EXPERIMENTAL STUDY ON THE INHIBITION OF KININ RELEASE IN ENDOTOXIN SHOCK BY GLUTATHIONE, PROTEINASE INHIBITORS, HYDROCORTISONE AND HYPERBARIC OXYGEN

SAPIO SUMIDA, M.D. AND HIROSHI YAGI, M.D.

Glutathione, proteinase inhibitors, steroids and hyperbaric oxygen are significantly effective to improve the survival of rats and to inhibit the liberation of plasma kinin in endotoxin shock. The combination of those anti-shock agents resulted in a decrease of kinin release in endotoxin shock in contrast with the treatment with each agent alone.

SUDDEN and simultaneous activations in the blood coagulation-fibrinolysis, complement and kinin systems have been increasingly recognized as complications of septic shock due to a variety of gram-negative bacteria (Fig. 1). Catecholamines stimulate all the three essential plasma cascades by the following sequence: microcirculation spasm, hypoxia, acidosis, sludging, lysosomal degradation, and then the proteinase activation.

Severe hypotension and respiratory distress in the course of septic shock were believed to be due to liberation of kinins and related vasoactive peptides into the circulation as hemodynamic mediators. This paper is concerned with the changes of plasma kinin level in endotoxin treated rats and with the influence of inhibitors: glutathione, aprotinin and methanesulfonate (FOY) hydrocortisone and hyperbaric oxygen.

METHODS

Endotoxin shock was produced in rats by intravenous injection of Escherichia coli endotoxin preparation (Difco B4 1 mg in 1.0 ml saline) in the volume of 0.5 ml/100g body weight. Rats ranging in weight from 350 to 450 anesthetized lightly by intraperitoneal administration of thiopental sodium, 30 mg/100g body weight were placed in the supine position on the operating table. To sample mixed venous blood, a polyethylene tube (21 gauge) was inserted into the right jugular vein until its tip lay in the right atrium.

Test Drugs: Glutathione 100 mg intraperitoneally per 100g body weight, aprotinin 10^5 KIU intravenously, hydrocortisone 1 mg intravenously, FOY (0.5 mg) + hydrocortisone (1 mg), and FOY (0.5 mg) + hydrocortisone (1 mg) + heparin (0.1 mg) were used before the endotoxin (Table 1). In the hyperbaric oxygen group, the rats were treated in the hyperbaric chamber at a pressure of 2.5 atmospheres absolute (ATA), using 100 per cent oxygen for 20 min after endotoxin.

In about 40 min after endotoxin, blood pressure decreased abruptly to 60 mmHg or less, the renal blood flow decreased, central venous pressure increased, nasal sanguineous discharge and diarrhea occurred, and the rats fell in shock as mentioned elsewhere.

Sixty min after endotoxin, 5 ml of blood was drawn without anti-coagulant through the

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cannula with a polyethylene disposable syringe. Blood sample to estimate the normal value of plasma kinin was collected from other rats anesthetized without endotoxin. The blood was immediately inactivated within 10 sec by forcibly ejecting it through the needle into 15 ml of chilled 80% (v/v) ethanol in a stoppered polyethylene centrifuge tube, which was then shaken to give adequate dispersion to extract kinin in whole blood according to the method reported by Brocklehurst and Zeitlin. Proteins and enzymes are precipitated and inactivated at this concentration of ethanol. Diethylstilbestrol (10 μg/100g body weight), a synthesized potent estrogenic preparation, was intraperitoneally injected into rat 18 hours before use. One horn of the isolated rat uterus was used for determination of free kinin. In our hands, the methods of Brocklehurst and Zeitlin worked with a 68 ± 11 (n = 5)% yield. The contraction of uterus was recorded using a force-displacement transducer (Nihon Kohden Kogyo Co., Ltd., Japan). Synthetic bradykinin (Nakarai Chemical Ltd., Japan) was used as the standard. The concentration of plasma kinin levels in venous blood was expressed in ng bradykinin equivalent/ml plasma.

In order to observe the survival, unanesthetized rats were used, and test drugs except gluta-

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (per 100g body weight)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Endotoxin Shock)</td>
<td></td>
<td>4% (2/52)</td>
</tr>
<tr>
<td>Glutathione</td>
<td>100 mg intraperitoneally</td>
<td>40% (4/10)</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>10³ KIU intravenously</td>
<td>31% (5/16)</td>
</tr>
<tr>
<td>FOY</td>
<td>0.5 mg intravenously</td>
<td>33% (6/18)</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1 mg intravenously</td>
<td>77% (17/22)</td>
</tr>
<tr>
<td>FOY + Hydrocortisone</td>
<td>0.5 mg + 1 mg</td>
<td>69% (9/13)</td>
</tr>
<tr>
<td>FOY + Hydrocortisone + Heparine</td>
<td>0.5 mg + 1 mg + 0.1 mg</td>
<td>73% (11/15)</td>
</tr>
<tr>
<td>Hyperbaric Oxygen</td>
<td>2.5 ATA, 20 min</td>
<td>33% (3/9)</td>
</tr>
</tbody>
</table>

FOY: Methanesulfonate, ATA: atmosher absolute, *: p < 0.01

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TABLE II  EFFECTS OF TEST DRUGS ON FREE PLASMA KININ IN THE BLOOD OF ENDOTOXIN SHOCK (RATS)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>0.2</th>
<th>0.0</th>
<th>0.0</th>
<th>2.5</th>
<th>0.0</th>
<th>0.5 ± 1.1 (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Endotoxin Shock)</td>
<td>20.0</td>
<td>46.7</td>
<td>73.3</td>
<td></td>
<td></td>
<td></td>
<td>46.7 ± 21.8 (3)</td>
</tr>
<tr>
<td>Endotoxin Shock + Glutathione</td>
<td>12.3</td>
<td></td>
<td>2.1</td>
<td>18.5</td>
<td>7.4</td>
<td>4.1</td>
<td>8.8 ± 5.9 (5) p &lt; 0.05</td>
</tr>
<tr>
<td>Endotoxin Shock + Hyperbaric Oxygen</td>
<td>22.6</td>
<td>22.5</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
<td>18.1 ± 6.3 (3) N.S.</td>
</tr>
<tr>
<td>Endotoxin Shock + FOY</td>
<td>2.2</td>
<td>2.4</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td>2.0 ± 0.5 (3) p &lt; 0.05</td>
</tr>
<tr>
<td>Endotoxin Shock + Aprotinin</td>
<td>0.4</td>
<td>1.3</td>
<td>1.8</td>
<td>0.6</td>
<td>1.6</td>
<td></td>
<td>1.2 ± 0.5 (5) p &lt; 0.05</td>
</tr>
</tbody>
</table>

FOY: methanesulfonate, Aprotinin: trasylol. Free plasma kinin levels were expressed as ng bradykinin equivalent/ml plasma.

Thione were administered into the tail vein immediately after endotoxin. In the glutathione group, glutathione was injected intraperitoneally before and after endotoxin, and the survival of the 48 hours period was determined. The control rats received same volume of saline instead of test drugs.

Student’s t test and chi square test were employed where appropriate to determine significance which was set at the 95% confidence level.

RESULTS

Effect of Test Drugs on the Survival of Rats Endotoxin Shock
The control rats did not survive longer than those rats treated with either glutathione, hydrocortisone, proteinase inhibitors, or hyperbaric oxygen (Table I).

Plasma Kinin Levels in Venous Blood in Normal Conditions and in Endotoxin Shock
The maximum value of free kinin in normal rat plasma was 2.5 ng/ml plasma (Table II). The release of plasma kinin in the state of shock and the therapeutic effect of test drugs are shown in Tables II and III. The kinin release was significantly increased in endotoxin shock from corresponding normal values before the endotoxin, however the kinin release was markedly inhibited when the test drugs were prophylactically and

TABLE III  EFFECTS OF TEST DRUGS ON FREE PLASMA KININ IN THE BLOOD OF ENDOTOXIN SHOCK (RATS), PREVIOUSLY REPORTED BY THE AUTHOR

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>34.5 ± 18.3 (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>16.5 ± 11.6 (3)</td>
<td></td>
</tr>
<tr>
<td>Aprotinin</td>
<td>21.3 ± 8.8 (3)</td>
<td></td>
</tr>
<tr>
<td>FOY</td>
<td>18.4 ± 9.4 (7)</td>
<td></td>
</tr>
<tr>
<td>FOY + Hydrocortisone</td>
<td>14.9 ± 8.3 (3)</td>
<td></td>
</tr>
<tr>
<td>FOY + Hydrocortisone + Heparine</td>
<td>11.0 ± 7.0 (3)</td>
<td></td>
</tr>
</tbody>
</table>

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intravenously administered. Although the combined uses of FOY + hydrocortisone, and FOY + hydrocortisone + heparin did not increase the survival in contrast with the single use of hydrocortisone, the release of kinin was more remarkably prevented when the combination of these anti-shock agents was used (Table III).

DISCUSSION

Plasma kinins produce vasodilation, edema, pain and hypotension. Clinical importance of plasma kinins was first recognized in septic shock, but now their role in various forms of shock has been acknowledged. In 1971 the International Symposium on Proteinase Inhibition in Shock Therapy was held in Wiesbaden, and recently many interesting works have appeared from the clinical field. Proteinase inhibitors have been used to suppress activation of kallikrein-trypsin system and to treat the state of shock. However, glutathione has never been evaluated on the inhibition of kinin release.

Beck and Linkenhmeier firstly reported that the level of liver sulf-hydryl as a result of the administration of endotoxin had declined, and this study was supported by Jeffries and Szymanski et al. After that, glutathione, a tripeptide, r-glutamylcysteinyglycine (GSH), came in focus in the treatment of shock. There are two kinds of glutathione: the reduced (GSH) and the oxidized (GSSG), of which GSH is used in this experiment. Thiol-compounds and GSH have an inhibitory effect on the histamine and serotonin release in a concentration of 5–10 mM. He suggested the inhibitory effect of thiol-compounds above described was due to the reduction of the natural antibodies against endotoxin or other component macromolecules, as he observed that SH-compound inhibited the guinea-pig complement in sensitized sheep’s red blood cells. We also recognized recently that glutathione inhibited the activation of rabbit complement in endotoxin shock unpublished data. A marked inhibition of anaphylactic reaction by various thiol-compounds including glutathione had been confirmed by Edman. Cook reported that methyl-prednisolone and cysteine prevented the lead-induced endotoxin lathality in rats. Galvin et al. observed that cysteine significantly maintained arterial blood pressure in cats subjected to cardiogenic shock. Cysteine also significantly prevented the plasma accumulation of the lysosomal hydrolase, catepsin D, and the toxic factor, myocardial depressant factor (MDF). In addition, cysteine maintained hepatic and cardiac non-protein sulf-hydryl concentrations in cardiogenic shock. They suggested that cysteine exerted beneficial effects in shock primarily by modulation of cellular metabolism and stabilization of lysosomal membranes rather than by direct hemodynamic actions. Brown observed the decrease in the level of liver glutathione (GSH) in endotoxin treated mice in part due to formation of glutathione disulfide (GSSG), and explained that the inadequate supply of nicotinamide adenine dinucleotide phosphate (NADP) for glutathione reductase activity and the inhibition of "tripeptide" synthesis appeared to account for the loss of liver glutathione in endotoxin shock. The decrease of glutathione level can result in a variety of metabolic derangements.

In this study, glutathione significantly improved the survival of rats in endotoxin induced shock. As the plasma kinin values in endotoxin shock were suppressed by the intravenous injection of glutathione, we considered that glutathione can be used without any hesitation for the purpose of proteinase inhibition therapy not only in endotoxin but in any forms of shock. In our clinic, large doses of glutathione (200 mg/kg body weight daily for 3–5 days, intravenously) is routinely administered for those purposes.

Stiff lung, fall in lung compliance, is usually seen in the patients with endotoxin shock, which was slight when pretreatment with proteinase inhibitors and steroids had been applied. The stiff lungs are more difficult to be inflated and more easily to be deflated than the normal. The morphologic changes of interstitial edema, vascular congestion and alveolar collapse were also observed. Alveolar edema was seen in severe cases which will be jeopardized by kinin, therefore, inhibition of the release by use of glutathione, steroids, proteinase inhibitors, hyperbaric oxygen and any means is clinically essential.

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