Neutrophil Elastase as a Predicting Factor for Development of Acute Lung Injury

Tadatomo Kodama¹,², Hidekazu Yukioka², Takayuki Kato³, Noboru Kato², Fumihiko Hato³ and Seiichi Kitagawa¹,³

Abstract

Objective To determine whether the development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) associated with systemic inflammatory response syndrome (SIRS) can be predicted by the plasma neutrophil elastase level.

Patients and Methods Patients were sequentially enrolled after obtaining informed consent. Twenty-three adult patients with SIRS were classified into the following groups; SIRS alone (5 patients), Group A of ALI/ARDS with SIRS (9 patients) that did not require mechanical ventilation, and Group B of ALI/ARDS with SIRS (9 patients) that required mechanical ventilation. Blood samples were obtained after the diagnosis of SIRS, and the sequential sampling was performed.

Results The plasma neutrophil elastase level was significantly elevated in all patient groups as compared with healthy controls (43.7 ± 5.4 ng/ml). The elastase levels in SIRS alone, Group A of ALI/ARDS, and Group B of ALI/ARDS were 126.9 ± 11.0 ng/ml, 316.2 ± 68.9 ng/ml, and 458.4 ± 132.8 ng/ml, respectively. The elastase level in ALI/ARDS with SIRS was significantly greater than that in SIRS alone. The maximal level in 13 of 18 patients with ALI/ARDS with SIRS was more than 220 ng/ml. The level in all patients with SIRS alone was consistently less than 220 ng/ml over the study period. The serum levels of inflammatory cytokines were elevated in these patients, but no statistical significance was detected among the groups.

Conclusion The critical level of plasma neutrophil elastase is 220 ng/ml, and the SIRS patients with more than 220 ng/ml neutrophil elastase are highly likely to develop ALI/ARDS.

Key words: acute respiratory distress syndrome, systemic inflammatory response syndrome, inflammatory cytokines

(DOI: 10.2169/internalmedicine.46.6182)

Introduction

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is an acute inflammatory lung injury that may be associated with systemic inflammatory response syndrome (SIRS) and develops after a latent period of hours or days subsequent to various predisposing conditions, including multiple trauma, burns, and acute pancreatitis (1, 2). Neutrophils and their products, especially reactive oxygen species and serine proteases such as elastase, are implicated in the pathogenesis of ALI/ARDS (2-5). In fact, neutrophil elastase is elevated in the circulation of ARDS patients (6-8). In addition, it has been reported that sivelestat, a specific elastase inhibitor, is efficacious in a variety of animal models of ALI (4). These findings indicate that elastase could be a therapeutic target for the treatment of ALI/ARDS. However, the clinical trial of sivelestat failed to improve the survival rate of ALI/ARDS, although some beneficial effects such as reduced duration of mechanical ventilation were observed (9-11). In the previous studies, sivelestat was always administered after the development of ALI/ARDS, implying that the lung injury is already established before the administration of sivelestat. The failure of sivelestat to improve the

¹ Department of Medicine, Osaka City University Graduate School of Medicine, Osaka, ² Department of Emergency and Critical Care Medicine, Osaka City University Graduate School of Medicine, Osaka and ³ Department of Physiology, Osaka City University Graduate School of Medicine, Osaka

Received for publication August 15, 2006; Accepted for publication January 8, 2007
Correspondence to Dr. Seiichi Kitagawa, kitagawas@med.osaka-cu.ac.jp
survival rate of ALI/ARDS might be ascribed to the late administration of this inhibitor. The timing of any neutrophil elastase-directed intervention may be critically important for prevention of the development of ALI/ARDS. It is expected that early administration of an elastase inhibitor may provide a chance of success. For appropriate administration for appropriate patients, it is essential to determine the signs or factors predicting the imminent development of ALI/ARDS. A possible predicting factor may be the critical level of plasma neutrophil elastase, which may determine the development of ALI/ARDS in certain patients. In this study, the sequential blood sampling was performed immediately after the diagnosis of SIRS, and the levels of neutrophil elastase and proinflammatory cytokines were monitored over the period of 5-7 days after the onset of SIRS. The results indicate that the critical level of plasma neutrophil elastase may be 220 ng/ml, and the SIRS patients with more than 220 ng/ml neutrophil elastase are highly likely to develop ALI/ARDS.

Materials and Methods

Patients

Twenty-three adult patients with SIRS were sequentially enrolled. Their characteristics are shown in Table 1. The patients were classified into the following groups: SIRS alone (5 patients), Group A of ALI/ARDS with SIRS (9 patients) that did not require mechanical ventilation, and Group B of ALI/ARDS with SIRS (9 patients) that required mechanical ventilation (1). The diagnosis was based on the observations over the study period. SIRS alone indicates that the patient showed the signs and symptoms of SIRS without pulmonary dysfunction over the study period. If ALI/ARDS developed during the course of SIRS, the patient was diagnosed as having ALI/ARDS with SIRS. Blood samples were obtained immediately after the diagnosis of SIRS, and the sequential sampling was performed over the period of 5-7 days. The onset of the accident was determined by the date when the patient suffered from burn or trauma. The onset of underlying diseases was determined by the date when the apparent symptoms or signs were recognized for the definitive diagnosis. For determination of the concentration of plasma neutrophil elastase, the plasma samples were obtained from venous blood using ethylenediaminetetraacetic acid (EDTA) as the anti-coagulant. The blood samples were centrifuged at 3,000 rpm for 10 min at room temperature immediately after obtaining the venous blood with EDTA. The plasma samples were carefully harvested to avoid the contamination of blood cells, and were stored at -80°C until analysis. The serum was used for determination of the concentrations of cytokines, and the samples were stored at -80°C until analysis. Written informed consent was obtained from all subjects or their surrogates. The study was approved by the Ethics Committee of our medical school.

Determination of neutrophil elastase and proinflammatory cytokines

Plasma neutrophil elastase was determined by ELISA (HyCult Biotechnology, Uden, The Netherlands). Interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12 and tumor necrosis factor α (TNF-α) in the serum were determined by the cytometric bead array (CBA) (BD Biosciences, San Di-
Figure 1. The concentrations of neutrophil elastase and inflammatory cytokines in the circulation of the patients with SIRS alone, and in Group A and Group B of ALI/ARDS with SIRS. For the statistical analysis, all samples obtained within 5 days after the onset of SIRS were used for SIRS alone and Group A of ALI/ARDS with SIRS. For Group B of ALI/ARDS with SIRS, all samples obtained before the development of ALI/ARDS were used, because the intensive treatments, including mechanical ventilation, were performed for these patients after the development of ALI/ARDS and the intensive treatments could affect the inflammatory parameters. The same blood samples were used for determination of the concentrations of neutrophil elastase and cytokines. The data are expressed as the mean ± SE. *Significantly elevated as compared with healthy controls (*p < 0.05). #Significantly greater as compared with SIRS alone (#p < 0.05).

Statistical analysis

The statistical analysis was performed using Kruskal-Wallis test followed by Mann-Whitney test with adjustment of Bonferroni.

Results

The plasma neutrophil elastase level in healthy controls was 43.7 ± 5.4 ng/ml (n = 6; mean ± SE). The plasma neutrophil elastase levels in SIRS alone, Group A of ALI/ARDS with SIRS, and Group B of ALI/ARDS with SIRS were 126.9 ± 11.0 ng/ml (n = 20 from 5 patients; mean ± SE), 316.2 ± 68.9 ng/ml (n = 35 from 9 patients; mean ± SE), and 458.4 ± 132.8 ng/ml (n = 18 from 8 patients; mean ± SE), respectively. The neutrophil elastase level in each patient group was significantly elevated as compared with healthy controls, and the elastase level in Group A or Group B of ALI/ARDS with SIRS was significantly greater than that in SIRS alone. Although the neutrophil elastase level in Group B was apparently greater than that in Group A, the difference was not statistically significant (Fig. 1).

The serum concentrations of IL-6 and IL-8 were elevated in all patient groups as compared with healthy controls with no statistical significance being observed among the patient groups (Fig. 1). The serum IL-1β level in SIRS alone and the serum IL-10 level in Group A were also elevated as compared with healthy controls. On the other hand, the serum concentrations of TNF-α and IL-12 were not significantly elevated in all patient groups as compared with healthy controls. These findings indicate that the level of neutrophil elastase, but not proinflammatory cytokines, reflects the severity of pulmonary dysfunction associated with SIRS (Fig. 1), raising the possibility that the imminent development of ALI/ARDS could be predicted by the plasma neutrophil elastase level.

The neutrophil elastase levels in each patient over the study period are shown in Fig. 2. The neutrophil elastase level was significantly elevated in all patients as compared with healthy controls. It should be noted that the level of neutrophil elastase in all patients with SIRS alone was always less than 220 ng/ml over the study period. By contrast, the maximal level of neutrophil elastase in most patients (13 of 18 patients) with ALI/ARDS with SIRS reached more than 220 ng/ml (Fig. 2). In other words, all patients with SIRS expressing the elastase level of more than 220 ng/ml finally developed ALI/ARDS. The blood sampling in 5 patients with ALI/ARDS (Patients 7, 10, 16, 17 and 18), that expressed the elastase level of less than 220 ng/ml, started at 1 or 2 days after the accidents, because the diagnosis of SIRS was made at that time. Therefore, we cannot exclude the possibility that the neutrophil elastase level might have been higher than 220 ng/ml before the onset of SIRS or the start of sampling in these 5 patients. The remarkable increase (more than 500 ng/ml) was transient in all patients (Fig. 2).

Most patients developed SIRS within 2 days (15 of 18 patients) and pulmonary dysfunction within 3 days (16 of 18 patients) after the accidents (Fig. 2 and Fig. 3A and B). As a result, most patients (11 of 18 patients) developed pulmonary dysfunction within 1 day after the onset of SIRS. It
Figure 2. The plasma neutrophil elastase levels in each patient over the study period. The plasma neutrophil elastase levels in each patient belonging to the group of SIRS alone, and Group A and Group B of ALI/ARDS with SIRS are shown over the study period. In each set, the elastase levels at the indicated days after the onset of SIRS (Left column) and the initial accident (Right column) are shown, and the identical patient is represented by the same symbol. Closed symbols represent the elastase levels at the days when pulmonary dysfunction developed. Closed symbols are not shown for some patients, in whom blood samples at the days of the development of pulmonary dysfunction were not available, because pulmonary dysfunction developed before the onset of SIRS. Shaded area represents the normal range of the elastase level. Note that the vertical scale of the elastase concentrations is different.

should be noted that some patients (4 of 18 patients) developed pulmonary dysfunction before the onset of SIRS (Fig. 3C). These findings indicate that SIRS is not necessarily a predisposing condition for the development of pulmonary dysfunction or ALI/ARDS, and in some patients pulmonary dysfunction precedes the onset of SIRS. In most patients (11 of 13 patients), the elastase level of more than 220 ng/ml was detected within 3 days after the accident (Fig. 3D). Furthermore, in most patients (10 of 13 patients), the neutrophil elastase level reached more than 220 ng/ml concomitantly with the onset of SIRS (Fig. 3E), and pulmonary dysfunction was observed within 2 days after the detection of an elastase level of more than 220 ng/ml (Fig. 3F). Some patients (2 of 13 patients) showed more than 220 ng/ml elastase before the onset of SIRS (Fig. 3E), indicating that SIRS is not necessarily a predisposing condi-
The major findings in the present experiments are as follows. The plasma neutrophil elastase level was significantly elevated in all patients with SIRS alone and ALI/ARDS with SIRS as compared with healthy controls. The elastase level of ALI/ARDS with SIRS was significantly greater than that of SIRS alone. The level of neutrophil elastase in all patients with SIRS alone was consistently less than 220 ng/ml over the study period. By contrast, the maximal level of neutrophil elastase in most patients (13 of 18 patients) with ALI/ARDS with SIRS was more than 220 ng/ml, and all patients with SIRS expressing the elastase level of more than 220 ng/ml finally developed ALI/ARDS. These findings indicate that 220 ng/ml is the critical level of plasma neutrophil elastase. The patients presenting SIRS with more than 220 ng/ml neutrophil elastase are highly likely to develop ALI/ARDS, which develops within 2 days after the detection of more than 220 ng/ml elastase in most patients. We suggest that these patients are good candidates for early administration of elastase inhibitors to prevent the development of pulmonary dysfunction.

Although the level of neutrophil elastase in all patients with SIRS alone was consistently less than 220 ng/ml over the study period, this level does not indicate the intact pulmonary function, and ALI/ARDS developed in some SIRS patients with less than 220 ng/ml elastase. It is possible that, in addition to neutrophil elastase, other factors such as reactive oxygen species may play an important role in the lung injury in these patients. In fact, a massive amount of superoxide is released from neutrophils by the adhesive interaction with endothelial cells stimulated by proinflammatory cytokines (IL-1β and TNF-α) (12, 13). In addition, it has been reported that a significant part of active elastase is associated with the plasma membrane on activated neutrophils, which may contribute to the tissue injury in concert with reactive oxygen species (3, 14). It is also possible that in some patients neutrophils are not primarily involved in the lung injury, as indicated by the findings that ALI/ARDS develops in certain patients with severe neutropenia (15).

Inflammatory cytokines such as IL-1β, IL-6, IL-8, and IL-10 were also elevated in the circulation of some patients. However, no statistical significance in the levels of these cytokines was observed among the patient groups, indicating that the level of these cytokines is not a reliable predicting factor for the development of pulmonary dysfunction or ALI/ARDS. Although it is possible that these cytokines, especially IL-1β and TNF-α, may play an important role in the development of lung injury, consistent elevation of these cytokines at the high level was not detected in the circulation, and the serum levels were not correlated with the severity of pulmonary dysfunction. No remarkable elevation of IL-1β and TNF-α in the circulation may be ascribed to rapid binding of these cytokines to a variety of cells, including endothelial cells and neutrophils, and degradation of these cytokines by neutrophil elastase (14). By contrast, neutrophil elastase forms the complex with protease inhibitors such as α1-protease inhibitor and α2-macroglobulin in the plasma (3), allowing the easy detection of the elevated level of neutrophil elastase.

The pathogenesis and the underlying diseases of the patients with pulmonary dysfunction or ALI/ARDS are heterogeneous, and neutrophil elastase might not be a primary causative agent for the lung injury in some patients (16). The heterogeneity of the underlying diseases and the late
administration of sivelestat may partly explain why the clinical trial of sivelestat in a large scale failed to improve the survival rate of ALI/ARDS (11). The present experiments indicate that the critical level of plasma neutrophil elastase may be 220 ng/ml, and the SIRS patients with more than 220 ng/ml neutrophil elastase are highly likely to develop ALI/ARDS. In most patients, a level of greater than 220 ng/ml of elastase and the development of ALI/ARDS were observed within 3 days after the accident. In addition, in most patients the level of more than 220 ng/ml elastase was already detected when the diagnosis of ALI/ARDS with SIRS was made. These findings indicate that the sequential monitoring of plasma neutrophil elastase for at least 3 days after the accident may be useful for determination of the patients susceptible to the development of ALI/ARDS. Further clinical study is necessary to determine whether early administration of neutrophil elastase inhibitors such as sivelestat can prevent the development of ALI/ARDS in selected patients, in whom neutrophil elastase may play a major role in the lung injury.

This work was supported by Grant-in-Aid for Scientific Research, Japan. We thank Dr. M. Fukui, Laboratory of Statistics, Osaka City University Medical School, for kind help for the statistical analysis.

References