Significance of Intravascular Coagulation in Canine Endotoxin Shock

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The contribution of disseminated fibrin clot formation to the pathogenesis of canine endotoxin shock was explored in control dogs and in those defibrinated with a purified fraction of Malayan pit viper venom. The hemodynamic and hormonal responses after the administration of an intravenous challenge dose of Escherichia coli endotoxin were comparable as was mortality. It is concluded that, although the role of the coagulation sequence in canine endotoxin shock is unclear, it does not appear to be determinate.

Bacterial endotoxins activate many humoral pathways in vivo, including complement (8,11), kinin (24), and coagulation (9) sequences. Although some of the consequences of kinin (24) and complement (8) activation in canine endotoxin shock have been described recently, the importance of disseminated intravascular coagulation in this situation is unclear.

Anticoagulant pretreatment of dogs has in some instances markedly increased survival after endotoxin challenge (9,14) and in others had no effect on mortality (22,25). Because heparin, the anticoagulant used in most such studies, may have other effects than those on coagulation system components (7,19), the variable results may be independent of the anticoagulant properties of heparin. Therefore, the effects of endotoxin were studied in dogs with prior defibrination induced by a purified fraction of Malayan pit viper (MPV) venom, an agent known to act only upon the terminal effector portion of the coagulation system (1).

(A preliminary report of these studies was published previously: Circulation 42(Suppl. 3):118, 1970.)

MATERIALS AND METHODS

Dogs weighing 10 to 12 kg were anesthetized with 30 mg of pentobarbitol per kg administered intravenously. Hemodynamic changes were continuously monitored during the experiments. Aortic pressure was measured with a strain-gauge transducer via a catheter inserted in the carotid artery. The pressure was recorded with an oscillographic system. Average pressure was obtained with an electrical averaging circuit. A jugular venous catheter was inserted to inject drugs and obtain venous blood samples for the determination of complement levels (20), Lee-White clotting times (23), and fibrinogen levels (13). Blood leukocyte and platelet counts (23) were also obtained in several experiments.

In seven control experiments, after a 60-min period during which base line measurements were obtained, an approximately 80% lethal dose of purified Escherichia coli endotoxin (0.75 mg/kg) (17) was injected intravenously as a bolus, and pressure measurements and blood samples were obtained over a 4-h period. In six other animals a chromatographically purified fraction of MPV venom (2) was infused over a 60-min period (0.39 to 0.78 mg/dog). Over the next several hours clotting times were frequently determined until the blood became incoagulable. Endotoxin was then administered as described above, and the animals were monitored for an additional 4 h.

RESULTS

MPV venom is a potent defibrinating agent (Table 1). Three hours after the start of the infusion, the blood was incoagulable in glass, as demonstrated by the Lee-White clotting times. The plasma fibrinogen was markedly reduced to approximately 10% of control levels. These changes were highly significant ($P < 0.005$; Student's $t$ test). The MPV venom infusion caused no discernible hemodynamic changes, as has been reported previously (15). Figure 1 demonstrates the hemodynamic changes after endotoxin injection in the control and defibrinated animals. There was no real difference between the two groups with respect to the mean arterial blood pressure response in either the immediate postchallenge period or the later periods (except for several intermediate time intervals where there were slight statistically significant differences). The extremely close hemodynamic correspondence of the control and experimental groups during the first hour after challenge is seen in Fig. 2, in which the
arterial blood pressure response to an endotoxin challenge in decomplemented dogs (8) is also shown. Table 2 shows the changes in plasma complement after the various experimental interventions. It is seen that endotoxin caused a significant early decrease in complement levels in both control and defibrinated animals ($P < 0.05$; Student's $t$ test) but that MPV venom treatment alone caused no significant drop in complement. Figure 3 shows the response of the leukocyte and platelet counts after MPV venom and then endotoxin challenge in two experiments. Neither was remarkably altered by MPV venom alone, but endotoxin caused a rapid and marked decline of both. This response is identical to that reported previously in endotoxin-challenged animals (27). We have also seen this response in all control animals so studied (not shown). The mortality in both groups of animals was similar, with all animals in the control group dying within 72 h and five out of six in the defibrinated group also dying during this period.

**DISCUSSION**

Endotoxin may interact with the coagulation sequence in at least two ways: (i) through direct activation of Hageman factor (21) or possibly indirectly through the induction of endothelial damage (10), and (ii) by causing platelet aggregation and release (5) in the presence of a heat-labile serum factor (26), which may (12) or may not (4) be complement. Hence, endotoxin interaction with coagulation system components can potentially alter homeostasis through the generation of kinins resulting from Hageman factor activation, through the release of serotonin (3) or other vasoactive substances (26) from platelets, through the formation of platelet plugs (G. Evans and J. F. Mustard, J. Clin. Invest. 47:31a, 1968), or as a result of disseminated fibrin clot formation (14).

In contrast to heparin, the anticoagulant action of which stems from the ability of that agent to inhibit the formation and action of thrombin (presumably by electrostatically binding to the relevant clotting factors) (6), MPV venom induces the conversion of fibrinogen to an unstable form of fibrin without altering other coagulation factors or platelet number or function. Degradation of MPV venom-formed fibrin is presumably achieved by the fibrinolytic and reticuloendothelial systems (1). It has also been demonstrated in dogs that administration of a more rapidly defibrinating

![Table 1. Coagulation before and after MPV venom infusion](image)

<table>
<thead>
<tr>
<th>Determination</th>
<th>Plasma fibrinogen (mg/100 ml)</th>
<th>Lee-White clotting time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ......</td>
<td>$357 \pm 23^a$</td>
<td>$24 \pm 3$</td>
</tr>
<tr>
<td>3 h after infusion ......</td>
<td>$38 \pm 3$</td>
<td>Unclopted at 120 min</td>
</tr>
<tr>
<td>Control vs. 3 h after infusion ......</td>
<td>$P &lt; 0.005^b$</td>
<td>$P &lt; 0.005$</td>
</tr>
</tbody>
</table>

$^a$ Mean $\pm$ standard error of the mean.

$^b$ Student's $t$ test.

![Fig. 1. Mean aortic blood pressure responses for control animals and those with MPV venom-induced defibrination](image)

**Fig. 1.** Mean aortic blood pressure responses for control animals and those with MPV venom-induced defibrination. The only statistically significant differences between the two groups are at 120 and 180 min. SEM, Standard error of the mean.
dose of MPV venom than used in these experiments does not itself cause microscopically detectable fibrin clot formation even after pretreatment with epsilon aminocaproic acid, an inhibitor of fibrinolysis (18). Therefore, in the present experiments MPV venom made disseminated fibrin clot formation improbable by removing the terminal effector, fibrinogen, without modifying the ability of the platelet, leukocyte, or earlier clotting factors to react to endotoxin and presumably without itself inducing such clot formation.

The current data, therefore, indicate that disseminated fibrin clot formation does not play a determinant role in the hemodynamic response or mortality attendant upon canine endotoxin shock. Furthermore, subsequent to our preliminary report of this work, similar data were obtained in a comparable feline endotoxin shock model. In that report, no differences were noted between the control, shocked animals and the defibrinated, shocked animals with respect to hemodynamic changes, hematological changes, or survival (16). This does not imply that disseminated fibrin clot formation plays no role in modifying the response to endotoxin, but rather that under the conditions of a near

**TABLE 2. Effect of MPV venom pretreatment on lipopolysaccharide (LPS)-induced decomplementation**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Plasma complement (CHs units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPS (n = 7)</td>
</tr>
<tr>
<td>Control</td>
<td>42.5 ± 3.0a</td>
</tr>
<tr>
<td>3 h after MPV venom treatment</td>
<td></td>
</tr>
<tr>
<td>10 min after LPS treatment</td>
<td>33.2 ± 2.0</td>
</tr>
<tr>
<td>Control vs. 3 h after MPV venom treatment</td>
<td>NS</td>
</tr>
<tr>
<td>Control vs. 10 min after LPS treatment</td>
<td><em>P &lt; 0.05</em></td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.
* Student’s t test.

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**FIG. 2.** Early mean aortic blood pressure responses for control animals, MPV venom-defibrinated animals, and cobra venom factor (CVF) decomplemented animals (8) are shown. There is no alteration of the early hemodynamic response caused by MPV venom pretreatment in marked contrast to the modifications induced by decomplementation.

**FIG. 3.** Platelet and leukocyte changes after MPV venom and then endotoxin treatment. Although MPV venom causes only a moderate rise in the leukocyte count and a similar moderate fall in the platelet count, endotoxin causes precipitous drops in both.
maximal endotoxin challenge (i.e., 80% lethal dose) other factors assume dominance in determining the characteristics of the response.

These findings are not in conflict with previous data; rather, they may clarify some of the apparently contradictory findings of the past. The variable response to heparin anticoagulation (9, 14, 22, 25) may be dose related and dependent upon properties of heparin other than its ability to prevent the formation of fibrin. For example, it has been suggested that heparin may activate the reticuloendothelial system and that this may be important in the defense reaction against endotoxin (7). Large doses of heparin can also inhibit antigen-antibody reaction-induced aggregation of platelets (19), and it is possible they may interfere with endotoxin-induced aggregation. There is also no conflict with the suggestion that Hageman factor activation or platelet activation and dissolution may be an important feature of the endotoxin response, since it has been shown that MPV venom does not alter early clotting factors (1), and our data show that MPV venom does not alter the usual platelet or leukocyte response to endotoxin (27). It is also of interest that the MPV venom does not alter the usual complement response to endotoxin (8, 11) or the early hemodynamic response to endotoxin thought to be a consequence of complement activation (8). It is concluded that, although the role of much of the coagulation sequence in the endotoxin response is not yet settled, there seems to be no evidence that disseminated fibrin clot formation plays a major role in canine endotoxin shock.

ACKNOWLEDGMENTS

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LITERATURE CITED