Original Article

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Surfactant protein-D and polymorphonuclear leukocyte elastase concentrations in patients with septic acute respiratory distress syndrome

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Abstract

Purpose: **Inhibition of surfactant activities by the protein in pulmonary edematous fluid plays a role in the occurrence of respiratory failure in acute respiratory distress syndrome (ARDS). Polymorphonuclear (PMN) elastase may be involved in surfactant-mediated damage. Accordingly, the concentrations of surfactant protein-D (SP-D) and PMN elastase were determined in patients with sepsis, and the associations of these two factors with the occurrence of ARDS and prognosis were examined.**

Methods: **Blood samples from 33 patients with sepsis and with or without ARDS were assayed. The SP-D and PMN elastase levels were determined using an enzyme-linked immunosorbent assay.**

Results: **SP-D levels in groups with and without ARDS were 493.9 Ø 373.3 ng/ml and 91.8 Ø 30.1 ng/ml, respectively. The level in the ARDS group was significantly higher than that in the group without ARDS ($P = .0002$). The PMN elastase levels in the groups with and without ARDS were 845.1 Ø 294.0 ng/ml and 424.9 Ø 81.1 ng/ml, respectively. The level in the ARDS group was significantly higher than that in the group without ARDS ($P < .0001$). The SP-D level in patients who survived was 157.0 Ø 127.4 ng/ml and that in those who died was 625.5 Ø 433.2 ng/ml. The level in the latter group was significantly higher than that in the former ($P < .0001$). The PMN elastase level in patients who survived was 493.7 Ø 145.8 ng/ml and that in those who died was 980.9 Ø 300.9 ng/ml. The level in the latter group was significantly higher than that in the former ($P < .0001$). A significant correlation was observed between SP-D and the PMN elastase levels ($r = 0.818$, $P < .0001$).**

Conclusion: **In the presence of ARDS, SP-D and PMN elastase served as good indicators of severity.**

Keywords: ARDS, sepsis, SP-D, elastase

Introduction

Acute respiratory distress syndrome (ARDS) is an acute pulmonary disorder that develops when the body is exposed to excessive stress. When neutrophils activated by inflammatory cytokines accumulate in the lungs, these neutrophils adhere to the endothelium of pulmonary capillary blood vessels, and invade the alveolar septum and the alveolar space. During this process, protease, active oxygen species and leukotrienes are released from the neutrophils and induce extensive non-specific inflammatory reactions in endothelial cells of pulmonary capillary vessels and type I cells of the alveolar septum, thereby inducing pulmonary edema resulting from accelerated permeability [1].

PMN elastase is one of the lysosomal enzymes contained in the azurophilic granules of neutrophils, and is believed to play an important role in the pulmonary tissue damage associated with neutrophils. We previously reported PMN elastase to be involved in the development of ARDS [2-3].

Pulmonary surfactant prevents alveolar collapse and maintains gas exchange in the lungs. Apoproteins are essential for manifestation of the functions of pulmonary sur-

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factant [4]. They are divided into four types, i.e., surfactant protein (SP) -A, SP-B, SP-C and SP-D. All are synthesized in and secreted by type II cells [5]. SP-A and SP-D are hydrophilic glycoproteins. Together with the mannan-binding protein, they are referred to as the collectin family of glycoproteins. They bind to bacteria, fungi and viruses, and are involved in host defense mechanisms [5,6-8]. Since the surface activity function of the pulmonary surfactant collected from bronchoalveolar lavage fluid (BALF) of ARDS patients is markedly low, damage to alveolar type II cells and inactivation of surfactant are considered to occur in the presence of ARDS and the associated alveolar pulmonary edema [9]. A quantitative abnormality of surfactant is also observed in ARDS [9-10]. We have reported blood SP-A and SP-D to be elevated in the presence of septic ARDS [11]. In this study, we determined plasma PMN elastase and SP-D levels in patients with sepsis, and examined the associations of these two factors with the occurrence of ARDS and prognosis.

Patients and Methods

This study was approved by the Ethical Committee of Iwate Medical College. Informed consent was obtained from all of the patients or their family. Thirty-three patients with sepsis (24 men and 9 women; mean age, 59.3Ø15.4 years) who had been receiving treatment at our center (between January and December 1999) since an early stage of their disease were enrolled in the study. The age difference between the men (56.7Ø14.1 years) and the women (66.2Ø17.1 years) was insignificant

Table 1. Clinical characteristics of the 33 patients with sepsis

Patient no.	Age (year)	Sex	Cause of disease	ARDS	Outcome
1	67	male	Multiple trauma	+	died
2	54	male	Peritonitis	+	died
3	77	male	Burn	+	died
4	80	female	Peritonitis	+	survived
5	72	female	Acute pancreatitis	+	survived
6	15	male	Peritonitis	+	survived
7	90	female	Multiple trauma	+	died
8	56	male	Poisoning	+	died
9	40	male	Burn	+	survived
10	64	male	Multiple trauma	+	survived
11	54	male	Peritonitis	+	survived
12	60	male	Peritonitis	+	survived
13	80	female	Acute pancreatitis	+	survived
14	38	female	Poisoning	+	survived
15	60	male	Pneumonia	-	died
16	64	male	Pneumonia	-	died
17	75	female	Peritonitis	-	survived
18	63	male	Head injury	-	survived
19	76	male	Head injury	-	survived
20	48	male	Brain infarction	-	survived
21	64	male	Head injury	-	survived
22	34	male	Multiple trauma	-	survived
23	57	female	Burn	-	survived
24	68	male	Multiple trauma	-	survived
25	54	female	Poisoning	-	survived
26	51	male	Cervical spinal cord injury	-	survived
27	72	male	Pneumonia	+	survived
28	48	male	Cervical spinal cord injury	+	died
29	52	male	Multiple trauma	+	died
30	65	male	Peritonitis	-	survived
31	42	male	Acute pancreatitis	-	survived
32	50	female	Peritonitis	-	survived
33	66	male	Peritonitis	-	survived

(p=0.1126). The clinical characteristics of the patients are shown in **Table 1**. A diagnosis of sepsis was made based on the criteria of the College of Chest Physicians/ Society of Critical Care Medicine [12]. Diagnosis of ARDS was on the basis of the criteria of the American-European consensus conference [13].

Blood was serially obtained from patients from the admission until the outcome. Blood samples collected in heparinized, endotoxin-free sampling tubes were centrifuged at 3,000 rpm for 40 sec to obtain platelet rich plasma and stored at ˘80 °C until use for the measurement of SP-D. Blood samples collected in sampling tubes containing EDTA were centrifuged at 3,000 rpm for 10 min and stored at ˘80 °C until use for the measurement of PMN elastase. The SP-D levels were determined using an enzyme-linked immunosorbent assay (ELISA; Teijin Bio Medicine, Tokyo, Japan) with a cutoff value of 109.8 ng/ml. The PMN elastase levels were also determined using an ELISA (Merck, Darmstadt, Germany); the normal PMN elastase level is 21 to 165 ng/ml.

All data are expressed as the mean ØSD. Differences were evaluated statistically using a Student *t* -test, and the presence of correlations was examined by calculating Pearson correlation coefficients. A value of p<.05 was considered to be significant.

Results

The group with ARDS consisted of 17 patients (12 men and 5 women), with a mean age of 59.2 Ø 18.4 years. The group without ARDS consisted of 16 patients (12 men and 4 women), with a mean age of 59.3 Ø 12.0 years. There was no significant difference in the age between the two groups (*P* = .9888). The SP-D level during the course of ARDS in the ARDS group was 493.9 Ø 373.3 ng/ml. The maximum SP-D level noted during the clinical course in the group without ARDS was 91.8 Ø 30.1 ng/ml. The level in the ARDS group was significantly higher than that in the group without ARDS (*P* = .0002, **Figure 1**). Similarly, the PMN elastase level in the ARDS group was 845.1 Ø 294.0 ng/ml, that in the group without ARDS 424.9 Ø 81.1 ng/ml. The level in the ARDS group was significantly higher than that in the group without ARDS (*P* < .0001, **Figure 1**).

Twenty-four patients (15 men and 9 women) survived and their mean age was 64.0 Ø 12.9 years. Nine patients (8 men and 1 woman) died and their mean age was 57.2 Ø 16.1 years. There was no significant difference in the age between the two groups (*P* = .2498). The maximum SP-D level noted during the clinical course in patients who survived was 157.0 Ø 127.4 ng/ml and that in the patients who died was 625.5 Ø 433.2 ng/ml. The level in the patients who died was significantly higher than that in those who survived (*P* < .0001, Fig. 2). Similarly, the PMN elastase level in patients who survived was 493.7 Ø 145.8 ng/ml, that in patients who died, 980.9 Ø 300.9 ng/ml. The level in patients who died was significantly higher than that in those who survived (*P* < .0001, **Figure 2**).

A significant correlation was noted between the SP-D level and the PMN elastase level (r = 0.818, *P* < .0001, **Figure 3**).

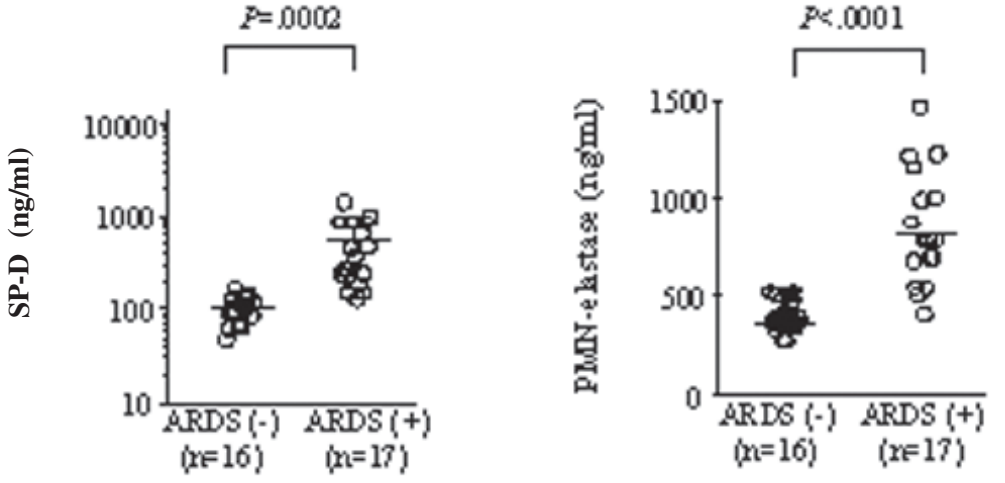

FIGURE 1. SP-D AND PMN ELASTASE LEVELS IN SEPTIC PATIENTS WITH ARDS AND WITHOUT ARDS. SP-D AND PMN ELASTASE LEVELS ARE SIGNIFICANTLY HIGHER IN PATIENTS WITH ARDS THAN IN PATIENTS WITHOUT ARDS. HORIZONTAL BAR SHOWS MEAN.

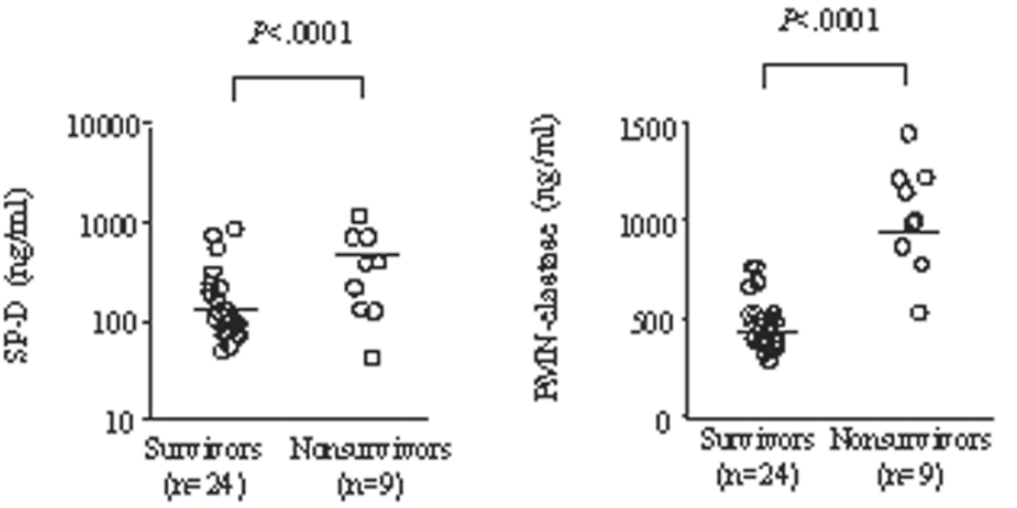

FIGURE 2. SP-D AND PMN ELASTASE LEVELS IN THE SURVIVORS AND NONSURVIVORS. SP-D AND PMN ELASTASE LEVELS ARE SIGNIFICANTLY HIGHER IN NONSURVIVORS THAN IN SURVIVORS. HORIZONTAL BAR SHOWS MEAN.

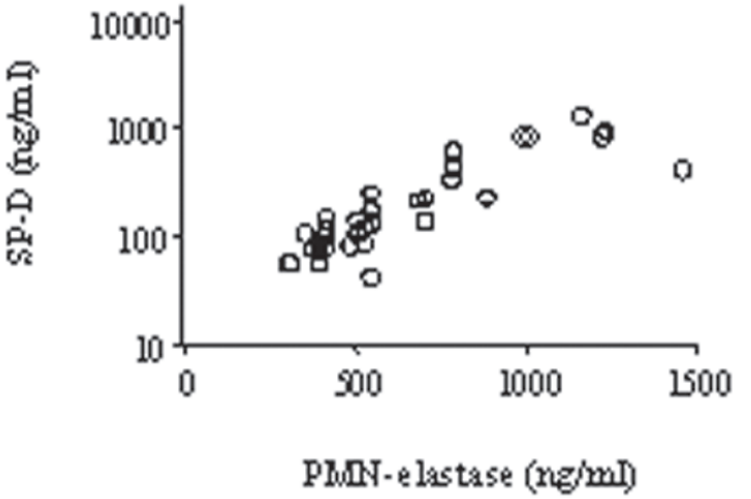

FIGURE 3. RELATION BETWEEN SP-D AND PMN ELASTASE LEVELS. A SIGNIFICANT CORRELATION IS OBSERVED. (N=33, R=0.818, *P*<.0001)

Discussion

Neutrophils have numerous neutral proteases such as elastase, cathepsin G and collagenase. Among them, elastase has extremely strong proteolytic activity. It is able to decompose almost all systemic proteins. Moreover, it is present in great abundance. On the other hand, an antiprotease mechanism is also present in the body. PMN elastase is inhibited by the α1-protease inhibitor (α1-PI) which is also present in great abundance in blood. Therefore, it is impossible to detect the elastase activity in blood in healthy people. When the body is exposed to excessive stress such as in cases of sepsis and acute pancreatitis, inflammatory reactions throughout the body are accelerated, and excessive amounts of inflammatory cytokines are produced from monocytes and macrophages. When neutrophils are activated by these inflammatory cytokines, PMN elastase appears in blood [2,3]. Upon activation, neutrophils produce active oxygen species and myeloperoxidase which in turn activates elastase, and inactivates α1-PI [14]. In our study, the PMN elastase level was significantly higher in the group with than in the group without ARDS, and was significantly higher in patients who died than in those who survived. These results suggest PMN elastase to play a role in the onset and exacerbation of ARDS.

The SP-D of rats binds to CD14, which is a cell membrane receptor for LPS, in a concentration-dependent manner [15]. In the mice in which excess SP-D has been induced, pulmonary damage can be inhibited by administration of LPS. Thus, SP-D plays an important role in host defense against the early stage of pulmonary damage caused by endotoxin. Abnormal accumulation of surfactant was observed in SP-D knock-out mice, while accumulation of surfactant lipid was inhibited when SP-D was specifically manifested in the lungs of SP-D defective mice [16]. These observations suggest SP-D to play an important role in the metabolism of surfactant. SP-D has been revealed to appear at high concentrations in the blood of patients with such pulmonary diseases as interstitial pneumonia and pulmonary proteinosis [17,18]. High concentrations have also been reported in ARDS [11,19]. There is a report indicating the PMN level to be increased in BALF from ARDS patients and that PMN elastase activity, which is not detectable in healthy people, was measurable [19-20]. Another report showed the SP-D level in BALF from ARDS patients who died to be lower than that in those who survived [10]. In our present study, the level in patients who died was significantly higher than that in those who survived. Moreover, a significant correlation was observed between the SP-D and PMN elastase levels. We speculate that SP-D have leaked into the circulation, thereby increasing its blood level, due to alveolar epithelial damage produced by PMN elastase.

Acknowledgments

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