High-dose heparin fails to improve acute lung injury following smoke inhalation in sheep


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ABSTRACT

Thrombin is involved in various inflammatory responses. In sepsis, coagulation abnormalities are major complications. Acute lung injury is one of the most life-threatening problems that can result from sepsis. We hypothesized that high-dose heparin might be effective in attenuating acute lung injury in our sepsis model. Female sheep (n = 16) were surgically prepared for the study. After a tracheotomy, 48 breaths of cotton smoke (< 40 °C) were insufflated into the airway. Afterwards, live Pseudomonas aeruginosa (5 × 10^11 colony-forming units) bacteria were instilled into the lung. All sheep were ventilated mechanically with 100% O_2, and were divided into three groups: a heparin infusion group (n = 6), a Ringer's lactate infusion group (n = 6), and a sham-injury group (n = 4; surgically prepared in the same fashion but receiving no inhalation injury or bacteria). The treatment was started 1 h after the insult, and was continued thereafter for 24 h. The dose of heparin was adjusted by monitoring to target an activated clotting time of between 150 and 150 s (baseline). Sheep exposed to lung injury presented with typical hyperdynamic cardiovascular changes, including an increased cardiac output and a fall in systemic vascular resistance. There was a decrease in the arterial partial pressure of O_2. In conclusion, high-dose heparin did not prevent lung dysfunction in this model, in which acute lung injury was induced by combined smoke and septic challenge.

INTRODUCTION

Activated protein C has proven to be beneficial in the treatment of sepsis in a recent clinical trial [1]. However, the mechanism of action of this material is still not well understood. It is considered that an anticoagulant effect is one of the mechanisms. Therefore we were interested in determining whether or not the inhibition of coagulation abnormalities is beneficial in a model of sepsis. Heparin is an inexpensive anticoagulant and is widely used for the treatment of coagulation disorders. In the present study, we sought to clarify the beneficial aspects of using intravenous high-dose heparin in the treatment of sepsis. To this end, we chose an animal model of sepsis, which mimics clinical situation, and investigated the effects of post-treatment heparin.

Thrombin is a serine protease that activates fibrinogen to form fibrin. This is a final step of haemostasis. In addition, thrombin binds to its receptor, termed a protease-activated receptor (PAR). Of the four types of PARs, PAR-1 is the most important in the inflammatory response.

Key words: anticoagulant, haemodynamics, infection/inflammation, Pseudomonas aeruginosa, septic shock, thrombosis.

Abbreviations: ACT, activated clotting time; ALI, acute lung injury; FDP, fibrin degradation products; Fio_2, fraction of inspired oxygen; IL-1 (etc.), interleukin-1 (etc.); NO_2, total amount of nitric oxide metabolites (i.e. nitrates plus nitrites); PaO_2, arterial partial pressure of oxygen; PAR, protease-activated receptor.

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PAR that have been identified, thrombin activates PAR-1, -3 and -4 [2]. It has been reported that endothelial cells are activated by thrombin via PARs, and that various inflammatory responses are subsequently induced. Protein kinase C or nuclear factor-κB is activated [3]; as a consequence, adhesion molecules such as P-selectin, or inflammatory cytokines such as interleukin-1 (IL-1) or IL-6, are induced [4]. In addition, another report has demonstrated that nitric oxide is released when thrombin binds to a PAR [5]. Therefore we hypothesized that the inhibition of thrombin would be beneficial in reversing hyperdynamic sepsis and acute lung injury (ALI). The aim of the present study was to establish whether or not the inhibition of thrombin by high-dose heparin was effective in treating sepsis. We elected to use heparin because our previous study [6] showed that aerosolized heparin, nebulized into the airway, improved ALI in the same sepsis model by inhibiting airway obstruction. In that paper [6], we reported that intravenous heparin improved neither ALI nor the haemodynamic changes seen in this model. However, the dosage we gave in the previous study was only sufficient to attenuate the hypercoagulable state in sepsis – this dosage was not able to prolong the activated clotting time (ACT). Therefore we developed a high-dose intravenous heparin treatment, in which ACT would be doubled compared with baseline levels. In the present study, we sought to determine whether or not high-dose intravenous heparin treatment would attenuate hyperdynamic sepsis and ALI in an ovine sepsis model.

**MATERIALS AND METHODS**

**Animal preparation**

The Animal Care and Use Committee of The University of Texas Medical Branch approved the experiments reported in this paper. All animals were handled within the guidelines established by the U.S. National Institutes of Health (NIH Publication No. 85-23; revised 1996).

A total of 16 female sheep (body weight 37.0 ± 0.9 kg) were surgically prepared as described previously [7]. Briefly, catheters were placed in the femoral artery and vein, as well as in the left atrium, and a Swan–Ganz catheter was placed in the jugular vein and passed into the pulmonary artery. The catheter was flushed continuously with saline containing heparin (3000 units/l). The saline was connected to the pressure bag so that animals received approx. 1 unit · kg⁻¹ · h⁻¹ heparin. We confirmed that this dose of heparin is sufficient to prevent clot formation in the catheters, but does not affect systemic haemostasis. The animals were studied after a 5–7-day recovery period. After the measurement of baseline data, 12 animals received a smoke and bacterial challenge, and four animals received a sham injury. The technique for the induction of inhalation injury was as described previously [8]. After smoke inhalation, animals were ventilated mechanically with 100% O₂ throughout the 24-h study. At 30 min after smoke inhalation, an experimental bacterial solution was instilled into the lung lobes using a bronchoscope. *Pseudomonas aeruginosa* (2–5 × 10¹⁰ colony-forming units) were suspended in 30 ml of saline and instilled into the right lower and middle lobes (10 ml each), as well as the left lower lobe (10 ml). Ringer’s lactate (5–7 ml · kg⁻¹ · h⁻¹) was infused intravenously to prevent haemoconcentration. The sham-injury group (n = 4) received tracheostomy and 48 breaths of sham smoke (room air) while under halothane anaesthesia. After inhalation, sham-group animals were mechanically ventilated with 100% O₂ in the same way as the injured group. The concentration of NOₓ (total amount of nitric oxide metabolites, i.e. nitrates plus nitrites) in plasma was measured using a nitric oxide chemiluminescent detector (Antek model 7020; Antek Instruments Inc., Houston, TX, U.S.A.) [9].

Of the 12 animals that received a smoke and bacterial challenge, six received heparin (from beef lung; Pharmacia & Upjohn Co., Kalamazoo, MI, U.S.A.) and the other six received lactate Ringer’s solution. Each solution was injected 1 h after the insult (100 units/kg; bolus), followed by a continuous infusion (50–60 units · kg⁻¹ · h⁻¹). We decided to begin the treatment 1 h after the injury to mimic the clinical situation: in cases of smoke inhalation due to building fire, we think it is likely that treatment is started within 1 h of the injury. This is the concept of this study. ACT was monitored during the experiment (Hemocheck model 801; International Technidyne Co. Edson, NJ, U.S.A.) and the rate of infusion of heparin was adjusted to maintain the ACT between 300 and 400 s. Plasma levels of fibrin degradation products (FDP) were measured at baseline (0 h) and 24 h with a latex agglutination assay kit (FDP Plasma Kit; Diagnostica Stago, Parsippany, NJ, U.S.A.). The bloodless lung wet/dry weight ratio was calculated using Pearce’s method [10]. Plasma antithrombin activity was measured using a colorometric assay kit. A complete blood count was performed using a Hemavet (automatic veterinary CBC analyser; CDC Technologies, Oxford, CT, U.S.A.) with the species setting set for sheep.

After the animals were killed, the right lower lobe from each animal was excised and inflated with 10% (v/v) formalin. Fixed samples were embedded in paraffin, sectioned into 6 μm slices, and stained with haematoxylin/cosin. A pathologist who was unaware of the group assignment analysed the samples. We established a scoring system to evaluate the histology [11]. A total of 24 areas of lung parenchyma were graded on a scale of 0–4 (0, absent, appears normal; 1, light; 2, moderate; 3, strong; 4, intense) for congestion, oedema, inflammation and haemorrhage. A mean score for each of the parameters was then calculated, and the sum (maximum = 16) was taken as a histology score.
Table 1  Lung histology scores

Data are expressed as means ± S.E.M. Pathological changes were scored as described in the Materials and methods section, and the total scores were compared with a non-parametric Kruskal–Wallis test. *P < 0.05 compared with sham injury group. No statistical difference was found between the sepsis control and heparin groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Histology score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham injury</td>
<td>3.33 ± 0.72</td>
</tr>
<tr>
<td>Sepsis control</td>
<td>9.03 ± 1.30*</td>
</tr>
<tr>
<td>Sepsis with heparin</td>
<td>8.56 ± 0.74*</td>
</tr>
</tbody>
</table>

Data analysis

Data are expressed as means ± S.E.M. The statistical software StatView 5.0 (SAS Institute, Cary, NC, U.S.A.) was used to perform the analysis. For haemodynamic and blood gas data, the parameters over 24 h (five time points shown in Table 1) were analysed by a two-way, repeated-measurements ANOVA, which takes into account the multiplicity of measurements over time. In other studies, ANOVA for multiple comparisons and Scheffe’s post hoc test were used. Between-group comparisons at individual time points were made using the Student Newman–Keuls test for unpaired data where appropriate. P < 0.05 was considered statistically significant.

RESULTS

Peak levels of carboxyhaemoglobin in the saline-treated and heparin-treated groups were 58.6 ± 8.2% and 62.7 ± 4.7% respectively (P > 0.05). Thus all groups received very similar amounts of smoke using our procedure.

ACT

We started giving heparin 1 h after injury, so that ACT began increasing immediately after the bolus injection of heparin (Figure 1). The target of ACT in the heparin-treated group was about double the baseline level (300–350 s). Since we adjusted the heparin infusion speed after that, ACT reached the target range by 6 h after insult, and was maintained at that level for the remainder of the study (Figure 1). Control animals showed stable ACT values during the 24 h study. The mean total dose of heparin given in 24 h was 56100 units. In the Ringer’s
K. Murakami and others

Figure 4 Changes in PaO\textsubscript{2}/FiO\textsubscript{2} ratio (A) and pulmonary shunt fraction (B)

The PaO\textsubscript{2}/FiO\textsubscript{2} ratio (P/F) and the pulmonary shunt fraction (Qs/Qt) were calculated. Data are expressed as means ± S.E.M. *Sepsis control group (n = 6); ●, heparin-infusion group (n = 6). No differences were found between the groups.

lactate-treated control group, ACT did not change from the baseline level after the insult (Figure 1).

**Blood count**
The white cell count decreased markedly after the animals were subjected to smoke inhalation and pneumonia (Figure 2A). Heparin treatment did not prevent the fall in leucocyte numbers (Figure 2A). The platelet count also decreased significantly in control animals, but heparin infusion prevented this fall ($P < 0.05$; Figure 2B).

**Lung wet/dry weight ratio**
In saline-nebulized animals, the lung wet/dry weight ratio increased significantly 24 h after smoke inhalation followed by bronchial instillation of bacteria. This increase was not attenuated by high-dose heparin infusion (Figure 3).

**Lung histology**
By 24 h after the smoke and bacterial challenge, there was a marked inflammatory reaction in the lungs, characterized by cellular infiltrates in the interstitium and the air spaces. The infiltrates were composed predominantly of neutrophils. Interstitial oedema, vascular congestion and haemorrhage were also observed. The total histology score, based on the scores for congestion, oedema, inflammation and haemorrhage, was increased significantly after exposure to smoke and bacteria (Table 1). The score was not improved by heparin injection (Table 1).

**Changes in pulmonary gas exchange**
The arterial partial pressure of oxygen (PaO\textsubscript{2})/fraction of inspired oxygen (FiO\textsubscript{2}) ratio decreased markedly in the saline-treated group (Figure 4A). This decrease was not improved by heparin treatment (Figure 4A). The pul-

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**Figure 4** Changes in PaO\textsubscript{2}/FiO\textsubscript{2} ratio (A) and pulmonary shunt fraction (B)

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**Table 2** Changes in haemodynamic parameters, arterial pH and body temperature

Data are expressed as means ± S.E.M. Two-way, repeated-measurements ANOVA was performed to test the differences between the treatment groups. We were unable to show that there was a statistical difference in any of these variables between the treatment group and the control group. HR, heart rate; CI, cardiac index; MAP, mean arterial pressure; PAP, pulmonary artery pressure; LAP, left atrial pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Baseline</th>
<th>6 h</th>
<th>12 h</th>
<th>18 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>Control</td>
<td>82 ± 5</td>
<td>102 ± 8</td>
<td>116 ± 12</td>
<td>112 ± 12</td>
<td>118 ± 8</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>96 ± 5</td>
<td>122 ± 5</td>
<td>134 ± 6</td>
<td>132 ± 9</td>
<td>131 ± 8</td>
</tr>
<tr>
<td>CI (litres · min\textsuperscript{-1} · m\textsuperscript{-2})</td>
<td>Control</td>
<td>6.1 ± 0.3</td>
<td>6.2 ± 0.5</td>
<td>7.1 ± 0.9</td>
<td>7.1 ± 0.5</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>6.4 ± 0.4</td>
<td>6.6 ± 0.4</td>
<td>7.3 ± 0.6</td>
<td>8.5 ± 0.7</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>Control</td>
<td>97 ± 3</td>
<td>86 ± 13</td>
<td>70 ± 10</td>
<td>72 ± 9</td>
<td>71 ± 9</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>101 ± 2</td>
<td>97 ± 14</td>
<td>80 ± 11</td>
<td>71 ± 9</td>
<td>62 ± 8</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>Control</td>
<td>21 ± 1</td>
<td>28 ± 2</td>
<td>27 ± 2</td>
<td>29 ± 2</td>
<td>30 ± 2</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>21 ± 1</td>
<td>27 ± 2</td>
<td>29 ± 1</td>
<td>30 ± 1</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>LAP (mmHg)</td>
<td>Control</td>
<td>7 ± 0</td>
<td>11 ± 2</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>6 ± 0</td>
<td>12 ± 1</td>
<td>14 ± 1</td>
<td>15 ± 2</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>Control</td>
<td>7.47 ± 0.01</td>
<td>7.59 ± 0.03</td>
<td>7.56 ± 0.02</td>
<td>7.48 ± 0.02</td>
<td>7.52 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>7.48 ± 0.01</td>
<td>7.58 ± 0.02</td>
<td>7.53 ± 0.04</td>
<td>7.45 ± 0.03</td>
<td>7.42 ± 0.05</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>Control</td>
<td>39.1 ± 0.1</td>
<td>40.6 ± 0.3</td>
<td>39.9 ± 0.3</td>
<td>39.8 ± 0.4</td>
<td>39.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>39.2 ± 0.1</td>
<td>40.8 ± 0.3</td>
<td>40.2 ± 0.4</td>
<td>39.8 ± 0.6</td>
<td>39.4 ± 0.7</td>
</tr>
</tbody>
</table>
monary shunt fraction increased in the saline-treated group, reaching 50–60% at 24 h. Heparin treatment did not affect the change in shunt (Figure 4B). Arterial blood pH was not different between the treatment groups (Table 2).

**Haemodynamic changes**

Cardiac index increased significantly in the saline-treated group, which indicates that our model is a hyperdynamic septic model (Table 2). Intravenous heparin did not affect the changes in cardiac output (Table 2). The mean arterial pressure decreased to a significantly lower level in both groups; heparin administration did not attenuate this decrease (Table 2). Pulmonary artery pressure and the left atrial pressure rose significantly in both the heparin- and saline-treated groups in a similar fashion (Table 2).

The systemic vascular resistance index and the left ventricular stroke work index dropped markedly in both saline- and heparin-treated groups (Figures 5A and 5B). Whereas the pulmonary vascular resistance index and the right ventricular stroke work index increased slightly in the saline-treated group, there was a trend for these variables to be elevated to a lesser extent in the group treated with intravenous heparin (Figures 5C and 5D). There were several points of significant difference between groups.

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**Figure 5** Changes in systemic vascular resistance index (A), pulmonary vascular resistance index (B), left ventricular stroke work index (C) and right ventricular stroke work index (D)

Systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI) are given in units of dyn s cm⁻¹ m⁻². Left ventricular stroke work index (LVSWI) and right ventricular stroke work index (RVSWI) are given in units of g m⁻¹ m⁻². Data are expressed as means ± S.E.M.

@, Sepsis control group (n = 6); ●, heparin-infusion group (n = 6); * denotes a difference compared with the sepsis control group (P < 0.05).

**Figure 6** Changes in peak airway pressure (A) and pause airway pressure (B)

Peak airway pressure and pause airway pressure were measured during mechanical ventilation. Data are expressed as means ± S.E.M. (changes from baseline values; cmH₂O).

@, Sepsis control group (n = 6); ●, heparin-infusion group (n = 6).
Changes in airway pressures

We measured ventilatory peak airway pressure and pause airway pressure during mechanical ventilation. The former is an index of airway resistance and the latter is an index of compliance of the lung. Both peak and pause airway pressures increased during the study period; heparin treatment did not affect these increases (Figures 6A and 6B).

Changes in haematocrit, plasma NO\textsubscript{x} levels and antithrombin activity

When animals become septic, vascular permeability increases markedly and animals show haemoconcentration and hypovolaemic shock. To prevent these responses, we measured the haematocrit every 4 h and adjusted the pump settings of fluid resuscitation (Ringer’s lactate). We usually began the study period with an infusion rate of 2 ml kg\textsuperscript{-1} h\textsuperscript{-1}, and, after the insult, 5–7 ml kg\textsuperscript{-1} h\textsuperscript{-1} was required to keep the haematocrit stable. Since we treated all animals in the same fashion, the haematocrit was almost the same in the two groups (Figure 7A). Plasma NO\textsubscript{x} levels increased significantly after the insult, and no difference was demonstrated between the saline- and heparin-treated groups (Figure 7B). Plasma antithrombin activity decreased severely in the saline-treated group, dropping below 50% at 24 h (Figure 7C). Heparin treatment did not inhibit the fall in antithrombin activity (Figure 7C).

Fluid net balance

Fluid net balance (total fluid intake/kg minus total urine output/kg) at 24 h was calculated. The balances in the control and heparin-treated groups were $+85.8 \pm 17.0 \text{ ml/kg}$ and $+87.1 \pm 10.3 \text{ ml/kg}$ respectively (not significant).

FDP

Plasma FDP were determined semi-quantitatively by means of a commercially available assay (positive, $>20 \mu g/ml$; negative, $<5 \mu g/ml$; $\pm$, 5–20 $\mu g/ml$). All determinations were negative at baseline. In the saline-treated group, four animals tested positive and two tested negative for FDP at 24 h. The heparin-treated group all displayed negative values, suggesting that high-dose infusion of heparin inhibited the intravascular coagulation/fibrinolytic abnormalities associated with sepsis.

Body temperature

Body temperature rose significantly after the insult and then gradually decreased back to baseline levels. Heparin treatment did not affect the changes in body temperature (Table 2).

DISCUSSION

In the present study, we aimed to clarify whether or not intravenous high-dose heparin was effective in treating sepsis. We chose heparin as an anticoagulant for reasons of practicality. Even though hirudin and argatroban are specific thrombin inhibitors, heparin is more widely used in the clinical setting, and we had no previous data on the use of heparin treatment in septic patients. In addition, heparin has some anti-inflammatory properties other than the inhibition of thrombin, e.g. inhibiting selectins [12] and chemokines [13]. Once chemokines bind to heparin, they no longer activate their receptors; thus heparin also acts as an anti-chemokine [13]. We wanted to clarify the effects of heparin on sepsis when used at a relatively high dosage.

Thrombin, a serine protease, is a key enzyme of the blood coagulation system. Moreover, thrombin is known to be involved in inflammatory reactions, such as P- and E-selectin expression [14], IL-1 and IL-6 production [15], and nitric oxide production [5]. Most of these responses are mediated by thrombin receptors called PARs. The PAR-1 receptor of thrombin is coupled to various G-proteins, so its activation triggers a number of
intracellular signal transduction pathways [17]. In many
cells, activation of PAR-1 by thrombin results in ac-
tivation of phospholipase C and protein kinase C.
Activation of this receptor also results in tyrosine
phosphorylation and the activation of nuclear factor-κB
[17]. Therefore thrombin is considered as a pro-inflam-
matory factor.

Heparin is a common antithrombotic agent. Although
heparin itself does not have a direct antithrombotic effect,
it significantly potentiates the action of antithrombin III
[18]. Besides this antithrombotic effect, heparin might
prevent the adhesion of neutrophils to endothelial cells,
because heparin is very similar to the ligand of selectins
[12].

On the basis of these various reports, high-dose
heparin was considered as a good treatment strategy for
sepsis. Several previous studies have shown a beneficial
effect of heparin. Cox et al. [19] demonstrated that
intravenous heparin attenuated lung injury after smoke
inhalation in sheep. Griffin et al. [20] showed an
improvement in a heparin-treated group in haemo-
dynamic profiles in a Gram-negative sepsis model in
piglets. On the other hand, Uchiba and Okajima [21]
reported that heparin did not attenuate endotoxin-in-
duced ALI in rats. Moreover, Hoffmann et al. [22]
demonstrated that the thrombin antagonist hirudin failed
to inhibit endotoxin-induced leucocyte–endothelial-cell
interaction and microvascular reperfusion failure. There-
fore it is still not well established whether or not
intravenous heparin is beneficial in treating sepsis.

We have established an ovine model of sepsis induced
by a combination injury of smoke inhalation and
P. aeruginosa pneumonia [11]. This animal model mimics
human hyperdynamic sepsis, in that, in clinical settings,
most septic patients have some type of underlying disease
or diseases. In addition, pneumonia is one of the most
frequent infectious disease states and is considered as a
focus of sepsis [23]. On the other hand, when animals are
given intrabronchial bacteria without smoke inhalation,
they do not show coagulation abnormalities or a severe
drop in the PaO$_2$/FiO$_2$ ratio and mean arterial pressure.
Therefore the ‘double hit’ with smoke inhalation and
pneumonia induces the animal systemic inflammatory
response and septic lung. In the present study, we
investigated the effects of intravenous heparin on ALI in
this ovine sepsis model. The advantages of using such
large animals were that the situation allowed us to be able
to treat the animal in the same manner as a patient in
an intensive care unit, and that we were able to monitor
the ACT frequently during the study. We adjusted the
dose of heparin by monitoring the ACT. The purpose of
our heparinization was not just to normalize the
hypercoagulable state following the septic response, but
also to keep the ACT between 300 and 400 s, which is
approximately double the normal value. Even though
plasma antithrombin activity decreased significantly in
this study (Figure 7C), the high-dose heparin prolonged
the ACT and kept it in the stable target range, thus
suggesting that heparin management was successful. As
seen in Figure 2, the consumption of platelets due to
coagulation abnormalities was inhibited by heparin.
Also, FDP, a marker of fibrinolysis and evidence of
intravascular fibrin formation, was negative in the
heparin-treated group. These facts clearly show that the
heparin dosage was adequate to prevent the coagulation
abnormalities associated with sepsis.

Sepsis-induced changes in gas exchange, shown as a
reduced PaO$_2$/FiO$_2$ ratio and an increased lung wet/dry
weight ratio, were not attenuated by heparin (Figure 4),
suggesting that the inhibition of systemic thrombin by
heparin is not effective in the treatment of lung injury.
Also, hyperdynamic cardiovascular responses were not
affected by heparin. Consequently, we believe that
systemic thrombin does not play an important role in the
changes in haemodynamics or in ALI in sepsis induced
by smoke inhalation and pneumonia. As shown in Table
1, body temperature rose significantly after the injury in
both heparin-treated and control groups, suggesting that
the inflammatory reaction was not much different be-
tween the groups. This result is consistent with the other
findings in the present study.

We might speculate that thrombin does not play an
important role in IL-1 or prostaglandin E$_2$ synthesis,
which are the main mediators of fever elevation. The
measurement of cytokine or prostaglandin E$_2$ levels
in a sheep model is our next step. On the other hand,
heparin does not inhibit thrombin directly. In order to act
as an agonist of thrombin, heparin must augment the
activity of antithrombin. Antithrombin is markedly re-
duced in our model. We have determined in preliminary
studies that restoration of the antithrombin levels will
reduce much of the cardiopulmonary dysfunction seen
in our model [24]. Perhaps our next study should
be one in which both antithrombin and heparin are
increased during resuscitation. However, one aspect of
concern is a bleeding tendency. In a recent randomized
clinical trial of high-dose antithrombin, it was reported
that, in patients receiving antithrombin and concomitant
heparin, a significantly increased incidence of bleeding
was observed [25].

In contrast with the other variables, the pulmonary
vascular resistance index and the right ventricular stroke
work index were lower in heparin-treated animals (Figure
5). Since thrombin stimulates platelets to release throm-
boxane, which is a strong vasoconstrictor, this could be
the cause of the increase in vascular resistance. We noted
that the platelet count decreased in the saline group, but
not in the heparin group. Platelets have an adhesion
molecule – P-selectin – and pulmonary endothelial cells
and neutrophils express its ligand [26], so that many
platelets accumulate in the lung in the septic condition
[27]. Furthermore, in the septic condition, inflammatory
cytokines such as tumour necrosis factor or IL-1 induce tissue factor to form on the surface of endothelial cells and monocytes/macrophages. Since tissue factor is an initiator of the coagulation cascade, thrombin is formed in the intravascular space. Therefore there is a possibility that heparinization might reduce pulmonary vascular resistance, in part, through preventing the formation of pulmonary artery thrombi. However, antithrombin in the plasma usually inactivates thrombin immediately, so that the thrombus formed in the vessel is rarely seen. Furthermore, we have not observed intravascular fibrin by electron microscopy in this animal model. We assume that the thrombus formed in the vessel is rarely seen.

In conclusion, ALI and hyperkinetic haemodynamic changes were not attenuated by high-dose heparin in an ovine model of sepsis. These results suggest that high-dose heparin is not a substitute for activated protein C in the treatment of sepsis.

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