disease. N Engl J Med. 1995;332:647–55.

21. Juillard L, Lerman LO, Kruger DG, Haas JA, Rucker BC, Polzin JA, Riederer SJ, Romero JC. Blood oxygen level-dependent measurement of acute intra-renal ischemia. Kidney Int. 2004;65:944–50.

22. Whitehouse T, Stotz M, Taylor V, Stidwill R, Singer M. Tissue oxygen and hemodynamics in renal medulla, cortex, and cortico-medullary junction during hemorrhage-reperfusion. Am J Physiol Renal Physiol. 2006;291:F647–53.

23. Endo S, Inada K, Taki K, Hoshi S, Yoshida M. Inhibitory effects of ulinastatin on the production of cytokines: implications for the prevention of septicemic shock. Clin Ther. 1990;12:323–6.