Clinical and pathological features of fat embolism with acute respiratory distress syndrome

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ABSTRACT

FES (fat embolism syndrome) is a clinical problem, and, although ARDS (acute respiratory distress syndrome) has been considered as a serious complication of FES, the pathogenesis of ARDS associated with FES remains unclear. In the present study, we investigated the clinical manifestations, and biochemical and pathophysiological changes, in subjects associated with FES and ARDS, to elucidate the possible mechanisms involved in this disorder. A total of eight patients with FES were studied, and arterial blood pH, $P_{aO_2}$ (arterial partial pressure of $O_2$), $P_{aCO_2}$ (arterial partial pressure of $CO_2$), biochemical and pathophysiological data were obtained. These subjects suffered from crash injuries and developed FES associated with ARDS, and each died within 2 h after admission. In the subjects, chest radiography revealed that the lungs were clear on admission, and pulmonary infiltration was observed within 2 h of admission. Arterial blood pH and $P_{aO_2}$ declined, whereas $P_{aCO_2}$ increased. Plasma PLA$_2$ (phospholipase A$_2$), nitrate/nitrite, methylguanidine, TNF-α (tumour necrosis factor-α), IL-1β (interleukin-1β) and IL-10 (interleukin-10) were significantly elevated. Pathological examinations revealed alveolar oedema and haemorrhage with multiple fat droplet depositions and fibrin thrombi. Fat droplets were also found in the arterioles and/or capillaries in the lung, kidney and brain. Immunohistochemical staining identified iNOS (inducible nitric oxide synthase) in alveolar macrophages. In conclusion, our clinical analysis suggests that PLA$_2$, NO, free radicals and pro-inflammatory cytokines are involved in the pathogenesis of ARDS associated with FES. The major source of NO is the alveolar macrophages.

INTRODUCTION

FES (fat embolism syndrome) can be induced by various causes [1–4]. Although the precise mechanisms of FES remain unclear, intravasation of fat or fatty acids from long-bone fractures and other sources is the primary cause leading to FES [1,3–5]. In a previous study [6], we reported six cases who died of ARDS (acute respiratory distress syndrome) with fat embolism due to a fracture of the femur and/or tibia. The occurrence of ARDS associated with FES suggests that the lung is one of the target organs following intravasation of

Key words: acute respiratory distress syndrome (ARDS), alveolar macrophage, fat embolism syndrome, inducible nitric oxide synthase (iNOS), pro-inflammatory cytokine.

Abbreviations: ARDS, acute respiratory distress syndrome; FES, fat embolism syndrome; H&E, haematoxylin and eosin; HR, heart rate; IL-1β, interleukin-1β; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; MBP, mean blood pressure; MG, methylguanidine; NOx, nitrate/nitrite; Paco$_2$, arterial partial pressure of $CO_2$; PAF, platelet-activating factor; Pao$_2$, arterial partial pressure of $O_2$; PAP, pulmonary arterial pressure; PLA$_2$, phospholipase A$_2$; TNF-α, tumour necrosis factor-α.

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Table 1 Characteristics of patients who died from FES associated with ARDS

Values are means ± S.E.M. (range).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Sex (n) (male/female)</td>
<td>5/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 5 (38–64)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72 ± 6 (64–78)</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>175 ± 14 (162–184)</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>116 ± 8 (108–122)</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>1432 ± 24 (1236–1628)</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>41 ± 5 (34–48)</td>
</tr>
<tr>
<td>Traumatic injury (n)</td>
<td></td>
</tr>
<tr>
<td>Fracture of tibia</td>
<td>2</td>
</tr>
<tr>
<td>Fracture of femur</td>
<td>1</td>
</tr>
<tr>
<td>Fracture of tibia and femur</td>
<td>3</td>
</tr>
<tr>
<td>Fracture of multiple pelvic bones</td>
<td>2</td>
</tr>
</tbody>
</table>

fat emboli [1,3,5,7]. In addition to the presence of fatty droplets in the lungs, chemical mediators including PAF (platelet-activating factor), PLA₂ (phospholipase A₂), cGMP, serotonin and NO have been implicated in the pathogenesis of FES [5,6,8]; however, the detrimental and/or beneficial roles of these factors remain unclear.

Following long-bone fracture and other causes, a wide spectrum of symptoms and signs might occur. ARDS only develops in a certain number of patients [2–4]. The risk factors leading to the development of ARDS due to FES are important clinical issues.

Following our study in six cases of FES associated with ARDS [6], we have encountered an additional eight cases suffering from FES following a serious crash injury. In the present study, we reported the clinical features, and biochemical and pathological changes, in these eight subjects, with the aim of investigating the pathogenetic mechanisms of ARDS associated with FES due to long-bone fracture.

MATERIALS AND METHODS

Patients

A total of eight patients were studied, and each of these died from ARDS following a crash injury. Table 1 summarizes the characteristics of these subjects, including the traumatic injury they suffered. The patients were immediately taken to the hospital after the crash injury.

Clinical studies, including autopsy and protocols, were approved by the Institutional Review Board of the University Hospital. Written consent was obtained from patients when they were still conscious or from their relatives upon admission.

Clinical observation and examination

Upon admission, chest radiography was taken, along with routine physical examinations, including body weight, height and MBP (mean blood pressure). Clinical signs and symptoms were also monitored.

Arterial blood samples (4–6 ml) were taken to determine pH, PaO₂ (arterial partial pressure of O₂) and PaCO₂ (arterial partial pressure of CO₂) using pH and blood gas analysers (178 pH and Blood Gas Analyser respectively; Corning). Plasma concentrations of PLA₂ were measured on a spectrofluorimeter using a method described by Kittsioulis et al. [9]. Briefly, the standard incubation mixture contained 10 mmol/l Tris/HCl buffer (pH 7.4) with 2 mmol/l Ca²⁺ and 5 μmol/l C₁₂-NBD-PC {1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-sn-glycero-3-phosphocholine} as the substrate. The absorbance of the reaction mixture was measured with excitation and emission wavelengths at 475 and 535 nm respectively.

Plasma concentrations of NOx (nitrate/nitrite) and MG (methylguanidine) were determined by HPLC [10,11]. TNF-α (tumour necrosis factor-α), IL-1β (interleukin-1β) and IL-10 (interleukin-10) were measured by ELISA using a commercial antibody pair, recombinant standards and a biotin–streptavidin–peroxidase detection system (Endogen) [12]. All agents, samples and standards were prepared at room temperature according to the manufacturer’s instructions. Absorbance was measured at 450 and 540 nm by automated ELISA-plate readers.

Pathological examinations

Autopsy specimens of the lung, kidney and brain were obtained from the eight subjects who died from FES associated with ARDS. Samples were fixed in formaldehyde, embedded in paraffin and the tissue blocks were sectioned (4 μm in thickness). Tissue sections were stained with H&E (haematoxylin and eosin) for histopathological examination. Fat staining with various techniques was employed for detection of fatty droplets [13].

Immunohistochemical staining with labelled streptavidin and antibody was used to detect iNOS (inducible nitric oxide synthase). The sections were placed on poly(L-lysine)- and silane-coated slides and incubated at 70 °C for 20 min, followed by rehydration in water and digestion with antigen-retrieval solution (Dako) for 20 min at 99 °C. The slides were incubated in 30% (v/v) H₂O₂ for 30 min to quench endogenous peroxidase activity and rinsed in deionized water. Non-specific antibody binding was blocked by incubating specimens with a mixture of goat serum and avidin-blocking reagent (Vector Laboratories) for 30 min. The anti-iNOS antibody (Dako) was added to the sections at a dilution of 1:100 in biotin-blocking reagent and incubated at 4 °C overnight. The sections were then incubated with...
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Figure 1 Chest radiographs on admission (A) and at 1 h (B) and 2 h (C) after admission. On admission, the lungs were clear, whereas pulmonary infiltration (arrows) was observed at 1 and 2 h after admission.

Table 2 Arterial blood pH, PaO\textsubscript{2} and PaCO\textsubscript{2} in the subjects over time following admission

Values are means ± S.E.M. (n = 8) The pH and PaO\textsubscript{2} progressively decreased over time following admission, whereas PaCO\textsubscript{2} increased. *P < 0.05 compared with at admission; and †P < 0.05 compared with 1 h after admission.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Admission</th>
<th>1 h</th>
<th>2 h</th>
<th>Before death</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.38 ± 1.24</td>
<td>6.21 ± 0.09*</td>
<td>5.78 ± 0.08†</td>
<td>4.03 ± 0.06†</td>
</tr>
<tr>
<td>PaO\textsubscript{2} (mmHg)</td>
<td>94 ± 5</td>
<td>83 ± 4*</td>
<td>67 ± 3†</td>
<td>53 ± 4†</td>
</tr>
<tr>
<td>PaCO\textsubscript{2} (mmHg)</td>
<td>39 ± 3</td>
<td>44 ± 4*</td>
<td>52 ± 5†</td>
<td>64 ± 6†</td>
</tr>
</tbody>
</table>

Table 3 Plasma concentration of PLA\textsubscript{2}, NOx and MG

Values are means ± S.E.M. (n = 8). Each of these factors increased progressively and significantly over time following admission. *P < 0.05 compared with at admission; and †P < 0.05 compared with 1 h after admission.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Admission</th>
<th>1 h</th>
<th>2 h</th>
<th>Before death</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA\textsubscript{2} (pg/ml)</td>
<td>0.33 ± 0.08</td>
<td>0.48 ± 0.09*</td>
<td>0.66 ± 1.01†</td>
<td>0.79 ± 1.03†</td>
</tr>
<tr>
<td>NOx (pmol/ml)</td>
<td>19.42 ± 0.32</td>
<td>42.83 ± 0.44*</td>
<td>56.92 ± 0.46†</td>
<td>64.64 ± 0.51†</td>
</tr>
<tr>
<td>MG (pmol/ml)</td>
<td>1.46 ± 0.05</td>
<td>1.67 ± 0.06*</td>
<td>3.02 ± 0.08†</td>
<td>3.54 ± 0.11†</td>
</tr>
</tbody>
</table>

In the time following admission, subjects developed signs of respiratory distress and progressive loss of consciousness. Chest radiography showed lung infiltration (Figure 1), and gross inspection revealed diffuse petechial rashes. MBP decreased from an average of 116 ± 8 mmHg to 72 ± 4 mmHg within 1 h, and to 46 ± 5 mmHg at 2 h after admission. HR (heart rate) was 82 ± 4 beats/min on admission, but tachycardia (HR, 142 ± 6 beats/min) was observed at 1 h, with severe bradycardia (HR, 38 ± 5 beats/min) occurring after 2 h. Tachypnoea, cyanosis, generalized weakness, loss of consciousness and cardiac arrest developed. Despite intensive treatments with endotracheal ventilation and intravenous vasoconstrictors, the patients died. Arterial blood pH and PaO\textsubscript{2} were decreased at 1 and 2 h and before death, whereas an increase in PaCO\textsubscript{2} was observed (Table 2).

Before death, measurement of PAP (pulmonary arterial pressure) using a Swan–Ganz catheter revealed a high PAP in all patients (Table 1) compared with the normal range of 12–17 mmHg [6].

PLA\textsubscript{2}, NOx and MG levels

Table 3 shows the changes in plasma PLA\textsubscript{2}, NOx and MG upon admission and in the time period before death. Each
of these factors were markedly and significantly increased at 1 and 2 h after admission and before death compared with those on admission.

**TNF-α, IL-1β and IL-10 levels**

Plasma levels of TNF-α, IL-1β and IL-10 were also increased significantly at 1 and 2 h after admission and before death compared with the levels upon admission (Table 4).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Admission 1 h</th>
<th>2 h</th>
<th>Before death</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>424 ± 28 3126 ± 124*</td>
<td>4634 ± 116†</td>
<td>5868 ± 120‡</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>48 ± 6 138 ± 8*</td>
<td>186 ± 9†</td>
<td>224 ± 12‡</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>96 ± 9 352 ± 12*</td>
<td>602 ± 18†</td>
<td>726 ± 21‡</td>
</tr>
</tbody>
</table>

**Pathological findings**

Histopathological micrographs revealed pathological changes in the lung, kidney and brain. H&E staining showed alveolar haemorrhagic oedema with fat droplet depositions and fibrin thrombi. Fat staining demonstrated multiple fatty droplets in the lung (Figures 2A–2C). Marked iNOS levels were detected in the alveolar macrophages as determined by immunohistochemical staining (Figure 2D). Fat-specific staining with Oil Red, Sudan Black and Sudan III indicated the presence of fat droplets in the pulmonary arteriole lumen (Figures 2E–2H). In the kidney, multiple fat deposits were found in the glomeruli by H&E and fat staining (Figures 2I and 2J). Fat droplets were also detected in the cerebral capillaries (Figure 2K). In fact, specific fat staining with Oil Red, Sudan Black and Sudan III also showed fat droplets in the arterioles of the kidney and brain similar to the distribution found in pulmonary arterioles (Figures 2I–2K). However, pathological involvement, including fat droplets in tissues and arterioles, was only found in two cases with renal lesions (25.0%) and five cases in the brain (62.5%) compared with all subjects (n = 8; 100%) with lesions in the lung.

**DISCUSSION**

In the present study, we have examined eight subjects who suffered from a traumatic injury and who developed FES with fulminant ARDS shortly after admission. ARDS was demonstrated by lung infiltration on a chest radiograph, high PAP and increased lung weight. Respiratory insufficiency included acidosis (decreased pH), hypoxia (reduced PaO₂) and hypercapnia (increased PaCO₂). Clinical features, such as respiratory, neurological and cutaneous symptoms, were consistent with the Gurd criteria for FES [15,16].

Rae et al. [8] found that the PLA2 propeptide was involved in patients with acute injury. They proposed that the PLA2 propeptide caused neutrophil sequestration and activation in the pulmonary vasculature and interstitium. A recent study [5] has shown that PAF and PLA2 levels increased in bronchoalveolar lavage fluid obtained from patients with FES. In the present study, we also found that plasma PLA2 concentrations increased significantly. This result supports the involvement of PLA2 in the pathogenesis of ARDS associated with FES. Our previous study [6] indicated that NO may also be involved. In the present study, plasma levels of NOx and MG were greatly increased. In addition, pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-10, were increased remarkably. The formation of MG has been considered as a biochemical marker of hydroxyl radicals [17], and our present results indicate that free radicals and pro-inflammatory cytokines participate in ARDS and other changes associated with FES. Studies from our laboratory suggest that free radicals and pro-inflammatory cytokines are responsible for the pathophysiological and biochemical changes in conscious rats and rats with sepsis [12,18]. In this regard, FES may be considered as a systemic inflammatory reaction. Indeed, Fabian [19] has proposed that the action of pulmonary lipases results in the release of non-esterified fatty acids that induce an inflammatory response. However, the interaction between fat-emboli-induced mediators requires further investigation.

The present study probably provides the most comprehensive histopathological images of fat emboli in the lung, kidney and brain. Although experimental studies and clinical reports on cerebral fat embolism due to fat emboli or FES have been demonstrated and the neurological disorders described [20–23], fat embolism in the kidney has rarely been reported. However, intravasation of fat emboli should involve multiple organs [2,19,24,25]. In the present study, fat emboli were found in the kidney in only two of the eight subjects. On the other hand, Brondén et al. [26] used tritium-labelled triolein to induce lipid microembolization in pigs. The radioactivity reflected the density of microemboli, and they found higher amounts of radioactive lipid particles in the kidney and spleen than in the lung and brain. The severity and extent of involvement may depend on sequestration of fat emboli in an organ, whereas subsequent mediator release might cause multiple organ damage.

One major and important finding in the present study was the presence of iNOS in alveolar macrophages, as shown by immunohistochemical staining. Our previous studies have indicated that pro-inflammatory cytokines provoke iNOS mRNA expression and lung injury in...
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Figure 2  Histopathological micrographs of lung (A–H), kidney (I and J) and brain (K) tissue from subjects with FES associated with ARDS

(A) H&E staining showing alveolar oedema and haemorrhage with fat droplet depositions (arrows). (B) Fibrin thrombi (arrows). (C) Fat staining revealing multiple fat droplets in lung tissue (arrows). (D) Immunochemical staining showing marked iNOS in alveolar macrophages (arrows). (E) H&E staining showing fat droplets (white colour). Fat staining with Oil Red (F), Sudan Black (G) and Sudan III (H) showing fat droplets [reddish colour in (F), brown to black colour in (G) and orange colour in (H)] in the pulmonary arteriolar lumen. H&E staining (I and K) and fat staining (J) demonstrating fat droplets [arrows in (I), and brown colour in (J)] in the renal glomeruli and cerebral capillaries [arrows in (K)]. Original magnification, ×200 in (A–D) and ×400 in (E–K).

rats following administration of LPS (lipopolysaccharide) [27]. We have also identified the lung as the major site that produces NO, which is toxic to the lung [28]. Several experimental studies have indicated that the release of NO via iNOS is responsible for oxidative stress and lung injury following smoke inhalation [29], exposure to ozone [30], carrageen treatment [31], acute hypoxia [32], acid aspiration [33] and endotoxaemia [34–37]. In a murine model of sepsis, Razavi et al. [38] proposed that up-regulation of iNOS in the lung caused neutrophil infiltration. Recruitment of iNOS-producing neutrophils enhanced further oxidative stress in the lung. Activated iNOS also enhanced the release of pro-inflammatory cytokines [39]. These interactions of cytotoxic mediators thus create a vicious cycle in the inflammatory process. A previous clinical report from our laboratory revealed that iNOS mRNA was up-regulated in patients who died from enterovirus infection [40]. In the present study, we suggest that NO produced via iNOS in alveolar macrophages caused lung injury in FES.

Although neutrophil activation plays an important role in the pathogenesis of acute lung injury or ARDS,
histopathological examination in the present study did not reveal significant neutrophil infiltration. It is likely that the time (within 2 h) is too short for significant neutrophil activation and infiltration. On the other hand, massive arteriolar obstruction in the pulmonary circulation is able to produce ischaemia/reperfusion and platelet–neutrophil interaction following pulmonary capillary blockage. Further experimental studies in animals are required to investigate these possible events.

In conclusion, the present study has examined the clinical features, and biochemical and pathophysiological changes, in subjects who suffered from FES and died of ARDS. We suggest that NO, PLA₂, free radicals and pro-inflammatory cytokines (TNF-α, IL-1β and IL-10) play a role in the pathogenesis of FES-induced ARDS. Alveolar macrophages were probably the major source of iNOS in producing NO in the lung. Renal and cerebral lesions were also found in some cases.

ACKNOWLEDGMENTS

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