Protective Effects of Urinary Trypsin Inhibitor in Experimental Shock

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Accepted June 11, 1985

Abstract—The effects of human urinary trypsin inhibitor (UTI) were studied in experimental shock models. Administration of 50,000 U/kg, i.v., of UTI protected against mortality from shock induced by burn, endotoxin or trauma. Aprotinin at a dose of 50,000 U/kg improved only endotoxin shock and showed a moderate but not significant effect on burn and traumatic shock. Administration of 50,000 U/kg, i.v., of UTI protected against the aggravation in systemic hemodynamics in canine hemorrhagic shock. Furthermore, in rat traumatic shock, 50,000 U/kg, i.v., administration of UTI significantly reversed the increased serum $\beta$-glucuronidase and trypsin activities and the decreased hepatic ATP level, and it moderately suppressed the increased serum uric acid level. Aprotinin failed to affect all these biochemical changes induced by drum trauma. These results suggest that the protective effect of UTI against experimental shock is possibly exerted through lowering the elevated enzyme activities in the serum during shock.

It has been shown that the activities of trypsin (1), kininogenase (2-4), plasmin (5), cathepsins (6) and CPK (7) in the blood increase and the activity of serum plasmin inhibitor decreases (5) in animals during shock. These findings strongly suggest that protease activities possibly play an important role in the pathogenesis and/or development of shock. In this regard, aprotinin, a protease inhibitor extracted from bovine lung, has been already shown to possess antishock activity (8, 9).

Human urinary trypsin inhibitor (UTI), an acidic glycoprotein with a molecular weight of 67,000–70,000 (10, 11), is known to be significantly excreted into urine during shock (12), although its physiological role has not yet been well clarified (13). Our previous study (14) demonstrated that UTI differs from aprotinin in its spectrum of inhibitory activity on various enzymes other than proteases.

Moreover, the authors have found out that UTI shows a beneficial effect on the treatment of experimental acute pancreatitis in rats and dogs (14, 15) and operative stress in mice (16). Therefore, it is of great interest whether UTI is an endogenous substance for the treatment of shock. The present paper describes the effect of UTI on experimental shock, in comparison to that of aprotinin.

Materials and Methods

Drugs and animals
UTI was extracted and purified from fresh human urine by the method of Proksh and Routh (17). Its specific activity was 2,613 U/mg protein, one unit being the amount necessary to inhibit the activity of 2 $\mu$g trypsin (3,200 NFU/mg, Canada Packers) by 50%. The UTI preparation showed a single band in polyacrylamide gel electrophoresis, and its molecular weight, measured by gel filtration with Sephadex G-100, was 67,000. Aprotinin (Repulson®, Mochida Pharmaceutical), a different type of trypsin inhibitor, was used as a reference drug. UTI and aprotinin were diluted in 0.9% physiological saline. E. coli endotoxin (0127B8) was purchased from Difco Co., Ltd.

Male and female ddY mice weighing 17.0
to 32.0 g (Shizuoka Laboratory Animal Center) and ICR mice weighing 22.0 to 28.0 g (Clea Japan), male Wistar strain rats weighing 160 to 200 g (Shizuoka Laboratory Animal Center), and male and female mongrel dogs weighing 17.0 to 32.0 kg were used.

**Methods**

**Effect on traumatic shock in rats:** This experiment was carried out according to the method of Noble and Collip (18). After a fast of 24 hr, rats were placed in a drum and rotated at 45 rpm for 10 min and observed for the following 4 hr to determine the number of surviving animals. Drugs were administered 3 times i.v. at 1 min before and 1 and 10 min after the tumbling.

A similar experiment was conducted to investigate the effects of drugs on the biochemical parameters during traumatic shock. Each rat was sacrificed 30 min after the tumbling to collect its serum sample. β-Glucuronidase activity was measured by the method of Fishman et al. (19), trypsin activity by the method of Anson (20), glucose level by the method of Hultman (21) and uric acid level by the methods of Caraway (22) and Henry et al. (23).

Furthermore, other rats were sacrificed 2 hr after the tumbling, and their livers were extirpated for the measurement of ATP and ADP levels. The livers were immediately frozen with dry ice and kept at −65°C until assayed. The livers were homogenized for 2 min with 10 ml of 1 N perchloric acid per 2.5 g of liver. After centrifugation at 3,000 rpm at 4°C for 10 min, ATP and ADP levels in the supernatant were measured by the methods of Bücher (24) and Jaworek et al. (25), respectively.

**Effect on burn shock in mice:** According to the method by Hechter et al. (26), each mouse was immersed in hot water at 65°C for 10 sec to the axillar line under ether anesthesia, and observed for the following 4 hr to determine the number of surviving animals. Drugs were administered 3 times i.v. at immediately before, and 15 min and 1 hr after immersion in hot water.

**Effect on endotoxin shock in mice:** Mice were administered i.p. with 25 mg/kg endotoxin dissolved in physiological saline and observed for the following 24 hr to determine the number of surviving animals. Drugs were administered 4 times i.v. at 5 min and 3, 6 and 12 hr after the injection of endotoxin.

**Effect on hemorrhagic shock in dogs:** Under anesthesia with 30 mg/kg pentobarbital sodium, i.v., a tracheal cannula connected to a respirator (SN-480-4, Shinano) was inserted for the artificial ventilation and left thoracotomy was performed in the fifth intercostal space. A catheter (PE 205, Intramedic) was inserted into the right femoral artery and connected to a reservoir containing 10 ml of 500 units/ml heparin solution. Mean aortic blood pressure (MABP) was adjusted to 40 mmHg by 10 min of bleeding, and the blood was kept in a reservoir for 20 min. Then, the total blood shed in the reservoir was reinfused into the right femoral vein for 1 hr.

MABP, mean aortic blood flow (MABF) and heart rate (HR) were recorded using a polygraph (RM 8000, Nihon Kohden). MABP was measured with a pressure transducer (LPU-0.5–290, Nihon Kohden) connected to a catheter which was inserted into the carotid artery and advanced to the aortic arch. MABF was measured with an electromagnetic blood flow meter (MF-27, Nihon Kohden) connected to a blood flow probe (8–12 mm, Nihon Kohden) attached to the origin of the aorta. Left femoral arterial pressure was measured with a pressure transducer connected to a catheter placed in the left femoral artery. Cardiac work (CW) was calculated using the following equation:

\[
CW (g\cdot m/min) = MABF (ml/min) \times MABP (mmHg) \times 13.6/1,000
\]

Infusion of UTI through the left femoral vein was connected simultaneously with the blood transfusion for 1 hr.

**Statistical analysis:** Statistical significances of the changes from the value at onset of the blood transfusion were examined by Student’s two-tailed t-test in comparison to that of the control group in hemodynamic variables, and significant differences from that of the control group were examined using Student’s two tailed t-test in evaluating the biochemical variables and by Fisher’s exact method to evaluate the survival rate. All values were
expressed as the mean±S.E.

**Results**

**Effect on traumatic shock in rats:** UTI at a total dose of 50,000 U/kg significantly increased the number of survival animals. Aprotinin at a dose of 50,000 U/kg also tended to increase the number of surviving animals, although it was less effective than UTI (Table 1).

Trypsin and β-glucuronidase activities and uric acid level were significantly increased, but glucose level was decreased in the serum of traumatized rats 30 min after the tumbling. Moreover, hepatic ATP level was markedly decreased, and ADP level remained unchanged in the rats 2 hr after the tumbling. UTI at a dose of 50,000 U/kg significantly suppressed both the enzyme accumulations in the serum and the decreased hepatic ATP level and suppressed moderately but not significantly the increased serum uric acid level, although it did not affect the serum glucose level and hepatic ADP level. On the contrary, aprotinin at a dose of 50,000 U/kg did not show any significant effect on these parameters (Table 2).

**Effect on burn shock in mice:** UTI at a total

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### Table 1. Effect of urinary trypsin inhibitor (UTI) on the traumatic shock by the Noble-Collip drum method in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (U/kg i.v.)</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time after shock procedure (hr)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>4/10</td>
</tr>
<tr>
<td>UTI</td>
<td>10,000</td>
<td>3/10</td>
</tr>
<tr>
<td></td>
<td>30,000</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>9/10*</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>50,000</td>
<td>6/10</td>
</tr>
</tbody>
</table>

Significant difference by Fisher's exact method from that of the control group is marked: * (P<0.06). Rats were injected intravenously with drugs, and 1 min later, whole body trauma was induced by the tumbling for 10 min at 45 rpm. Further, drugs were injected 1 and 10 min after the tumbling, and the survival rate was determined for the following 4 hr.

### Table 2. Effects of urinary trypsin inhibitor (UTI) on the changes in serum β-glucuronidase and trypsin activities, the glucose and uric acid levels, and hepatic ADP and ATP levels during traumatic shock induced by the Noble-Collip drum method in rats

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Control (traumatized)</th>
<th>UTI (50,000 U/kg i.v.)</th>
<th>Aprotinin (50,000 U/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>β-Glucuronidase (U/ml)</td>
<td>43.5±3.2</td>
<td>278.8±41.3††</td>
<td>152.3±22.6</td>
<td>203.8±27.1</td>
</tr>
<tr>
<td>Trypsin (U/ml)</td>
<td>6.35±0.75</td>
<td>10.06±1.22†</td>
<td>6.07±0.76*</td>
<td>8.96±1.14†</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>131.0±11.9</td>
<td>70.6±5.7††</td>
<td>76.4±5.4</td>
<td>66.9±7.7</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.78±0.22</td>
<td>5.30±0.98†</td>
<td>3.66±0.27</td>
<td>5.21±0.97</td>
</tr>
<tr>
<td>ADP (μg/g wet weight)</td>
<td>31.7±3.3</td>
<td>38.5±3.4</td>
<td>33.7±2.2</td>
<td>38.8±4.3</td>
</tr>
<tr>
<td>ATP (μg/g wet weight)</td>
<td>115.9±19.0</td>
<td>54.1±9.0†</td>
<td>86.7±6.1**</td>
<td>73.1±7.0</td>
</tr>
</tbody>
</table>

Each value represents the mean±standard error. Significant differences by Student's t-test from that of the traumatized control group are marked: * (P<0.05), ** (P<0.01), and significant differences from that of the normal group by Student's t-test in the traumatized control group are marked: † (P<0.05), †† (P<0.01). Rats were injected intravenously with drugs, and 1 min later, whole body trauma was induced by the tumbling for 10 min at 45 rpm. Further, 1 and 10 min after the tumbling, drugs were injected. Thirty min after the tumbling, the animals were sacrificed, and the serum enzyme activities and the glucose and uric acid levels were measured. Two hr after the tumbling, animals were sacrificed and the hepatic ADP and ATP levels were measured.
dose of 50,000 U/kg significantly increased the number of surviving animals at 2 hr after the burn shock. Aprotinin at a dose of 50,000 U/kg showed a lesser, non-significant effect on the survival rate of animals (Table 3).

**Table 3. Effect of urinary trypsin inhibitor (UTI) on the burn shock in mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (U/kg i.v.)</th>
<th>Survival rate</th>
<th>Time after shock procedure (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>10/10</td>
<td>4/10</td>
</tr>
<tr>
<td>UTI</td>
<td>10,000</td>
<td>10/10</td>
<td>4/10</td>
</tr>
<tr>
<td>UTI</td>
<td>30,000</td>
<td>10/10</td>
<td>6/10</td>
</tr>
<tr>
<td>UTI</td>
<td>50,000</td>
<td>10/10</td>
<td>9/10*</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>50,000</td>
<td>10/10</td>
<td>8/10</td>
</tr>
</tbody>
</table>

Significant difference by Fisher's exact method from that of the control group is marked: * (P<0.05).

Drugs were injected intravenously just before and 15 min and 1 hr after immersion in water at 65°C for 10 sec.

**Table 4. Effect of urinary trypsin inhibitor (UTI) on the endotoxin shock in mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (U/kg i.v.)</th>
<th>Survival rate</th>
<th>Time after shock procedure (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>20/20</td>
<td>17/20</td>
</tr>
<tr>
<td>UTI</td>
<td>10,000</td>
<td>20/20</td>
<td>18/20</td>
</tr>
<tr>
<td>UTI</td>
<td>30,000</td>
<td>19/20</td>
<td>18/20</td>
</tr>
<tr>
<td>UTI</td>
<td>50,000</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>50,000</td>
<td>20/20</td>
<td>19/20</td>
</tr>
</tbody>
</table>

Significant difference by Fisher's exact method from that of the control group is marked: * (P<0.05).

Mice were injected intraperitoneally with 25 mg/kg endotoxin from *E. coli*, and drugs were injected intravenously 15 min and 3, 6 and 12 hr later.

dose of 50,000 U/kg significantly increased the number of surviving animals at 2 hr after the burn shock. Aprotinin at a dose of 50,000 U/kg showed a lesser, non-significant effect on the survival rate of animals (Table 3).

**Effect on endotoxin shock in mice**: UTI at a total dose of 50,000 U/kg significantly increased the survival rate 20 hr after the shock. Aprotinin at a dose of 50,000 U/kg showed equipotent activity to UTI on the survival rate of animals (Table 4).

**Effect on hemorrhagic shock in dogs**: Before bleeding, MABP of the control, 30,000 U/kg UTI and 50,000 U/kg UTI groups were 95.7±13.3, 104.8±8.4 and 97.5±5.6 mmHg, respectively. After bleeding, MABP of the control, 30,000 U/kg and 50,000 U/kg UTI groups were lowered to about 40% of the level obtained before bleeding: precisely, to 40.1±6.4, 40.6±0.8 and 39.1±1.1 mmHg, respectively. Further, there was a marked decrease in MABF and particularly CW, whose level decreased to 10% of that before the bleeding.

These changes were gradually recovered by the reinfusion of shed blood. By i.v. administration of UTI, the recovery of hemodynamic variables to the normal level were further enhanced as compared with those of the control group. When the statistical analysis was performed for each change from the value at onset of the blood transfusion, 50,000 U/kg UTI significantly increased the changed values of MABP, MABF and CW as compared with those of the control group. Significant increase of CW was also observed 20 min after the beginning of administration in the 30,000 U/kg UTI group. However, HR did not change markedly following the bleeding and UTI infusion (Fig. 1).

**Discussion**

It is well accepted that ischemia caused by circulatory deficiency is important in the pathogenesis of cell injury involving the disruption of lysosomes and zymogen
granules during shock. Thus, these subcellular alterations cause the release of lysosomal enzymes such as β-glucuronidase and cathepsins, zymogenic proteases such as

Fig. 1. Effect of urinary trypsin inhibitor (UTI) on the change in mean aortic blood pressure, mean aortic blood flow, cardiac work and heart rate induced by hemorrhagic shock in anesthetized dogs. Each value represents the mean±standard error. ○ ○: Saline; ●●●: UTI 30,000 U/kg, i.v.; ■■■: UTI 50,000 U/kg, i.v. Statistical significances of the changes from the value at the onset of the blood transfusion were examined by Student's t-test in comparison to that of the control group: *(P<0.05). Mean aortic blood pressure was adjusted to 40 mmHg by 10 min of bleeding, the blood from which was kept in a reservoir, and maintained at that level for 20 min. Then, total blood shed in the reservoir was reinfused into the right femoral vein for 1 hr. The infusion of UTI through the left femoral vein was conducted simultaneously with the blood transfusion for 1 hr.
trypsin and kininogenase, and the formation of toxic substances (27–29) by these enzymes to result in further tissue injury in shock. Changes in these enzymes are, therefore, believed to range from presumably reversible ones to those presumed to be irreversible (30, 31). In this regard, it can be proposed that an enzyme inhibitor possibly attenuates the development of shock through the inhibition of lysosomal enzyme and/or zymogenic protease activities.

UTI was more effective than aprotinin in increasing the survival rate of rats during traumatic shock and mice during burn shock; and even during endotoxin shock in mice, UTI was equipotent to aprotinin. The authors previously reported that UTI, which exerts markedly lower potentials than aprotinin as a protease inhibitor in inhibiting plasmin and kininogenase activities, inhibits the activities of several enzymes other than proteases (14) and that UTI is more potent than aprotinin in inhibiting various proteases released from pancreatic slices (15). It has been proposed that the inhibitory activity of aprotinin on plasmin and kininogenase is a major mechanism of the beneficial effect of aprotinin on the treatment of experimental shock (2, 3, 32). These findings suggest that the effectiveness of UTI may depend on its properties as an enzyme inhibitor which are different from those of aprotinin.

In traumatized rats, UTI, unlike aprotinin, clearly attenuated the accumulation in the serum of β-glucuronidase and trypsin, which are the marker enzymes of lysosomal enzymes and zymogenic enzymes, and reversed the decreased hepatic ATP storage. The present data on aprotinin are in good agreement with the result of Araki and Lefer (33), in which they describe that aprotinin does not attenuate β-glucuronidase accumulation in the plasma but improves the survival rate of traumatized rats. Our previous work demonstrates that UTI as well as aprotinin does not show any inhibition of β-glucuronidase activity and that UTI shows almost equipotent inhibition of trypsin to aprotinin in an in vitro study (14). Therefore, the attenuation by UTI of β-glucuronidase accumulation in the serum will reflect its suppression of the release of lysosomal enzymes from the splanchnic area. Moreover, the attenuation by UTI of trypsin accumulation in the serum may also reflect similar suppression of the release of zymogenic enzymes, since aprotinin at the same dose as UTI did not show significant effect on the trypsin activity in the present experiment. This suppressive effect of UTI, in addition to its moderate suppression of increased serum uric acid which suggests the increase in ATP degradation, appears to reveal the consequent improvement of the decreased hepatic ATP level. UTI did not affect the decreased serum glucose level, suggesting that UTI does not improve the suppression of glycolysis or glucose utilization during shock. The present finding that UTI unlike aprotinin is capable of the attenuation of the enzyme release from lysosomes and/or zymogen granules is also closely related to the beneficial action on the treatment of experimental shock.

In canine hemorrhagic shock, the blood transfusion did not cause an immediate return of the hemodynamic parameters to normal levels, suggesting that some metabolic alteration will proceed during bleeding in this shock model. It is generally known that ischemia after the bleeding can produce metabolic alteration in canine hemorrhagic shock (34–36). In this experiment, UTI significantly reversed CW and MABF, while it did not change HR in canine hemorrhagic shock. We ascertained that even when UTI at the higher dosage of 1,000,000 U/kg was infused, it virtually showed no effect on systemic hemodynamics in normal rabbits. The results obtained from canine hemorrhagic shock, therefore, suggest that UTI may be effective against metabolic alteration caused by circulatory deficiency. This improvement of metabolic alteration by UTI appears to be closely related to its preventive effect on the enzyme accumulation in the serum observed in traumatized rats, and its property as an enzyme inhibitor differed from that of aprotinin.

We have found out that the enzyme release can be induced not only by the addition of trypsin or phospholipase A₂, but also by the addition of such released enzymes to canine pancreatic slices (15). This suggests that the released enzymes may also have a triggering
activity of secondary-enzyme release during shock, although the primary release will be initiated by ischemia. Moreover, we described that UTI is more potent than aprotinin in inhibiting the activities of such released proteases (15). These findings suggest that UTI possibly attenuates the enzyme accumulation in the serum by inhibiting the released protease activities during shock. The beneficial effect of UTI on the treatment of various forms of shock, when UTI was injected in the early stage of shock, will be closely related to the property of UTI as an enzyme inhibitor.

References
10 Haberland, G.L.: Chlorine in distilled water as a


