A Therapeutic Effect of Ulinastatin on Endotoxin-Induced Shock in Dogs — Comparison with Methylprednisolone —

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ABSTRACT. The therapeutic effect of ulinastatin (25,000 U/kg, i.v.) on endotoxin-induced shock was compared with that of methylprednisolone (30 mg/kg, i.v.) in 17 anesthetized dogs. Both of these drugs had almost the same tendency to improve the hemodynamics, arachidonate cascade metabolites and pulmonary surface activity. There was little difference between the effectiveness of ulinastatin and that of methylprednisolone. It was newly confirmed that the release of 6-keto-PGF₁α, thromboxane B₂, and leukotriene B₄, arachidonate cascade metabolites and chemical mediators associated with endotoxin-induced shock, were significantly (p<0.01 and p<0.05) decreased by ulinastatin in the same way as methylprednisolone. These results suggest that ulinastatin is as useful as methylprednisolone for the treatment of endotoxin-induced shock. — KEY WORDS: arachidonic acid cascade, endotoxic shock, methylprednisolone, pulmonary surface activity, ulinastatin.


Antibiotics, adrenocorticosteroids and cardiotonics are usually used for the chemotherapy of endotoxin-induced shock [5, 9, 10, 12]. The elucidation of the pathology of endotoxin-induced shock has encouraged the clinical application of chemotherapy with proteolytic enzyme-inhibitors. These have recently contributed to an increase in the life-saving rate and are expected to be promising in the treatment of patients with endotoxin-induced shock [14, 15, 30].

Ulinastatin is a glycoprotein isolated and purified from human urine and has a molecular weight of approximately 67,000. It is a polyvalent enzyme-inhibitor having a strong inhibitory effect on some enzymes such as trypsin, chymotrypsin and enterokinase [23, 29]. The excretion of these enzymes into urine is known to increase in pregnancy, fever and shock [4]. It is also experimentally as well as clinically known to have an anti-pancreatic enzyme action and an anti-shock action [25, 27].

Methylprednisolone is extensively used for the treatment of shock, because of its therapeutic effects, such as to improve impaired circulation, stabilize cell membrane, inhibit the production of myocardial depressant factor (MDF), activate the reticuloendothelial system and enhance cell metabolism [21, 32, 34].

In this study, the effect of ulinastatin on hemodynamics, arachidonic acid cascade and pulmonary surface activity during endotoxin-induced shock in dogs was investigated and compared with the effect of methylprednisolone, an adrenocorticosteroid which is used commonly in the treatment of shock.

MATERIALS AND METHODS

1. Experimental animals and procedure

Seventeen clinically healthy Beagle dogs (ranging in age from 1 to 5 years and in weight from 8 to 14 kg) without filarial infection were used. The experiment was carried out under general anesthesia induced with atropine sulfate (0.05 mg/kg, i.v.) and pentobarbital sodium (30 mg/kg, i.v.). After endotracheal intubation, pancuronium bromide (0.1 mg/kg, i.v.) as administered to maintain respiration, with arterial carbon dioxide tension controlled at 30–40 mmHg. The dogs were laid in the right lateral recumbent position, and a 7 French catheter was inserted into the aorta via the femoral artery to monitor hemodynamics and to sample blood. A 5 French Swan-Ganz catheter was inserted into the pulmonary artery via the femoral vein to measurement pulmonary arterial pressure and cardiac output. During the experiment, an anesthetic and muscle relaxant were additionally administered as necessary to stabilize the anesthesia. The dogs were divided into 3 groups. Ulinastatin was administered at a dose of 25,000 U/kg to Group U (n=5). To Group M (n=5), methylprednisolone was administered at a dose of 30 mg/kg. Group C (n=7) was given physiological saline as a control. After all these were administered by single intravenous injections, 3 mg/kg of endotoxin (Escherichia coli 055:B5 Difco, Detroit, U.S.A.) was intravenously injected over 5 min. The changes in hemodynamics parameters and chemical mediators were observed until 360 min after the endotoxin administration.

A powder preparation of ulinastatin (50,000 unit, Mochida Pharmaceuticals, Tokyo) was used in this experiment. It was dissolved in 10 ml of physiological saline and administered at a dose of 25,000 U/kg. Methylprednisolone (sodium methylprednisolone succinate 125 mg, Upjohn, Tokyo) was dissolved in 10 ml of a solvent (distilled water and physiological saline) and administered at a dose

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of 30 mg/kg.

2. Measurement of parameters

(1) Hemodynamics

The following hemodynamic parameters were measured: heart rate (HR), mean aortic pressure (MAOP), mean pulmonary arterial pressure (MPAP), cardiac output (CO) and urinary volume (UV). Mean aortic pressure and MPAP were measured with a transducer (P23ID, GOULD, U.S.A.) and a Life-Scope 11 recorder (OMP-7201, Nihon Koden, Tokyo). A thermal dilution cardio- meter (MTC-6210, Nihon Koden, Tokyo) was used for measuring CO. From the values obtained, systemic vascular resistance (SVR), pulmonary vascular resistance (PVR) and cardiac index (CI) were calculated.

(2) 6-keto-PGF₁α, thromboxane B₂ and leukotriene B₄

Plasma concentrations of 6-keto-PGF₁α, thromboxane B₂ and leukotriene B₄ were measured with a 6-keto-PGF₁α, ¹²⁵I kit, thromboxane B₂, ¹²⁵I kit (Daichi RI, Tokyo) and leukotriene B₄ RIA kit (Amersham, Tokyo), respectively.

(3) Pulmonary surface activity

After the experiment, the dogs were sacrificed by euthanasia with a sufficient dose of pentobarbital sodium. The lungs were removed and the right middle lobe of the lung was inflated with air to 40 cmH₂O. The pressure-volume relation in deflation was recorded. The pulmonary surface activities obtained from the deflation curve, were expressed as Gruenwald’s stability index (SI) [6], Clements’ expansion index (EI) [1] and percent lung volume (VP₂₅cm) at 15 cmH₂O (which closely agrees with the γ-minimum according to Johnson [18]).

(4) Statistical analysis

The data were expressed as the mean±standard deviation. Statistical analysis of the data was carried out by Student’s t-test and p<0.05 was defined as statistically significant.

RESULTS

1. Effects of ulinastatin and methylprednisolone on hemodynamics

Table 1 shows the effects of ulinastatin and methylprednisolone on the hemodynamics. There were no significant differences between Groups U and M in the hemodynamic parameters. However, the MAOP was significantly (p<0.01) increased after 240 min in Group M compared with Group C. The heart rate tended to increase in Group C, but showed no significant change in Groups U and M. There were no significant differences in CI in groups U and M. However, it tended to be slightly higher in Groups U and M, than in Group C. Systemic vascular resistance was significantly (p<0.05) decreased at 30 min in Group U

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
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<tr>
<td>HR (U)</td>
<td>151±37.1</td>
<td>144±34.8</td>
<td>145±29.9</td>
<td>161±30.1</td>
<td>157±30.8</td>
<td>155±35.8</td>
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<tr>
<td>HR (M)</td>
<td>146±15.2</td>
<td>130±20.4</td>
<td>135±18.6</td>
<td>153±15.9</td>
<td>146±17.8</td>
<td>142±14.8</td>
<td>135±10.8</td>
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<td>C</td>
<td>154±31.0</td>
<td>160±19.5</td>
<td>165±22.2</td>
<td>166±25.3</td>
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<td>MAOP (U)</td>
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<td>59±16.6</td>
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<td>63±10.9</td>
<td>73±10.3</td>
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<td>126±15.9</td>
<td>83±31.9</td>
<td>80±37.5</td>
<td>79±30.9</td>
<td>97±27.4</td>
<td>115±13.4</td>
<td>114±17.4</td>
<td>121±8.5</td>
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<td>C</td>
<td>113±18.5</td>
<td>48±14.5</td>
<td>55±15.2</td>
<td>52±9.9</td>
<td>61±13.9</td>
<td>63±12.7</td>
<td>81±19.4</td>
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<td>8±3.2</td>
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<td>9±3.4</td>
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<td>10±2.8</td>
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<td>13±2.7</td>
<td>13±5.6</td>
<td>8±1.1</td>
<td>9±1.8</td>
<td>11±2.8</td>
<td>12±3.4</td>
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<td>14±6.4</td>
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<td>CI (U)</td>
<td>4.89±0.81</td>
<td>1.84±0.50</td>
<td>2.53±0.73</td>
<td>2.42±0.49</td>
<td>2.25±0.43</td>
<td>2.32±0.57</td>
<td>2.28±0.42</td>
<td>2.07±0.23</td>
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<td>CI (M)</td>
<td>3.99±0.51</td>
<td>1.83±1.04</td>
<td>2.15±1.33</td>
<td>2.69±1.05</td>
<td>2.46±0.82</td>
<td>2.55±0.75</td>
<td>2.30±0.36</td>
<td>2.13±0.42</td>
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<td>4.08±1.06</td>
<td>1.12±0.42</td>
<td>1.92±0.52</td>
<td>1.82±0.35</td>
<td>1.96±0.29</td>
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<td>1.90±0.30</td>
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<td>SVR (U)</td>
<td>3.01±0.46</td>
<td>3.74±0.57</td>
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<td>3.02±0.95</td>
<td>3.94±1.05</td>
<td>4.51±1.52</td>
<td>5.52±1.92</td>
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<td>6.48±1.32</td>
<td>5.40±1.05</td>
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<td>5.42±0.85</td>
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<td>6.63±4.44</td>
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<td>6.10±1.60</td>
<td>7.15±3.18</td>
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<td>PVR (U)</td>
<td>360±40</td>
<td>684±187</td>
<td>436±164</td>
<td>493±167</td>
<td>592±263</td>
<td>643±366</td>
<td>747±402</td>
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<tr>
<td>PVR (M)</td>
<td>427±117</td>
<td>839±278</td>
<td>774±357</td>
<td>576±270</td>
<td>618±277</td>
<td>691±363</td>
<td>754±278</td>
<td>864±294</td>
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<tr>
<td>C</td>
<td>449±100</td>
<td>2.21±843</td>
<td>762±346</td>
<td>823±281</td>
<td>797±288</td>
<td>1.00±361</td>
<td>1.13±374</td>
<td>1.30±732</td>
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<tr>
<td>UV (U)</td>
<td>1.8±0.8</td>
<td>NM</td>
<td>0.4±0.3</td>
<td>0.2±0.3</td>
<td>0.3±0.2</td>
<td>1.0±0.3</td>
<td>1.1±0.7</td>
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</tr>
<tr>
<td>UV (M)</td>
<td>1.5±1.2</td>
<td>NM</td>
<td>0.7±0.2</td>
<td>0.9±1.5</td>
<td>0.9±0.2</td>
<td>2.0±1.2</td>
<td>2.5±0.8</td>
<td>2.1±1.0</td>
</tr>
<tr>
<td>C</td>
<td>1.5±0.7</td>
<td>NM</td>
<td>0.5±0.4</td>
<td>0.5±0.3</td>
<td>0.4±0.3</td>
<td>0.1±0.1</td>
<td>0.3±0.4</td>
<td>0.4±0.5</td>
</tr>
</tbody>
</table>

Data are the mean±standard deviation.

a) heart rate (beat/min), b) mean aortic pressure (mmHg), c) mean pulmonary arterial pressure (mmHg), d) cardiac index (l/min/m²), e) systemic vascular resistance (dyne·sec·cm⁻²), f) pulmonary vascular resistance (dyne·sec·cm⁻²), g) urinary volume (ml/kg/h), h) ulinastatin 25,000 U/kg, i) methylprednisolone 30 mg/kg, j) control, k) p<0.05 versus control, l) p<0.01 versus control, m) not measured.
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compared with Group C. Pulmonary vascular resistance was significantly (p<0.05) decreased at 30 min in Groups U and M but not in Group C. Urinary volume in Groups U and M was significantly (p<0.01 or 0.05) increased after 240 min, while the dogs in Group C were almost anuric.

2. Changes in 6-keto-PGF₁α, thromboxane B₂ and leukotriene B₄

The increase in 6-keto-PGF₁α induced by the endotoxin administration was significantly (p<0.01) inhibited at 60, 180 and 360 min in Group U and at 60 and 360 min in Group M (Fig. 1). The increase in thromboxane B₂ was also significantly (p<0.01) inhibited at 180 and 360 min in Groups U and M (Fig. 2). Similarly, the enhancement of leukotriene B₄ was significantly inhibited (p<0.05) in Group U and (p<0.01) in Group M at 60 min (Fig. 3).

3. Changes in pulmonary surface activity

The decreases in SI, EI and VP₁₅₅m induced by the endotoxin administration were slowed in Groups U and M. In Group U the decrease was slowed slightly more than in Group M (Fig. 4). But, there was no significant difference among the three groups.

Fig. 1. Effects of ulinastatin and methylprednisolone on 6-keto-PGF₁α in dogs with experimentally-induced endotoxic shock. Data are the mean ± standard deviation. ●; ulinastatin, ○; methylprednisolone, □; control, ★; p<0.01 versus control.

Fig. 2. Effects of ulinastatin and methylprednisolone on thromboxane B₂ in dogs with experimentally-induced endotoxic shock. Data are the mean ± standard deviation. ●; ulinastatin, ○; methylprednisolone, □; control, ★; p<0.01 versus control.

Fig. 3. Effects of ulinastatin and methylprednisolone on leukotriene B₄ in dogs with experimentally-induced endotoxic shock. Data are the mean ± standard deviation. ●; ulinastatin, ○; methylprednisolone, □; control, ★; p<0.05 versus control, ★; p<0.01 versus control.

Fig. 4. Effects of ulinastatin and methylprednisolone on pulmonary surface activity in dogs with experimentally-induced endotoxic shock. Data are the mean ± standard deviation.
DISCUSSION

The effect of ulinastatin on endotoxin-induced shock was evaluated in comparison with that of methylprednisolone. The disturbance of hemodynamics induced by endotoxic shock was more effectively improved by methylprednisolone than by ulinastatin. Methylprednisolone may normalize the shock-induced contraction of smooth muscles, which diminishes cardiac strain and augments cardiac output [34]. Methylprednisolone also ameliorates the endotoxin-induced hemodynamic perturbation by inhibiting the production of MDF during shock [21]. The number of catecholamine receptors is decreased by catecholamine released by shock [11]. However, it has been suggested that steroids enhance the potentiality of catecholamine by inhibiting the decrease in catecholamine receptors and thereby improved the hemodynamics [7, 31]. The increase in urinary volume due to the administration of methylprednisolone seems to be attributable to the increased renal blood flow following the improvement in cardiac output. Although an increase in blood pressure and cardiac output have been demonstrated by urinary trypsin inhibitor in experimental shock [27], the administration of ulinastatin in this experiment had no significant effect on the hemodynamics without SVR and PVR at 30 min. The effect of ulinastatin in increasing urinary volume may involved in its direct action on renal function [16].

The present series of experiments had to be designed to use general anesthesia in consideration of the prevention of cruelty to animals and pulmonary vascular catheterization. As these data were directly extrapolated to the clinical condition (awake), we could not exclude the anesthetic effect, resulted in more complicated hemodynamics. Nevertheless, the validity of the above therapeutic effects could be assured at least in connection with the control.

It has been reported that arachidonic acid cascades considerably affected endotoxin-induced shock [8]. The suppressive effect of methylprednisolone on the production of 6-keto-PGF₁α, thromboxane B₂ and leukotriene B₄ (LTB₄) observed in the present study may be the result of the inhibition of phospholipase A₂ [2]. On the other hand, the suppressive effect on the increase in these metabolites caused by ulinastatin seems to inhibit the action of the proteolytic enzymes from vascular endothelial cells induced by endotoxin or to eliminate active oxygen [35], because this agent has hardly any inhibitory effect on phospholipase A₂ [26]. Both interleukin-1 and tumor necrosis factor are involved in the release of LTB₄. Ulinastatin has been reported to inhibit the release of cytokines [3]. It may be also presumed that the release of LTB₄ was indirectly inhibited by the inhibitory action of ulinastatin on the release of cytokine.

The stability indexes, EI and VP₂scm for the removed lungs, were examined to assess the influence of ulinastatin and methylprednisolone on pulmonary surface activity in endotoxin-induced shock. Lipoprotein lipase may be a factor affecting pulmonary surface activity [22]. An increase in blood lecithinase activity during shock has been found in association with this effect of lipoproteinlipase [20]. The improvement in pulmonary surface activity due to the administration of methylprednisolone is considered to result from the stabilization of lysosomal membranes by inhibiting lecithinase released from lysosomes [33], while that of ulinastatin seems to originate in the stabilization of lysosomal membranes [24]; and the reduction in the production of both granulocytic elastase [13] and active oxygen [35] are regarded as primary lung injury factors in endotoxin-induced shock.

We reported that the suppression of reticuloendothelial function was induced by the endotoxin-induced shock [28]. Although the effect of therapeutic drugs on reticuloendothelial function was not studied in this experiment, the steroid preparations seemed to have not only an activating effect on reticuloendothelial function but also a suppressive effect on arachidonate cascade [12, 17, 19]. The effect of ulinastatin on reticuloendothelial function may require further study in order to clarify it.

The results of this experiment suggest that ulinastatin as well as methylprednisolone should be a useful drug for the treatment of endotoxin-induced shock.

REFERENCES

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Saunders, Philadelphia.


