Assessment of Matrix Metalloproteinases-1 in Septic Acute Respiratory Distress Syndrome: A Report of Two Cases

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Abstract

The extracellular matrix (ECM) does not simply maintain the form of tissues; it is a dynamic factor that plays a major role in cell function. Matrix metalloproteinases (MMPs) are the most important enzymes in ECM degradation, and their activity is controlled strictly by specific inhibitors, that is tissue inhibitors of metalloproteinase (TIMPs). We assessed the clinical course of changes in ECM-degrading enzymes TNF-α, IL-6, IL-8, and nitrite/nitrate (NOx) in the blood of two septic acute respiratory distress syndrome (ARDS) patients. Negative correlation was found between the PaO₂/FIO₂ ratio (P/F ratio) and MMP-1, but positive correlation was found between the P/F ratio and both the TIMP-1/MMP-1 ratio and MMP-1 • TIMP-1 complex level. TIMP-1 was consistently maintained at high levels. These results suggest that both MMP-1 and TIMP-1 may be involved in septic ARDS, and that the balance between MMP-1 and TIMP-1 is important.

Introduction

The extracellular matrix (ECM) is composed of collagen, proteoglycans, and structural proteins, and it contributes greatly to tissue support functions as well as to the maintenance of cell functions including cell growth, differentiation, and adhesion [1,2]. Elastases which produced locally in the lungs are involved in ECM degradation in the lung [3]. Matrix metalloproteinases (MMPs) are the most important enzymes in ECM degradation, and their activity is controlled strictly by specific inhibitors, that is, the tissue inhibitors of metalloproteinase (TIMPs). It has been suggested that a breakdown in the balance between MMPs and TIMPs may be a factor in tissue destruction [4,5]. In this study we measured the concentrations of inflammatory cytokines, nitric oxide (NO) metabolites, i.e., NOx (nitrite/nitrate), MMP-1, and TIMP-1 in the blood of two septic ARDS patients to monitor the concentrations over the course of illness and to analyze these changes in relation to the severity and prognosis of the patients.

Methods

The study was conducted with the informed consent of the patients or their family, and the approval of the Ethical Committee of Iwate Medical College. The diagnosis of sepsis was based on the criteria of the consensus conference of the College of Chest Physicians/Society of Critical Care Medicine [6,7]. The diagnosis of ARDS was made according to the criteria of the American-European Consensus Conference [8].

Blood was drawn at 9-10 days after the diagnosis of septic ARDS for measurements of MMP-1, TIMP-1, TNF-α, IL-6, IL-8, and NOx and was collected into endotoxin-free heparinized blood specimen tubes. Platelet-rich plasma was obtained by immediate centrifugation at 4°C, 3000 rpm, for 40 sec and was stored at -80°C until use.

MMP-1, TIMP-1, and MMP-1 • TIMP-1 complex levels were determined by enzyme-linked immunosorbent assay (ELISA) (Amersham, Buckinghamshire, UK). The
detection limit for MMP-1 was 1.7 ng/ml, and levels of MMP-1 in healthy volunteer were below this limit. The detection limit for TIMP-1 was 1.25 ng/ml, and the normal value was 137 ± 33 ng/ml. The detection limit for MMP-1•TIMP-1 complex was 3 ng/ml, and levels in the healthy volunteer were below this limit. TNF-α was measured by ELISA (Medogenix, Fleurus, Belgium), and IL-6 and IL-8 were also measured by ELISA (TFB, Tokyo, Japan). The detection limits were TNF-α, 3 pg/ml; IL-6, 10 pg/ml; and IL-8, 3 pg/ml. NOx was measured by the Griess method with an autoanalyzer (TCI-NOX 1000; Tokyo Kasei Kogyo Co., Ltd., Tokyo),[9] and the normal value was 38.3 ± 19.1 mmol/l.

Case Reports

Case 1: The patient was a 56-year-old man who sustained a cerebral contusion and open fracture of the right femur in a motor vehicle accident. When he developed a fever of 38.7°C and his WBC count rose to 18,600/mm² 5 days after the injury, sepsis was suspected, and the patient was referred to us by his local physician On hospital day 1, his respiratory status suddenly deteriorated, and he was placed on artificial ventilation (Figure 1a). The source of the infection was the open fracture, and septic ARDS was cured by irrigating the wound and administering antibiotics. Marked improvement was seen on the x-ray film obtained 1 week after admission (Figure 1b). The chronological changes for each of the chemical mediators in this are shown in Figure 2. The PaO₂/FiO₂ ratio (P/F ratio), which is an expression of oxygenation ability, rose as the clinical manifestations improved. The MMP-1 values decreased as symptoms improved. The TIMP-1 values were already high in the early stage,
and they remained at high levels. The MMP-1\textbullet{}TIMP-1 complex values rose slightly, and the TIMP-1\textDash{}MMP-1 ratio increased. The TNF-\(\alpha\) levels decreased slowly, and the NOx levels decreased later. Internal fixation was performed, and the patient was discharged on hospital day 93 in good health.

**Case 2:** The patient was a 62-year-old woman who had undergone total hip replacement by a local physician for coxalgia caused by rheumatoid arthritis. The surgical site became infected, and the patient developed sepsis, for which she was referred to us for overall management, we admitted her. She was already in a state of septic shock upon arrival. On hospital day 1, she showed multiple organ failure, and she died on day 9 (Figure 3). Methicillin resistant *Staphylococcus aureus* (MRSA) was isolated from the wound site, and *Pseudomonas aeruginosa* and MRSA were isolated from the patient’s sputum. The changes in each of the chemical mediators over the course of illness are shown in Figure 4. The P/F ratio decreased, but the MMP-1 levels increased. The TIMP-1 levels were already high in the early stage, and they continued at high levels. The MMP-1\textbullet{}TIMP-1 complex values rose, and the TIMP-1\textDash{}MMP-1 ratio decreased considerably. The TNF-\(\alpha\), IL-6, and IL-8 values peaked when multiple organ failure was apparent and then decreased slowly, but the NOx levels continued to rise.

![Figure 3. Case 2. Chest X-rays](image)

![Figure 4. Case 2. Mediators levels over the course of illness.](image)
Discussion

Serine proteases and MMPs play a central role in decomposition of the ECM surrounding cells [1,2]. Since TIMPs inhibit active MMPs at a molar ratio of 1:1, the total MMPs (in moles) at a tissue site must exceed the total TIMPs for the tissue MMPs to act [4,5]. In our two patients, a negative correlation tended to be seen between the P/F ratios and MMP-1 levels and a positive correlation between the P/F ratios and both the TIMP-1/MMP-1 ratios and MMP-1•TIMP-1 complex levels. The MMP-1 values rose as the patients conditions worsened, and the TIMP-1/MMP-1 ratios decreased as the patients conditions worsened, suggesting that MMP-1 may be involved in the onset of septic ARDS, and that the balance between MMP-1 and TIMP-1 is important.

The decrease in MMP-1 in our patient in whom septic ARDS improved pointed to an increase in ECM, in other words, fibrosis of the lung, and this suggests a repair mechanism that continued in the presence of lung injury.

TNF-α is known to inhibit collagen and proteoglycan production of vascular endothelial cells at the transcription level and to stimulate MMP production [10,11]. In addition, it promotes production of various cytokines including IL-1, IL-6, and IL-8 [12-14]; it also promotes production of NO, which affects the regulation of tone in the blood vessel walls, endothelin-1, and platelet-derived growth factor [15-17]. NO has a particularly key function as a vasodilator in septic shock.

The fact that the changes in TNF-α, IL-6, IL-8, and NOx levels in this study paralleled the change in MMP-1 levels and that TIMP-1 was maintained continuously at high levels in the patient who survived and in the patient who died suggests that inflammatory cytokines and NO may be involved in increasing the production of both MMP-1 and TIMP-1 in septic ARDS. Because of the presence of a variety of different substances, it will be difficult to clarify whether the relations between NO, MMP-1, and TIMP-1 are indirect relations that are mediated by TNF-α or direct relations, in that they each stimulate production of the other. We will need to explore this question in future cases.

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