NOTES

Enhanced Endotoxin Effects in Plasma Fibronectin-Deficient Rats

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Bacterial sepsis and its attendant complications continue to result in major morbidity and increased mortality in hospitalized patients recovering from traumatic injury, burns, and surgical wounds (17). Numerous studies have documented the detrimental effects of accidental or surgical trauma on host defense mechanisms, such as subnormal leukocyte function, cutaneous anergy, and depressed function of tissue macrophages belonging to the mononuclear phagocyte system (MPS) (1). Depression of MPS phagocytic activity correlates with the severity of injury in these patients and may be the greatest risk factor for the development of endotoxemia during gram-negative infections (2, 3, 14). Fibronectin is a high-molecular-weight glycoprotein which appears to play a critical role in MPS clearance of autologous tissue debris, immune complexes, and bacteria. The concentrations of this glycoprotein in plasma may be decreased (by 40% or more) in patients after traumatic, burn, and surgical wounds (12, 15).

Recently, Everett et al. (5) reported that pretreatment of anesthetized dogs with purified human plasma fibronectin significantly ameliorated the effects of a lethal injection of Escherichia coli endotoxin on several clinically relevant cardiovascular, acid-base, and metabolic parameters. Saba et al. (12, 13) have reported that the infusion of fibronectin-rich cryoprecipitates in septic adult trauma and surgical patients increases the fibronectin concentration in plasma and is associated with clinical improvement. We have previously utilized a model of sublethal endotoxin administration to elucidate alterations in metabolism induced by endotoxemia in the rat (19) and have reported that impaired hepatic nitrogen metabolism with elevations in the concentrations of ammonia and urea in plasma is an early effect of endotoxin administration (9). Since fibronectin depletion has been associated with an increased risk of sepsis in burn patients and plasma fibronectin pretreatment reduces some of the effects of endotoxemia in animals, we hypothesized that transient plasma fibronectin deficiency might lead to enhanced endotoxin effects in our sublethal endotoxemic model. In this report, we compare the differences in the ammonia concentrations in plasma of endotoxin-challenged normal and plasma fibronectin-deficient rats.

Immunoreactive plasma fibronectin deficiency was induced by intravenous infusion of gelatin (denatured collagen). This technique has been shown by Deno et al. (3) to result in transient plasma fibronectin depletion, apparently through clearance of the gelatin-fibronectin complex by the liver. Briefly, 0.1 ml of blood was collected in EDTA-coated tubes from the tail vein of 20-250-g male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) for base-line determinations of plasma fibronectin. Animals (n = 10 for each group) were intravenously injected with a placebo (5% glucose in water) or 12 mg of gelatin (ICN Biochemicals Inc., Cleveland, Ohio) per kg of body weight (BW). Since endotoxin administration has been reported to increase the fibronectin concentration in plasma of the rat (11), we also quantified the fibronectin concentration in plasma of animals that were pretreated with placebo or gelatin and that then received 0.2 mg of E. coli O111:B4 endotoxin (phenol preparation from Sigma Chemical Co., St. Louis, Mo.) per kg of BW or 5% glucose in water intraperitoneally (Table 1). We previously determined (9) that this dose of endotoxin resulted in less than 1% mortality, and no animals died in any of the present experiments. Fibronectin concentrations in plasma were subsequently determined at 1, 2, 4, and 12 h by a competitive inhibition enzyme immunoassay as previously described (18), with a commercially prepared goat antibody to rat plasma fibronectin and a peroxidase-conjugated antibody to rabbit immunoglobulin (Cooper Biochemical, Inc., West Chester, Pa.). Statistically significant differences in test values were determined at the P < 0.05 level by the Student’s t test for two means.

Gelatin infusion resulted in a significant reduction in the fibronectin concentrations in plasma at 1 (40%), 2 (30%), and 4 (22%) h compared with those of control animals receiving a placebo injection (Table 1). The fibronectin concentration is plasma returned to normal 12 h after gelatin administration.

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TABLE 1. Fibronectin concentration in plasma of rats injected with gelatin or endotoxin and control animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean fibronectin concn ± SD (μg/ml) at:</th>
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<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Placebo</td>
<td>446 ± 22</td>
</tr>
<tr>
<td>Gelatin</td>
<td>456 ± 28</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>432 ± 21</td>
</tr>
<tr>
<td>Gelatin +</td>
<td>445 ± 19</td>
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* P < 0.05 for gelatin versus placebo.

Animals treated with endotoxin alone had significantly elevated fibronectin concentrations in plasma by 4 h, whereas rats treated with gelatin intravenously before endotoxin injection had significantly decreased values at 1 and 2 h, normal values by 4 h, and fibronectin concentrations at 12 h after treatment that were significantly greater than those from animals treated with endotoxin alone. Therefore, while endotoxin administration increases the fibronectin concentrations in plasma, it does not alter the gelatin-induced transient depression in the concentrations of immunoreactive plasma fibronectin.

To ascertain the effect of fibronectin depletion and endotoxin administration on ammonia concentrations in plasma, we studied animals that had been treated with gelatin or endotoxin and determined that ammonia concentrations in plasma were not significantly different from those in control animals.

Human plasma fibronectin was utilized since it is readily available and previous studies have shown that human and animal fibronectins show similar patterns of specificity and affinity for native and denatured collagen (4). A quantity of plasma fibronectin (200 μg/ml) was added to gelatin in vitro (7). Fibronectin concentrations in plasma decreased by 28 ± 8, 13 ± 15, and 7 ± 8% at 1, 2, and 4 h, respectively, after the addition of the gelatin-fibronectin complex. At 1 h, this percent decrease was significantly greater than that from rats given gelatin alone but greater than that obtained from control animals at 1 h. Six additional rats received the gelatin-fibronectin complex in vivo, followed by endotoxin (0.2 mg/kg of BW) intraperitoneally. Whereas gelatin infusion before endotoxin administration resulted in a 101% increase in ammonia concentrations in plasma, compared with that of placebo-treated rats, infusion of the gelatin-fibronectin complex before endotoxin injection resulted in a smaller, 44 ± 7% increment in ammonia concentration in plasma, nearly equivalent to that observed after endotoxin administration in these animals. Placebo-pre-treated animals were found to have ammonia concentrations of 5.98 ± 0.31 mM in plasma, whereas fibronectin-pre-treated animals had significantly lower ammonia concentrations of 3.70 ± 1.27 mM in plasma. The ammonia concentrations in plasma of the fibronectin-pre-treated animals were nearly equivalent to the ammonia concentrations (3.56 ± 0.37 mM) in plasma of normal fasted rats (9), suggesting that fibronectin infusion may have blunted the

effect of endotoxin on ammonia concentrations in plasma.
effect of endotoxin on nitrogen metabolism in these animals. The mechanism of gelatin-induced immunoreactive plasma fibronectin depletion is not well understood. Plasma fibronectin expresses a specific collagen binding site (2) but binds more avidly to denatured collagen (gelatin) than to native collagen (4). Ingham et al. (7) reported that the addition of gelatin to plasma fibronectin in vitro results in the formation of a high-molecular-mass fibronectin-gelatin complex. Recently, a high-molecular-mass (1,000-kilodalton) collagen-fibronectin complex was isolated from the plasma of patients after aortic surgery (10). The presence of this complex in the plasma of these surgical patients was associated with immunoreactive plasma fibronectin depletion. A similar collagen-fibronectin complex with associated plasma fibronectin depletion could be observed in porcine plasma after clamping of the abdominal aorta (10). Deno et al. (3) reported that the fibronectin concentration in plasma rapidly decreases after sublethal burns in anesthetized rodents, with the appearance of a gelatin-like ligand from burn skin that binds plasma fibronectin. 75Se-
radioabeled plasma fibronectin is rapidly sequestered in the area of burned skin and the liver of a burned animal. Intravenous gelatin (5 mg/kg) given to the dog. Circ. Shock 19(137-147) fibronectin concentrations in plasma (measured as the decrease in total isotopic 75Se-labeled plasma fibronectin content in plasma) by 50% during a 2-h period, and a gelatin-fibronectin complex similar to that seen in burned rodents was found in the rat plasma. Concomitantly, hepatic uptake of 75Se-labeled plasma fibronectin increased by 2.5-fold, suggesting that injected gelatin was interacting with plasma fibronectin to initiate rapid clearance by the hepatic MPS. These studies confirm the in vivo presence of collagenous material that is opsonized by plasma fibronectin and cleared by the MPS, as Kiener et al. (8) had proposed for MPS clearance of intravenously injected gelatin-coated colloids.

Previous studies have noted that decreases in immunoreactive fibronectin concentrations in plasma may not adequately indicate the extent of functional alterations in plasma fibronectin that occur in the presence of endogenously or exogenously generated circulating collagenous material (3, 10). Powell et al. (10) reported that decreases in immunoreactive plasma fibronectin below 190 μg/ml in patients with circulating collagen-fibronectin complexes were associated with the loss of all unbound, functionally normal plasma fibronectin, while functionally normal plasma fibronectin was detectable in plasma if the concentration in plasma was greater than 190 μg/ml. In this study, a decrease in the immunoreactive fibronectin concentration in plasma by approximately 30 to 40% for 2 h was associated with enhanced effects of endotoxin, resulting in greater ammonia concentrations in plasma. Smaller decreases in fibronectin concentration (13 to 28%) did not augment the effect of endotoxin. This study did not formally assess plasma fibronectin function after gelatin infusion, and therefore it is not clear if the apparently enhanced endotoxin effects are related solely to a decrease in the fibronectin concentration in plasma or to an alteration in the functional or immunoreactive behavior of the fibronectin molecule. The apparent amelioration of the effect of endotoxin on urea concentration in plasma in plasma fibronectin-pre-treated rats suggests that this adhe-
sive glycoprotein may play a role in the normal host response to endotoxin challenge. Further studies on the identification of circulating collagenous material with associated alterations in the fibronectin concentration and function in plasma may provide insight into the pathogenesis of sepsis and its detrimental metabolic sequelae in patients after traumatic, burn, and surgical wounds.

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