Combined anticoagulants ameliorate acute lung injury in sheep after burn and smoke inhalation


*Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX 77555, U.S.A., and †Shriners Hospital for Children, Galveston, TX 77550, U.S.A.

ABSTRACT

Burn and smoke inhalation-related multiple organ dysfunction is associated with a severe fall in the plasma concentration of antithrombin. Therefore the aim of the present study was to test the hypothesis that intravenous administration of recombinant human antithrombin in combination with aerosolized heparin will ameliorate acute lung injury in sheep exposed to cutaneous burn and smoke inhalation. Sheep were prepared operatively for study and, 7 days post-surgery, sheep were given a cutaneous burn (40% of total body surface area, third-degree burn) and insufflated with cotton smoke (48 breaths, < 40 °C) under halothane anaesthesia. After injury, sheep were placed on a ventilator and resuscitated with Ringer's lactate solution. The animals were divided into three groups: sham group (non-injured and non-treated; \( n = 6 \)), saline group (injured and received saline; \( n = 6 \)) and rhAT.iv. + Hep group [injured and treated with rhAT (recombinant human antithrombin) and heparin; \( n = 6 \)]. In the rhAT.iv. + Hep group, rhAT was infused continuously for 48 h starting 1 h post-injury with a dose of 0.34 mg · h\(^{-1}\) · kg\(^{-1}\) of body weight and heparin (10 000 units) was aerosolized every 4 h starting at 1 h post-injury. The experiment lasted 48 h. Haemodynamics were stable in sham group, whereas the saline-treated sheep developed multiple signs of acute lung injury, including decreased pulmonary gas exchange, increased inspiratory pressures, extensive airway obstruction and increased pulmonary oedema. These pathological changes were associated with a severe fall in plasma antithrombin concentration, lung tissue accumulation of leucocytes and excessive production of NO. Treatment of injured sheep with anticoagulants attenuated all of the pulmonary pathophysiology observed. In conclusion, the results provide definitive evidence that anticoagulant therapy may be a novel and effective treatment tool in the management of burn patients with concomitant smoke inhalation injury.

INTRODUCTION

Antithrombin, a potent endogenous anticoagulant, inactivates a number of proteinases in the coagulation cascade, especially thrombin and Factor Xa [1]. Beyond its anticoagulant effect, antithrombin has been also shown to exert anti-inflammatory properties. Importantly, it can modulate the inflammation by two different mechanisms: (i) through inhibiting the coagulation factors with pro-inflammatory potencies (thrombin or activated Factor X)
or (ii) by direct actions that are independent of its anticoagulant effects. There are numerous reports on the coagulation-independent anti-inflammatory effects of antithrombin. It has been suggested that antithrombin promotes prostacyclin production from endothelial cells [2], inhibits LPS (lipopolysaccharide)-induced IL-6 (interleukin-6) in multiple cell types [3] and reduces excessive production of iNOS [inducible NOS (NO synthase)]-derived NO through inhibiting TNF-α (tumour necrosis factor-α) [4]. Kaneider et al. [5] have described that antithrombin affects neutrophil migration via its heparin-binding site and action on cell-surface syndecan-4. Recently, Harada et al. [6] reported that antithrombin attenuates endotoxin-induced severe hypotension by enhancing pulmonary sensory neuron activation.

Of particular clinical interest is that this important molecule, which modulates both coagulation and inflammation to a physiologically meaningful level, is depleted in burn trauma. Burn patients develop an imbalance between pro- and anti-coagulant elements. It has been reported that the hypercoagulable state in burn patients during the initial 24 h was associated with high levels of activated Factor VII, thrombin/antithrombin complex, PAI-1 (plasminogen activator inhibitor-1), and low levels of antithrombin and protein C [7]. In addition, a number of investigators have documented that plasma concentrations of antithrombin are markedly reduced in burn patients [8,9]. Recently, Niedermayr and co-workers [10] reported that antithrombin deficiency in burn patients correlates strongly with total burned surface area, the presence of inhalation injury, a longer hospital stay and increased mortality.

In the present study, we hypothesized that restoration of the plasma concentration of antithrombin would alleviate the degree of ALI (acute lung injury). For this purpose, we have tested the effects of intravenously administered rhAT (recombinant human antithrombin) in combination with aerosolized heparin using our well-established ovine model of ALI induced by cutaneous burn and inhalation injury (48 breaths of cotton smoke, <40 °C). After injury, all of the sheep were awakened and placed on a ventilator with positive end-expiratory pressure set to 5 cmH₂O and tidal volume maintained at 15 ml/kg of body weight [11]. The inspiratory peak and pause airway pressures were recorded every 6 h. All animals were provided with fluid resuscitation with Ringer’s lactate solution (4 ml/percentage of total BSA/kg of body weight for the first 24 h and 2 ml/percentage of total BSA/kg of body weight for the second 24 h). The experiment was continued for 48 h. The experimental groups were: sham group (non-injured and non-treated; n = 6); saline group (injured and received saline; n = 6); and rhAT.iv. + Hep [injured and treated with intravenous rhAT in combination with aerosolized heparin (Hep); n = 6]. In the treatment group, continuous intravenous infusion of rhAT was started 1 h post-injury with a dose of 0.34 mg·h⁻¹·kg⁻¹ of body weight. The same animals were also aerosolized with heparin starting 1 h post-injury with a dose of 10,000 units per aerosolization, which was repeated every 4 h thereafter. Heparin (from porcine intestinal mucosa) was purchased from American Pharmaceutical Partners, and rhAT was kindly provided by GTC Biotherapeutics. The specific activity of the rhAT preparations used was 7 units/mg [8]. The plasma concentration of the antithrombin was determined using antithrombin assay kit (COAMATIC® Antithrombin; Chromogenix Instrumental Laboratory), and results are reported in activity (%). Plasma NOx (nitrite/nitrate) levels were determined using Griess reaction method [11]. Activated clotting time was determined by Hemocheck (International Technidyne).

The Animal Care and Use Committee of the University Texas Medical Branch approved the experimental protocol, and all of the animals were handled according to guidelines established by the American Physiology Society and the National Institutes of Health.

**Lung histology and lung wet-to-dry weight ratio**

Sheep were killed 48 h post-injury under anaesthesia. A pathologist examined the lung tissue and evaluated all bronchi in each section of the four tissue samples and, for each, estimated the percentage of the surface area of the lumen obstructed by cast material (0–100 %).
Table 1  Haemodynamics over the course of the experiment in the study groups

Values are means ± S.E.M. (n = 6). * P < 0.05 compared with the sham group; † P < 0.05 compared with the saline-treated control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (h)</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>Sham</td>
<td>93 ± 3</td>
<td>108 ± 6</td>
<td>109 ± 3</td>
<td>109 ± 4</td>
<td>110 ± 5</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>97 ± 6</td>
<td>126 ± 9</td>
<td>145 ± 12*</td>
<td>130 ± 4</td>
<td>124 ± 9</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>101 ± 4</td>
<td>121 ± 10</td>
<td>117 ± 6</td>
<td>137 ± 7</td>
<td>131 ± 6</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>Sham</td>
<td>95 ± 1</td>
<td>98 ± 1</td>
<td>96 ± 3</td>
<td>96 ± 3</td>
<td>95 ± 2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>100 ± 6</td>
<td>105 ± 5</td>
<td>99 ± 5</td>
<td>100 ± 4</td>
<td>101 ± 5</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>104 ± 2</td>
<td>103 ± 4</td>
<td>100 ± 3</td>
<td>103 ± 3</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>PAMP (mmHg)</td>
<td>Sham</td>
<td>20 ± 0</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
<td>28 ± 2</td>
<td>27 ± 2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>20 ± 0</td>
<td>25 ± 1</td>
<td>30 ± 1</td>
<td>33 ± 1*</td>
<td>32 ± 2</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>22 ± 1</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>Sham</td>
<td>6 ± 1</td>
<td>11 ± 1</td>
<td>9 ± 1</td>
<td>11 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>5 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
<td>11 ± 1</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>6 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1†</td>
</tr>
<tr>
<td>LVSWI (g·m·m⁻²)</td>
<td>Sham</td>
<td>80 ± 3</td>
<td>72 ± 4</td>
<td>64 ± 7</td>
<td>67 ± 4</td>
<td>69 ± 5</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>83 ± 7</td>
<td>56 ± 7</td>
<td>44 ± 4</td>
<td>50 ± 9</td>
<td>58 ± 7</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>74 ± 4</td>
<td>60 ± 5</td>
<td>60 ± 3</td>
<td>57 ± 9</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>Sham</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>23 ± 2</td>
<td>24 ± 2</td>
<td>22 ± 1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>27 ± 2</td>
<td>31 ± 2</td>
<td>34 ± 2*</td>
<td>36 ± 1*</td>
<td>34 ± 2*</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>26 ± 2</td>
<td>29 ± 2</td>
<td>27 ± 2*</td>
<td>27 ± 2*</td>
<td>27 ± 2*</td>
</tr>
<tr>
<td>Po (mmHg)</td>
<td>Sham</td>
<td>21.5 ± 1.1</td>
<td>20.7 ± 0.9</td>
<td>20.6 ± 0.3</td>
<td>21.2 ± 1.2</td>
<td>20.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>22.3 ± 0.9</td>
<td>14.4 ± 0.5*</td>
<td>11.8 ± 0.6*</td>
<td>10.9 ± 0.9*</td>
<td>10.8 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>23.9 ± 1.7</td>
<td>15.2 ± 1.0</td>
<td>14.7 ± 0.7</td>
<td>14.7 ± 0.6</td>
<td>15.2 ± 0.8†</td>
</tr>
<tr>
<td>FNB (ml/kg)</td>
<td>Sham</td>
<td>0 ± 0</td>
<td>23.4 ± 10.5</td>
<td>-2.0 ± 8.4</td>
<td>-7.2 ± 9.6</td>
<td>-9.3 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0 ± 0</td>
<td>62.9 ± 5.3*</td>
<td>101.5 ± 5.9*</td>
<td>124 ± 8.0*</td>
<td>139.9 ± 13.1*</td>
</tr>
<tr>
<td></td>
<td>rhAT + heparin</td>
<td>0 ± 0</td>
<td>56.8 ± 6.7</td>
<td>67.2 ± 10.4</td>
<td>61.8 ± 15.2†</td>
<td>61.5 ± 16.7†</td>
</tr>
</tbody>
</table>

[13]. The lower half of the right lower lobe was used for the determination of bloodless wet-to-dry weight ratio [14].

Lung MPO (myeloperoxidase) activity and VEGF (vascular endothelial growth factor) levels

MPO activity was evaluated in homogenized right lung samples using a commercially available assay (CytoStore) [15]. Lung tissue VEGF was determined by Western blot analysis, and results are expressed as arbitrary units [16].

Statistical analysis

Values are means ± S.E.M. Results were compared by ANOVA using either the Scheffe or Fischer post-hoc test. A value of P < 0.05 was accepted as statistically significant.

RESULTS

Haemodynamics

All animals survived in the three study groups throughout the experimental time period. The haemodynamics are summarized in Table 1. These variables were stable in sham animals, whereas HR (heart rate), PAMP (pulmonary artery mean pressure) and CVP (central venous pressure) were significantly elevated in saline-treated control animals (injured and non-treated control animals) at 24, 36 and 48 h respectively. Combined rhAT (intravenous) and heparin (aerosolized) therapy
had a slight tendency to decrease PAMP and HR, and significantly reduced the increase in CVP at 48 h post-injury. MAP (mean arterial blood pressure) was similar in all three groups throughout the study period. LVSWI (left ventricle stroke work index) was calculated using standard formula. LVSWI was significantly decreased in saline-treated control animals at 30 h post-injury compared with sham animals. Animals treated with anticoagulants had higher LVSWI values compared with the saline-treated control group; however, no statistically significant difference was found between the groups.

In Table 1, the effects of combined anticoagulant therapy on HCT (haematocrit), P0 (plasma oncotic pressure) and FNB (fluid accumulation) are summarized. Saline-treated control animals had signs of severe haemoconcentration and fluid accumulation, despite similar fluid resuscitation. The same animals also had a markedly decreased P0. All of these changes were significantly attenuated by intravenous rhAT in combination with aerosolized heparin. Activated clotting time was significantly increased in the saline-treated control group at 48 h (200 ± 7 min) compared with baseline (160 ± 5 min). The values were 171 ± 14 and 170 ± 6 min in the treated group at baseline and 48 h post-injury respectively.

**Plasma levels of antithrombin**

Combined burn and smoke inhalation injury resulted in a severe fall in plasma concentration of antithrombin. The plasma antithrombin concentration fell to almost 25 % at 12 h post-injury in saline-treated control animals compared with baseline values (100 %). However, the plasma antithrombin concentration was much higher in the treated group (approx. 70 % at 12, 24 and 36 h post-injury; Figure 1).

![Figure 1] Plasma concentration of antithrombin

Values are means ± S.E.M. (n = 6), and are shown as a percentage of baseline values. Open bars represent the sham group, striped bars represent the saline-treated control group, and closed bars represent the group treated with anticoagulants.

Pulmonary gas exchange

Pulmonary gas exchange [Pao2 (arterial partial pressure of oxygen)]/FiO2 (fraction of inspired oxygen) and Qs/Qt (pulmonary shunt fraction) was stable in sham animals. Combined burn and smoke inhalation caused a deterioration of pulmonary gas exchange, as shown by a reduction in the Pao2/FiO2 ratio (Figure 2A) associated with a marked increase in Qs/Qt (Figure 2B) in saline-treated control animals. The Pao2/FiO2 ratio in these animals reached a level below 200 at 24 h post-injury and steadily declined in the subsequent 48 h, indicating the presence of severe ARDS (acute respiratory distress syndrome). Treatment with combined rhAT and heparin prevented the impaired gas exchange caused by burn and smoke inhalation. The Pao2/FiO2 ratio stayed above 200 in treated animals throughout the experimental period (Figure 2A). The increase in Qs/Qt was markedly decreased by combined therapy (Figure 2B).
Airway obstruction and ventilatory pressures

Combined burn and smoke inhalation injury resulted in a massive formation of airway obstructive casts. Very little obstructive cast material was seen in sham animals (4.78 ± 0.6 %). The obstruction scores for the saline-treated control animals (31.6 ± 3.7 %) significantly increased compared with those in the sham group; however, this increase was significantly reduced by combined anticoagulant therapy (18.8 ± 2.4 %).

Inspiratory peak and pause airway pressures were recorded from the ventilator readout. Both peak (Figure 3A) and pause (Figure 3B) airway pressures were markedly elevated in saline-treated control animals compared with the pressures in sham animals. This increase in ventilatory pressures was especially pronounced in the second half of the experimental period and it continued to increase up to 48 h: in fact, the values were doubled at 48 h compared with baseline values.

However, combined rhAT and heparin therapy almost reversed these large increases in airway pressures.

Lung lymph flow and lung water content

Lung lymph flow, an index of pulmonary transvascular fluid flux, was unchanged in sham animals throughout the experimental time period. However, saline-treated control animals had a significant increase in lung lymph flow compared with those in the sham group. Combined rhAT and heparin therapy almost totally prevented the increase in transvascular fluid flux caused by the double insult (Figure 4A).

Lung water content was evaluated by measuring bloodless lung wet-to-dry weight ratio. The lung wet-to-dry weight ratio was significantly increased in saline-treated control animals; however, this increase was significantly reduced by combined anticoagulant therapy (Figure 4B).
Effect of anticoagulants on plasma levels of NOx (A), a stable metabolite of NO, and lung tissue VEGF (B). Values are means ± S.E.M. The VEGF protein level measured by Western blotting in lung tissue is shown as densitometric values. *P < 0.05 compared with the sham group; †P < 0.05 compared with the saline-treated control group.

**Lung tissue MPO activity**

The lung tissue MPO activity, a marker of leucocyte activation, was significantly increased by combined cutaneous burn and smoke inhalation; however, intravenous administration of rhAT in combination with aerosolized heparin significantly reduced the increase in MPO activity in lung tissue (results not shown). The values were 2.8 ± 0.2, 6.3 ± 1.0 and 2.7 ± 0.3 units/g of tissue in the sham, saline-treated control and treated groups respectively.

**Plasma levels of NOx and lung tissue VEGF**

Plasma NOx, a stable metabolite of NO, was significantly increased following injury compared with sham animals (at 12, 18, and 48 h; P < 0.05). Intravenous administration of rhAT in combination with aerosolized heparin significantly reduced the increase in NOx at 12 h post-injury (Figure 5A).

VEGF protein levels were significantly increased in animals exposed to combined injury; however, this increase was significantly attenuated by anticoagulant therapy (Figure 5B).

**DISCUSSION**

Antithrombin is the most potent endogenous anticoagulant, and it has a unique ability to modulate inflammation. This physiologically important molecule is deficient in pathological conditions such as thermal injury [7–9]. The resulting hypercoagulable state in burn patients leads to the impaired tissue perfusion, which may result in multiple organ derangements, including pulmonary dysfunction and delayed wound healing. The presence of smoke inhalation augments the severity of lung tissue injury, eventually leading to ARDS in burn victims. In the present study, we hypothesized that restoration of the plasma concentration of antithrombin by intravenously administered rhAT in combination with aerosolized heparin would ameliorate the severity of ALI caused by cutaneous burn and smoke inhalation in sheep.

The pathophysiology of ALI in the present model is characterized by severely deteriorated pulmonary gas exchange (decreased $P_{aO_2}/F_{iO_2}$ and increased $Q_s/Q_t$), pulmonary oedema formation (increased pulmonary transvascular fluid flux and water content) and increased airway obstruction (airway obstruction score and increased inspiratory pressures). Consistent with results from clinical studies [7–10], these pathological changes were associated with a severe fall in the plasma concentration of antithrombin, especially during the first 24 h post-injury. Previously, we have reported that excessive production of NO, a surge of neutrophils into the lung tissue and other inflammatory mediators play an important role in burn-related pathogenic process [11,17,18]. We have also demonstrated a potential role of airway coagulopathy in the pathogenesis of ALI in the same model [19]. Because combined burn and smoke inhalation injury is associated with both local airway coagulopathy and systemic inflammation, we administered the anticoagulants by a different route, assuming that intravenously administered rhAT may exert a maximum anti-inflammatory effect without interfering with concomitant heparin. Since combined burn and smoke inhalation injury is associated with both local airway coagulopathy and systemic inflammation, we administered the anticoagulants by a different route, assuming that intravenously administered rhAT may exert a maximum anti-inflammatory effect without interfering with concomitant heparin. Since combined burn and smoke inhalation injury is associated with vascular hyperpermeability and the airway epithelial barrier is impaired, we assumed that part of the intravenously administered rhAT would leak in to the airways. It is well known that heparin significantly enhances the ability of antithrombin to inhibit Factor Xa and thrombin [20,21]. Thus aerosolized heparin alone with rhAT would exhibit a local airway anticoagulant effect that prevents the formation of fibrin clots. At the same time, it is worth noting that concomitant heparin considerably prevented the anti-inflammatory effects of antithrombin in patients with sepsis [22]. We have reported previously that aerosolization of heparin into airways in sheep with sepsis with smoke inhalation and pneumonia ameliorated ALI without noticeable systemic effects [23]. Therefore we also assumed that aerosolized
heparin may not interfere significantly with systemic anti-inflammatory effects of intravenously administered rhAT.

Nevertheless, the combined anticoagulant therapy in the present model conceivably alleviated the cardiopulmonary morbidity seen after burn and smoke inhalation. In previous studies, we have reported several factors responsible for the impaired pulmonary gas exchange, such as massive airway obstruction composed partially of fibrin clots [19] and excessive production of NO, leading to loss of pulmonary hypoxic vasoconstriction, which results in increased shunt fraction [11]. The improvement in pulmonary gas exchange in the present study may be due to the effect of aerosolized heparin, which attenuated airway coagulopathy, and also the effect of intravenously administered rhAT, which inhibited excessive production of NO. Isobe et al. [4] demonstrated that rhAT ameliorates acute lung injury by inhibiting iNOS-derived excessive NO production in a rat model of sepsis. In support, Pata et al. [24] reported similar results in 2002. Although we were not able to measure directly the thrombin/fibrin deposition in the lung tissue in the present study, inhibition of these inflammatory sequelae may result in improved microcirculation and thus improve pulmonary function.

Of particular interest, combined anticoagulant therapy almost totally prevented the increase in lung lymph flow, a marker of pulmonary transvascular fluid flux, and significantly reduced the lung wet-to-dry ratio, a marker of lung water content, suggesting that the combined therapy had a potent effect on pulmonary vascular permeability. This notion was supported by the findings that combined anticoagulant therapy significantly attenuated the changes in HCT, , and fluid accumulation, indirect indices of vascular leakage. Although the exact mechanism is still not completely understood, we speculate that intravenously administered rhAT may reduce the vascular hyperpermeability by preventing the accumulation of neutrophils in lung tissue. Neutrophils accumulate at sites of lung injury and mediate inflammation via production of proteases, oxidants and cytokines [25]. In experimental models of ALI, the neutrophils that accumulate in the lungs have increased activation of NF-κB (nuclear factor κB) and produce large amounts of proinflammatory cytokines [26–28]. Accordingly, in the present study, we also showed that combined anticoagulant therapy significantly reduced the increase in lung tissue MPO activity. We have also shown previously that inhibition of L-selectin resulted in a marked reduction in pulmonary transvascular fluid flux and lung oedema [29]. Antithrombin suppresses leucocyte activation through inhibiting NF-κB [30] and affects neutrophil migration via its heparin-binding site interacting with cell-surface syndecan-4 [5,31], a transmembrane receptor expressed on multiple cell types including neutrophils. Thus intravenously administered rhAT may inhibit neutrophil activation and attenuate lung tissue inflammation, thereby reducing pulmonary oedema.

The other probable mechanism by which combined anticoagulant therapy reduced lung oedema might be related to the ability of antithrombin to inhibit excessive production of NO. In the present study, we show that the treatment group had significantly lower plasma NOx at 12 h post-injury compared with saline-treated control animals. It has been well documented that excessive production of NO causes vasodilation and increases vascular permeability [32]. In previous studies, we have reported that increases in pulmonary microvascular permeability are due to excessive production of NO, which is derived from iNOS in our ovine ALI model induced by cutaneous burn and smoke inhalation [11,12,17]. In addition, it was suggested that NOS contributes to VEGF-mediated vascular permeability [33]. VEGF is a potent permeability factor and has been shown to mediate morbidity and mortality in patients with sepsis [34]. Its ability to increase vascular permeability is 50-fold greater than histamine [35]. In the present study, we demonstrate that combined anticoagulant therapy markedly reduced lung tissue VEGF protein expression. Thus intravenously administered rhAT in combination with aerosolized heparin may reduce pulmonary vascular hyperpermeability through inhibiting excessive NO and VEGF. Although we did not directly measure it, we should not exclude the possible role of hypoxia itself in the pathological process. Our model is associated with severe hypoxia, and hypoxia, in turn, has been shown to cause vascular hyperpermeability [36,37]. It is worth noting that hypoxia is also a potent stimulator of VEGF, resulting in a 3–12-fold increase in gene expression [38]. Because thrombin, a potent procoagulant and pro-inflammatory substance, has been shown to provoke oedema formation by increasing endothelial permeability [39], it is also likely that combined anticoagulant therapy reduced the pulmonary vascular permeability via inhibition of thrombin formation. These possibilities should be investigated in future studies. Previous studies clearly demonstrated that anticoagulants, such as APC (activated protein C) and TFPI (tissue factor pathway inhibitor), had promising effects in ameliorating severity of ALI/ARDS and sepsis-related multiple organ dysfunction [40,41]. APC, the only available anticoagulant for treatment of severe sepsis, has been shown to ameliorate ALI by modulating endothelial barrier function and microvascular permeability [42].

Nevertheless, the present study is the first to report the beneficial effects of anticoagulants administered through different routes in the ovine model of ARDS induced by smoke inhalation and cutaneous flame burn. On the basis of the results of the present and previous studies,
we conclude that a combined anticoagulant therapy of intravenously administered rhAT and aerosolized heparin may represent a potential alternative in the management of burn victims, especially those who have a concomitant smoke inhalation injury.

ACKNOWLEDGMENTS

This study was supported by grants from the Shriners of North America (SBI 84504 and SBI 8954), and from the National Institutes of Health (GM066312 and GM060688).

REFERENCES

Vascular endothelial growth factor is an important determinant of sepsis morbidity and mortality. J. Exp. Med. 203, 1447–1458


