Ischemia-Reperfusion Injury of Canine Liver and Its Protection by Ulinastatin

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Summary Studies were made in dogs on ischemic injury of the liver induced by clamping the portal triad and inferior vena cava and on the influences of urinary trypsin inhibitor (Ulinastatin) on the ischemic changes. The increases in aspartate aminotransferase and alanine aminotransferase activities in the peripheral blood serum induced by ischemia and reperfusion were lowered by perfusion treatment of ischemic liver with ulinastatin for 60 min or its injection into the general circulation 5 min before reperfusion. The increase in lipid peroxides, measured by the TBA-method, in the post-ischemic liver was also decreased by administration of ulinastatin. α-Tocopherol in the liver decreased during ischemia-reperfusion, and its decrease was completely prevented by ulinastatin treatment. However, infiltration of neutrophils into post-ischemic liver tissue was not inhibited by ulinastatin. The effect of in vitro addition of ulinastatin on the formation of superoxide anion radicals by the xanthine-xanthine oxidase system and NADPH oxidase in polymorphonuclear leukocytes (PMNs) was also investigated. Ulinastatin did not affect xanthine oxidase activity or \( \text{O}_2^- \) formation. It also did not scavenge \( \text{O}_2^- \). On the contrary, it inhibited \( \text{O}_2^- \) formation by PMNs induced by N-formylmethionylleucylphenylalanine, concanavalin A plus cytocalasin B, or phorbol 12-myristate 13-acetate. The mechanisms of the protective effect of ulinastatin on ischemic liver injury are discussed from the view point of its antioxidant properties.

Key Words: liver transplantation, superoxide anion, lipid peroxide, antiprotease, ischemia

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Ischemic injury of various tissues such as liver, heart, digestive tract, and brain is thought to be due to active oxygens such as superoxide anion radicals and their reactions including lipid peroxidation [1-3]. Antioxidants such as CoQ_{10}, α-tocopherol, and SOD have been used for protection against ischemia-reperfusion injury [1, 4-6]. Recently we reported that vitamin E also has a protective effect on ischemic injury of the spinal cord and fetal distress [7-9], possibly by inhibiting lipid peroxidation.

Lie et al. [10] reported the protective effect of the protease inhibitor aprotinin on ischemic hepatocellular damage. They proposed that the protective effect of aprotinin on ischemia-reperfusion injury was due to its inhibition of proteolytic conversion of xanthine dehydrogenase to xanthine oxidase. Recently, many protease inhibitors have been reported to inhibit O_2^- generation by polymorphonuclear leukocytes (PMNs) [11-13]. Ulinastatin (Miraclid), a glycoprotein with a molecular weight of about 67,000 isolated from human urine [14], mainly inhibits proteolytic enzymes, such as trypsin, chymotrypsin, and leukocyte elastase [15], and has been widely used clinically in treatment of multiple organ failure, pancreatitis, and various types of shock [16].

In this study, we investigated the protective effect of ulinastatin on experimental liver ischemia associated with increases in aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities and pathological changes of the liver tissue. We examined its effects on the levels of lipid peroxides, estimated as TBA-reactive substances, and α-tocopherol in liver tissue and serum. We also studied its in vitro effect on superoxide anion release by a xanthine-xanthine oxidase system and by PMNs.

**MATERIALS AND METHODS**

*Reagents.* Ulinastatin was obtained from Mochida Pharmaceutical Co., Tokyo. One unit of Ulinastatin shows 50% inhibition of protease activities of 2 μg trypsin under the conditions described in reference [15]. Cytochalasin B (CytB), cytochrome c, and SOD were purchased from Sigma Chemical Co., St. Louis, MO; and concanavalin A (ConA), phorbol 12-myristate 13-acetate (PMA), N-formylmethionylleucylphenylalanine (fMLP), xanthine, xanthine oxidase (XO), and allopurinol, from Wako Pure Chemical Ind., Osaka.

*Animals.* Adult mongrel dogs weighing about 12 kg were anesthetized with pentobarbital and ventilated with a mechanical respirator. They were divided into 4 groups, which were subjected to treatment A (without liver washing) with or without ulinastatin and treatment B (with liver washing) with or without ulinastatin.

*Liver isolation and ischemia.* The total vascular exclusion technique is necessary for major hepatic resection [17], chemotherapy with liver isolation, or liver transplantation [18]. The technique was used in the present studies on ischemia-reperfusion injury of the liver. The abdomen was opened by an extended

right subcostal incision. The falciform ligament was cut to the vena cava and the lesser omentum was severed. The liver was completely mobilized by cutting triangular ligaments to the inferior vena cava on the right side, and the inferior vena cava was completely mobilized above and below the liver to allow the placement of vascular clamps. As shown in Fig. 1, complete liver ischemia was induced by clamping the portal triad to occlude inflow, the inferior vena cava below the liver but above the right adrenal vein and the suprahepatic inferior vena cava below the diaphragm. During the anhepatic period, the forced circulation method was applied for shunting systemic and splanchnic venous flow to the external jugular vein by use of a centrifugal pump (Bio-pump, Sarns, MI). Before clamping, 100 units/kg of heparin was injected. The liver was treated with ulinastatin in two ways (treatments A and B) to determine its protection site on the process of liver injury on ischemia and reperfusion. In treatment A, the liver was kept without washing for 60 min and 50,000 U/kg ulinastatin was injected into the general circulation 5 min before reperfusion. In treatment B, the portal vein was cannulated by venotomy and the cannula was kept patent by slow infusion of lactated Ringer's solution at room temperature. After the washing procedure, the liver was kept in the same solution with or without 50,000 U/kg ulinastatin for 60 min. A

Fig. 1. Diagrams of procedures for induction of ischemia by clamping the portal triad and inferior vena cava below the liver but above the right adrenal vein, and the suprahepatic inferior vena cava below the diaphragm. B, Common bile duct; A, common hepatic artery; P, portal vein; I, inferior vena cava; R, right adrenal vein; D, diaphragm; L, liver; S, splenic vein. The effect of ulinastatin was tested by two methods: For treatment A, ulinastatin was injected into the general circulation 5 min before reperfusion. In treatment B, after clamping, the liver was kept in lactated Ringer's solution with or without ulinastatin at room temperature.
silastic catheter was introduced into the infra-hepatic vena cava via the left femoral vein for removal of perfusate. After 60 min of ischemia, the liver was reperfused by removal of the clamps from the vessels. GOT and GPT in the serum, and lipid peroxide and \( \alpha \)-tocopherol in the serum and liver tissue were measured before ischemia, during ischemia, and after reperfusion. Pathological changes were also examined.

Hydrodynamic monitoring included measurements of pulse rate, and measurements of the femoral artery pressure and pulmonary artery pressure through a Swan-Ganz catheter (Baxter, Irvine). The hydrodynamics were kept stable during the operation. Metabolic acidosis was prevented by sodium bicarbonate infusion.

**Measurement of lipid peroxides.** Lipid peroxides in liver tissue were measured by the TBA-method and expressed as TBA-reactive substances (TBARS) in nmol equivalents of malondialdehyde [19]. Samples of about 1 cm\(^3\) of liver tissue were removed intact and chilled in ice-cold 0.9% NaCl until measurements could be made, within 2 h. For the assay, a 10% (w/v) tissue homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.5), and an aqueous solution of thiobarbituric acid, and heated at 95°C for 60 min. After cooling, the pink pigment was extracted with \( n \)-butanol-pyridine (15:1, v/v) mixture and measured as absorbance at 532 nm. Tetramethoxypropane was used as an external standard and the level of lipid peroxides was expressed as nmol equivalents of MDA. TBARS in the serum was measured by the method of Yagi [20]. The value of TBARS was measured before clamping, just before reperfusion, and 5, 30, 60 min after the start of reperfusion.

**Determination of \( \alpha \)-tocopherol content.** \( \alpha \)-Tocopherol was extracted with ethanol-hexane (2:5) from sera and homogenates of liver tissue. \( \alpha \)-Tocopherol in the hexane layer was separated by HPLC in a Hitachi 655A-11 apparatus with YMC-PACK A-600-3 (NH\(_2\) column, Yamamura Chem., Kyoto) and measured with a Hitachi-1100 fluorescence spectrometer [7].

**Preparation of PMN cells.** PMNs were obtained from male guinea pigs, weighing 300-400 g, by intraperitoneal injection of 40-50 ml of 0.9% NaCl solution containing 2% neutralized sodium caseinate and collection of the peritoneal exudate fluid 18 h later. The fluid was centrifuged at 135 \( \times \) g for 10 min at 0°C; and the cells were washed twice with 0.2% NaCl to remove contaminating erythrocytes by hypotonic lysis, and suspended in 50 mM KRP (Krebs Ringer Phosphate) buffer at 1 \( \times \) 10\(^6\) cells/ml. The preparation consisted almost entirely (>99%) of PMNs.

**Determination of \( O_2^- \) production by PMNs.** \( O_2^- \) generation by PMNs was assayed by measurement of the reduction of ferricytochrome \( c \) spectrophotometrically. The cell suspension was introduced into a 3-ml cuvette containing 5 mM glucose, 1 mM CaCl\(_2\), and 100 mM ferricytochrome \( c \) with or without 1,000 \( U/ml \) ulinastatin. The final cell concentration was 1 \( \times \) 10\(^6\) PMNs/ml. The reaction mixture in the cuvette was placed in a thermostatically controlled cuvette holder (37°C) of a spectrophotometer, and incubated for 4 min. Then Cyt B plus Con A, fMLP, or PMA was added to induce \( O_2^- \) production by the PMNs, and the
reduction of cytochrome c was followed at 550 nm. The final concentrations of Cyt B, Con A, fMLP, and PMA were 5 μg/ml, 50 μg/ml, 33 mmol/ml, and 2 μg/ml, respectively.

**Determination of** $O_2^-$ **consumption.** Oxygen consumption was monitored with a Clark-type electrode as described previously [21]. The consumption was calculated assuming an oxygen concentration of 217 nmol/ml in the initial incubation mixture at 37°C. The reaction mixture contained 5 mM glucose and 1 mM CaCl$_2$ with or without 1,000 U/ml ulinastatin. The final concentrations of Con A, Cyt B, fMLP, and PMA were 100 μg/ml, 5 μg/ml, 33 mmol/ml, and 2 μg/ml, respectively.

**Determination of** $O_2^-$ **production and** $O_2^-$ **consumption by xanthine-xanthine oxidase (XO).** XO activity and $O_2^-$ production were measured spectrophotometrically as the rates of urate formation and cytochrome c reduction, and $O_2^-$ consumption was measured with an oxygen electrode [21]. The final concentrations in the reaction mixture were 0.05 mM xanthine and $1 \times 10^{-5}$ M cytochrome c with 50 mM phosphate buffer (containing 0.1 mM EDTA-2Na). The reaction mixture was incubated with 1,000 U/ml of ulinastatin, 1 U/ml of SOD, or 400 μg/ml allopurinol for 2 min. Then 0.015 U/ml of XO was added and reduction of cytochrome c was measured at 550 nm. The amount of urate produced was determined from the increase in absorption at 290 nm.

**Statistical analysis.** All results were expressed as means ± SE. Statistical significance was determined by analysis of variance with Duncan’s multiple range test. Probability values of $p < 0.05$ were considered statistically significant.

**RESULTS**

*Changes during ischemia and reperfusion of canine liver*

Figure 2 shows the time courses of the changes in activities of GOT and GPT in the serum during ischemia and reperfusion. The serum GOT and GPT activities were markedly increased in the control groups for treatments A and B, 30 and 60 min after the start of reperfusion. But the increases in their activities were suppressed by ulinastatin treatment (Fig. 2). The serum GOT activities after a 60-min reperfusion in the control groups and ulinastatin-treated groups, respectively, were 4,596 ± 1,162 IU/liter and 1,551 ± 463 IU/liter after treatment A, and 2,757 ± 587 IU/liter and 1,515 ± 572 IU/liter after treatment B. The serum activities of GPT after the 60-min reperfusion in the control groups and ulinastatin-treated groups were 3,878 ± 542 IU/liter and 1,234 ± 504 IU/liter in treatment A, 2,779 ± 606 IU/liter and 1,154 ± 619 IU/liter in treatment B. The decrease in the β-ATP level in the liver during warm ischemia is reported to be inhibited by administration of ulinastatin before ischemia [22].

Figure 3 shows the time course of TBARS formation in liver tissue and the serum after 60 min of ischemia and during reperfusion. Data are shown as percentages of the value before ischemia. The levels of TBARS in the liver tissue after
Fig. 2. Effect of ulinastatin on the activities of GOT: ○ (n = 5), ● (n = 5), and GPT: □ (n = 5), ■ (n = 4 in treatment A and n = 5 in treatment B) in serum during ischemia and reperfusion. Black and white symbols indicate values of groups with and without ulinastatin treatment, respectively. Points and bars are means ± SE. The statistical significance of differences between values in 2 groups treated with and without ulinastatin were determined by analysis of variance with Duncan's multiple range test (*p < 0.01).

Fig. 3. Effect of ulinastatin on the levels of TBARS in the liver and serum during ischemia and reperfusion. Values before ischemia were taken as 100%. Treatment A: ○ (n = 7), ● (n = 5). Treatment B: □ (n = 5), ■ (n = 5). Black and white symbols indicate values for groups with and without ulinastatin treatment, respectively. The amounts of TBARS in liver tissue and serum before ischemia were 57.3 ± 2.7 nmol MDA/100 mg wet weight and 0.5 ± 0.05 nmol/ml, respectively. Points and bars are means ± SE. Significance of differences between values in the 2 groups treated with and without ulinastatin: *p < 0.01.
Liver reperfusion were higher in control groups than in the ulinastatin-treated groups for both treatments A and B, the TBARS values being $138 \pm 7.4\%$ (treatment A) and $119 \pm 7.3\%$ (treatment B) in the control groups after 30 min of reperfusion. However, the TBARS values were not increased by ischemia and reperfusion in the ulinastatin-treated groups. The serum value of TBARS after treatment B also increased during 30 min of reperfusion in the control group, but did not change markedly in the ulinastatin-treated groups or in the control for treatment A.

The changes in liver tissue and serum $\alpha$-tocopherol concentrations due to ischemia and reperfusion are shown in Fig. 4. Data are presented as percentages of the value before ischemia. The concentration of $\alpha$-tocopherol after ischemia decreased promptly on reperfusion in the control groups after both treatments A and B. The $\alpha$-tocopherol concentrations in the liver tissue after the 60-min reperfusion were $72.0 \pm 6.9\%$ and $77.1 \pm 1.8\%$ in the control groups after treatments A and B, respectively. However, the levels of $\alpha$-tocopherol did not change in the ulinastatin-treated groups after treatments A and B. The $\alpha$-tocopherol concentrations in the serum decreased on reperfusion after ischemia in all groups, and there were no significance differences between them.

Figure 5 shows the histological appearance of the liver tissue before and after ischemia. Neutrophil infiltration was observed in post-ischemic liver parenchyma (Fig. 5B). But the administration of ulinastatin did not inhibit the infiltration by
PMNs after either treatment A or B (Fig. 5C, D).

**O$_2^-$ generation by PMNs and by xanthine-xanthine oxidase**

As shown above, ulinastatin had a protective, anti-oxidant effect against ischemia-reperfusion injury. Suter and Chevallier [23] reported that eglin C, a protease inhibitor, has a SOD-like effect. Yoshikawa et al. [24] also reported that ulinastatin scavenged O$_2^-$ formed by the xanthine-xanthine oxidase system and by PMNs. On the contrary, Nomura reported that ulinastatin did not scavenge O$_2^-$ [13]. Therefore, we examined whether ulinastatin scavenged O$_2^-$ or not.

Table 1 shows the effects of ulinastatin on xanthine oxidase activity and O$_2^-$ formation by the xanthine-xanthine oxidase system. Allopurinol, a xanthine oxidase inhibitor, inhibited the reduction of ferricytochrome C, urate formation, and O$_2^-$ consumption induced by the xanthine-xanthine oxidase system, whereas SOD only suppressed the increase in ferricytochrome C reduction. On the contrary, ulinastatin did not affect O$_2^-$ production, urate formation or O$_2^-$ consumption by the xanthine-xanthine oxidase system.

Next, we investigated the *in vitro* effect of ulinastatin on O$_2^-$ production by J. Clin. Biochem. Nutr.
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Table 1. Effect of ulinastatin on \(O_2^-\) and urate formation and \(O_2\) consumption by xanthine oxidase.

<table>
<thead>
<tr>
<th></th>
<th>Reduction of ferricytochrome c ((\mu M/min))</th>
<th>Urate formation ((\mu M/min))</th>
<th>(O_2) consumption ((\mu M/2)min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5±0.23 (6)</td>
<td>3.82±0.07 (6)</td>
<td>132.6±2.5 (5)</td>
</tr>
<tr>
<td>Ulinastatin</td>
<td>3.1±0.2 (6)</td>
<td>3.02±0.17 (4)</td>
<td>128.0±3.4 (5)</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>0.33±0.01*(4)</td>
<td>0.21±0.03*(5)</td>
<td>25.5±1.0*(5)</td>
</tr>
<tr>
<td>SOD</td>
<td>1.6±0.14*(4)</td>
<td>3.9±0.07 (5)</td>
<td>131.3±0.6 (4)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate sample number. Values are means±SE. Significance of differences between test groups and control groups were analyzed by Student's \(t\)-test (*\(p<0.001\)).

Table 2. Inhibitory effect on ulinastatin on \(O_2^-\) generation and \(O_2\) consumption in PMNs.

<table>
<thead>
<tr>
<th></th>
<th>Reduction of ferricytochrome c ((\mu M))</th>
<th>(O_2) consumption ((\mu M))</th>
</tr>
</thead>
<tbody>
<tr>
<td>fMLP Control</td>
<td>7.5±0.8 (4)*</td>
<td>22.6±3.1 (5)*</td>
</tr>
<tr>
<td>Ulinastatin</td>
<td>4.4±0.6*(5)*</td>
<td>9.5±2.1*(6)*</td>
</tr>
<tr>
<td>PMA Control</td>
<td>9.2±0.4 (9)*</td>
<td>43.6±5.9 (6)*</td>
</tr>
<tr>
<td>Ulinastatin</td>
<td>6.4±0.7***(8)*</td>
<td>27.9±1.1***(6)*</td>
</tr>
<tr>
<td>ConA+CytB Control</td>
<td>11.7±0.8 (9)*</td>
<td>20.4±2.3 (6)*</td>
</tr>
<tr>
<td>Ulinastatin</td>
<td>3.7±0.6***(10)*</td>
<td>9.98±1.3***(5)*</td>
</tr>
</tbody>
</table>

Values are means±SE. Incubation times were \(a1\) min, \(b2\) min, \(c5\) min, \(d10\) min. Numbers in parentheses indicate sample numbers. *\(p<0.05\), **\(p<0.01\), vs. corresponding control.

PMNs obtained from the peritoneal cavity of male guinea pigs. Table 2 demonstrates the effects of ulinastatin on the reduction of ferricytochrome \(c\) and \(O_2\) consumption by PMNs stimulated with PMA, fMLP or Con A plus Cyt B. The \(O_2^-\) production by PMNs, measured as the reduction of ferricytochrome \(c\), induced by PMA, fMLP, or Con A plus Cyt B was inhibited by the addition of ulinastatin. Ulinastatin also had similar inhibitory effects on their \(O_2\) consumption stimulated by PMA, fMLP, or Con A plus Cyt B.

DISCUSSION

Oxygen-derived free radicals have been implicated as causes of cell injury in liver upon ischemia and reperfusion [1, 2, 25]. In this study ischemia and reperfusion injury of canine liver and its protection by ulinastatin were investigated. After ischemia we showed that the serum GOT and GPT activities increased markedly on reperfusion (Fig. 2), the content of lipid peroxides in the liver increased concomitantly (Fig. 3), and the \(\alpha\)-tocopherol level decreased (Fig. 4). These results suggest that lipid peroxidation and active oxygen radicals are involved in the process of liver injury induced by ischemia-reperfusion.

Ulinastatin lowered the increases in serum GOT and GPT activities on ischemia-reperfusion (Fig. 2). It also inhibited the increase in TBARS in the liver significantly. These results suggest that ulinastatin protects the liver from ischemic...
injury by its inhibitory effect on lipid peroxidation. The decrease in $\alpha$-tocopherol level induced by ischemia-reperfusion was completely inhibited by treatment with ulinastatin, indicating that ulinastatin does not scavenge but probably inhibits the production of active oxygen radicals such as $O_2^-$ and lipid peroxyl radicals derived from it.

$O_2^-$, a primary initiator of lipid peroxidation, which is associated with oxidative injury on ischemia and reperfusion, is mainly produced by two ways: (1), by increases in the amount of xanthine formed from ATP and xanthine oxidase level converted from xanthine dehydrogenase during ischemia [1, 2, 25], and (2), by PMNs and Kupffer cells due to activation of NADPH-oxidase present in the plasma membrane [26, 27]. Allopurinol, a xanthine oxidase inhibitor, was reported to protect the ischemia-reperfusion injury [28]. Grisham et al. [29] proposed that intestinal ischemia and reperfusion result in activation of xanthine oxidase in tissues, and then superoxide-dependent accumulation of inflammatory neutrophils occurs in the mucosa, where neutrophil-derived reactive oxygen metabolites mediate and/or exacerbate intestinal injury. In this study, injection of ulinastatin 5 min before reperfusion effectively inhibited ischemia-reperfusion injury. This observation indicates that tissue injury caused by oxidant stress may not be due to generation of $O_2^-$ by the xanthine-xanthine oxidase system, because less than 15% of the total xanthine dehydrogenase is reported to be converted to xanthine oxidase in rat liver after 30 min of ischemia, though the conversion was inhibited by a protease inhibitor such as soybean trypsin inhibitor [25]. This conclusion is supported by our finding that the production of urate, $O_2$ consumption and superoxide anion release by xanthine-xanthine oxidase were not inhibited by treatment with ulinastatin. Marubayashi et al. [30] reported that XO activity remained unchanged even after 90 min of hepatic ischemia and a subsequent 60-min reperfusion, although a marked increase in lipid peroxide in the liver tissue was observed. Similar slow conversion of xanthine dehydrogenase to xanthine oxidase during ischemia of rat kidney has been observed [31]. Metzger et al. [32] also concluded that oxidant stress induced by ischemia-reperfusion is not due to the generation of $O_2^-$ by xanthine oxidase, because the administration of allopurinol did not decrease the increase in glutathione disulfide release.

Administration of antibody against leukocytes was reported by Hess et al. [33] to protect the heart from ischemic injury. They concluded that oxygen free-radicals generated by neutrophils play an important role in the pathophysiology of ischemia-reperfusion injury. We observed marked infiltration of neutrophils into the liver parenchyma after ischemia-reperfusion. Grisham et al. [29] also reported a marked increase in neutrophil infiltration after reperfusion of ischemic intestine. Ulinastatin did not inhibit infiltration of PMNs (Fig. 5) but did inhibit $O_2^-$ production by the PMNs, indicating that $O_2^-$ production in neutrophils is activated after their infiltration into liver tissues, Moreover in our in vitro experiment we found that addition of ulinastatin effectively inhibited reduction of cytochrome $c$ and $O_2$ consumption on activation of PMNs induced by PMA.
fMLP, or Con A plus Cyt B. These findings suggest that $O_2^-$ production by the activation of PMNs is implicated in ischemia-reperfusion injury of the liver (Table 2).

Kitagawa et al. [11] reported that a chymotrypsin-type serine protease may be involved in $O_2^-$ production by human PMNs and monocytes, and be located at their cell surface. Utsunomiya et al. [34] supposed that this protease is also located in intracellularly, because gabexate mesilate (FOY), a low-molecular-weight protease inhibitor (M.W. 417), showed a stronger inhibitory effect on PMN $O_2^-$ production than aprotinin, a high-molecular-weight protease inhibitor (M.W. 6,500). Gabexate mesilate also has been reported to protect against active oxygen injury induced by low-blood flow (personal communication). Ulinastatin may inhibit this protease, which is possibly involved in a common process of NADPH-oxidase activation induced by various activators [11]. Another possible mechanism for inhibition of $O_2^-$ production by ulinastatin is inactivation of protease released from activated PMNs, by which PMNs are further activated resulting in a burst generation of $O_2^-$. Further investigations are required to assess these possibilities.

Recently, Kilpatrick et al. [35] reported that in highly purified serum antichymotrypsin (ACT) the region responsible for inhibition of superoxide anion production was structurally distinct from the protease inhibition site of ACT. A trypsin inhibitor polypeptide from soy beans was also recently separated into two subfractions having inhibitory activities toward PMN $O_2^-$ production and protease [36]. Thus the possibility remains that ulinastatin has different regions of antiprotease and inhibition of $O_2^-$ production.

The balance between released protease from PMNs and antiprotease is considered important in determining whether or not inflammation will damage connective tissue [37]. A recent report showed that active oxygens such as $O_2^-$ inactivates $\alpha_1$-proteinase inhibitor ($\alpha_1$-PI), which is an important component of the antiprotease system [38]. The protective effect of ulinastatin on damage of liver tissue induced by ischemia-reperfusion in this work is probably due to its suppression of $O_2^-$ production from PMNs and inhibition of increased protease activity by degradation of active oxygen-sensitive $\alpha_1$-PI.

Migration of PMNs has some time lag after reperfusion. Recently, Jaeschke [27] and Jaeschke et al. [39] reported that macrophage (Kupffer cell)-derived reactive oxygen species seem to be involved in the initial vascular and parenchymal cell injury by ischemia-reperfusion and that the PMN-dependent active oxygen injury is subsequent. Ulinastatin may inhibit the formation of $O_2^-$ derived from not only PMNs but also Kupffer cells.

Other factors such as recovery of liver tissue flow as pointed out by Hasselgren et al. [40] might also play an important role in the recovery of liver function induced by ischemia-reperfusion. Further investigation about this is necessary.
REFERENCES


