Excessive matrix metalloproteinase-9 in the plasma of community-acquired pneumonia

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Abstract

\textit{Background:} It has been shown that matrix metalloproteinase-9 (MMP-9) is involved in the pathogenesis of various pulmonary inflammatory diseases. We determined the MMP-9 concentration in the plasma of community-acquired pneumonia (CAP) patients before and after antibiotic treatment.

\textit{Methods:} Gelatin zymography and ELISA analysis were used to measure MMP-9 activity and MMP-9 level, respectively, in 35 control subjects and 46 CAP patients.

\textit{Results:} WBC counts, neutrophils, MMP-9 activity and MMP-9 level were significantly higher in CAP patients compared with that of control subjects ($P<0.001$), while MMP-9 activity and MMP-9 level were returned to normal after the antibiotic treatment ($P<0.001$). In addition, MMP-9 level correlated positively with WBC counts and neutrophils number both before and after the antibiotic treatment.

\textit{Conclusions:} MMP-9 may play an important role in the pathogenesis of CAP with a positive correlation with the number of neutrophils.

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\textit{Keywords:} Matrix metalloproteinase-9; Community-acquired pneumonia; Neutrophils

1. Introduction

Pneumonia is an inflammation of the lung caused by infection with bacteria, viruses or other organisms [1]. Pneumonia is often classified into two categories including community-acquired pneumonia (CAP) and...
hospital-acquired pneumonia (HAP) that may help predicting the organisms that are the most likely causes [2]. CAP, still an important cause of mortality in spite of effective antibiotics [3,4], affects nearly 4 million adults each year and *Streptococcus pneumoniae* is the most common pneumonia-causing bacteria, while other organisms, such as atypical bacteria called *Chlamydia* or *Mycoplasma pneumonia*, are also common causes of CAP [5].

In the processing of bacterial CAP, blood leukocytes respond to bacteria or bacterial products by secreting various substances, such as proinflammatory cytokines, chemokines, enzymes, oxygen and nitrogen radicals [4,6]. Among the enzymes secreted by leukocytes, matrix metalloproteinases (MMPs) play an important role in the pathogenesis of several inflammatory diseases [7,8]. MMPs are a large family of zinc and calcium-dependent endopeptidases with different substrate specificities, cellular sources and inducibility [8,9]. These enzymes are secreted as inactive proenzymes (or zymogens) and are autoactivated or activated by other proteolytic enzymes on site, resulting in the degradation of extracellular matrix. MMPs play an important role in physiological and pathological processes, including tumor migration, tissue remodeling and cell inflammation [10,11]. Of the MMP family, MMP-9 appears to be important for the migration of polymorphonuclear neutrophils (PMNs) across basement membranes [12,13].

Recently, various studies have shown that MMPs are implicated in the pathogenesis of various pulmonary inflammatory diseases like various pulmonary inflammatory diseases such as acute respiratory distress syndrome [14], bronchiectasis [15], cystic fibrosis [16], interstitial lung disease [17], chronic obstructive pulmonary disease [18] and hospital-acquired pneumonia [19]. Nevertheless, previous studies were focused on only bronchial lavage (BAL) fluid or sputum in various pulmonary inflammatory diseases. However, the MMP-9 activity and MMP-9 level in the plasma of community-acquired pneumonia have not yet been studied. In the present study, MMP-9 activity and MMP-9 level in the plasma of CAP patients and whether MMP-9 activity and MMP-9 level were related to the effectiveness of an antibiotics treatment was determined. Since PMNs are the main source of MMP-9 in the presence of pneumonia, the correlation between MMP-9 level and peripheral PMNs was also investigated.

## 2. Materials and methods

### 2.1. Subjects and specimen collection

Venous blood samples were obtained via routine venipuncture from CAP patients of the Armed-Force Taichung General Hospital, Taichung, Taiwan. A total of 81 subjects, including 35 control subjects and 46 CAP patients, were recruited into this study. Pneumonia was diagnosed on the basis of a lung radiographic opacity and at least two of the following conditions: fever (>38.5 °C), purulent expectoration, pleuritic chest pain or leukocytosis (white blood cell count of >10,000/mm³). Based the CAP patients conditions, the effective and commonly antibiotics, such as cefuroxime, ceftizoxime and clarithromycin, were used. For all patients, venous blood samples were obtained before and after the antibiotic treatment. The obtained blood was placed in tubes containing EDTA, immediately centrifuged and stored at −80 °C. Clinical characteristics and cell analysis of patients were summarized in Table 1.

### 2.2. Determination of MMP-2 and MMP-9 activities by gelatin zymography

Gelatin zymography was performed according to a protocol developed by Kleiner and Stetler-Stevenson [20]. Of each plasma sample, 20 µl of plasma containing 10 µg of total protein was loaded onto a precast sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) containing 0.1% gelatin. After electrophoresis, gels were processed as described by Kuo et al. [21]. Nonstaining bands representing the levels of latent form of MMP-2 and MMP-9 activities were quantitatively measured by spot density measurement using a digital imaging analysis system (Alpha Innotech, Mt. Prospect, IL) [22].

### 2.3. Measurements of MMP-9 level by enzyme-linked immunosorbent assay (ELISA)

The amount of MMP-9 level in the plasma was determined by a commercial available ELISA kit...
(R&D Systems, Abingdon, UK) according to the manufacturer’s instructions. Each sample was assayed in duplicate and the values were within the linear portion of the standard curve.

2.4. Statistical analysis

Values were expressed as means ± S.E. The statistical significance of the means for MMP-9 and cell counts was determined by Mann–Whitney rank sum test between groups. SigmaStat software (Jandel Scientific Software, San Rafael, CA) was used for all statistical analyses. Linear regression analysis was employed for the correlation between MMP-9 level, WBC counts and neutrophils counts. Statistical significance was set at \( P < 0.05 \).

3. Results

3.1. Characteristics and blood cell counts of subjects

The clinical characteristics of the normal subjects (including 20 men and 15 women, age 42.2 ± 3.4 years) and CAP patients (including 28 men and 18 women, age 48 ± 3.6 years) are summarized in Table 1. The total WBC counts of CAP patients were significantly higher than in controls \( (P < 0.001) \). Furthermore, the total WBC counts and the percentages of neutrophils of CAP patients were significantly reduced after treated with antibiotics \( (P < 0.001\) and \( P < 0.001\), respectively) (Table 1, Fig. 1A and B), while the percentage of lymphocytes was significantly enhanced \( (P < 0.001) \) (Table 1 and Fig. 1B). While, as indicated a total WBC count similar to that of control subjects, it was assured that CAP patients fully recovered after treated with antibiotics (Fig. 1A).

| Table 1: Clinical characteristics and blood cell analysis of all subjects |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Control                     | Untreated                   | Treated                     |
|                             | Subjects (n=35)             | CAP (n=46)                  | CAP (n=46)                  | \( P \)-value |
| Age                         | 42.2 ± 3.4                  | 48 ± 3.6                    | 48 ± 3.6                    | N.S.         |
| Sex (M/F)                   | 35 (20/15)                  | 46 (28/18)                  | 46 (28/18)                  | N.S.         |
| WBC (/mm\(^3\))             | 6338 ± 225                  | 13948 ± 676                 | 7030 ± 405                  | \(<0.0001\)  |
| \( DC \) counts*, %         |                             |                             |                             |              |
| Neutrophils                 | 58.1 ± 1.11                 | 78.4 ± 1.27                 | 58.3 ± 1.33                 | \(<0.0001\)  |
| Lymphocytes                 | 30.31 ± 0.99                | 11.64 ± 0.91                | 27.77 ± 1.24                | \(<0.0001\)  |
| Monocytes                   | 7.8 ± 0.32                  | 8.4 ± 0.66                  | 8.7 ± 0.53                  | =0.36        |
| \( DC \) counts*            |                             |                             |                             | =0.28        |
| Definition of abbreviations: C = control, UC = CAP patients before being treated, TC: CAP patients after being treated. |
| * DC counts: Differential cell counts. |

![Fig. 1. (A) WBC counts of patients with community acquired pneumonia and control subjects. (B) Differential cell counts of patients with community acquired pneumonia and control subjects. ***Refers to a significant difference while compared to that of control subjects, \( P < 0.001 \). **Refers to a significant difference while compared to that of CAP patients before the treatment \( P < 0.001 \).](image-url)
3.2. Gelatin zymographic and ELISA analysis for MMPs

Plasma activity of MMP-2 and MMP-9 of control subjects and CAP patients were assayed by gelatin zymography. The presence of MMP-2 and MMP-9 was indicated as a band of 72 and 92 kDa, respectively, as shown in Fig. 2A. The activity of MMP-2 was not significantly different between CAP patients and control subjects. The activities of MMP-9 of CAP patients being treated with antibiotics (144,011 ± 14,313) were significantly higher than after treated (40,916 ± 7285, P < 0.001) (Fig. 2B).

In order to quantitatively determine the MMP-9 level, an ELISA kit was used and the results, as shown in Fig. 2C, indicated that MMP-9 level of CAP patients before treated with antibiotics (396.58 ± 32.7 ng/ml) was significantly higher than after treated (131.79 ± 15.6 ng/ml, P < 0.001) and also much higher control subjects (149.47 ± 25.6 ng/ml, P < 0.001).

3.3. The correlation between MMP-9 level, WBC and neutrophil counts in CAP patients

Based on a linear regression analysis, either before or after an antibiotic treatment, MMP-9 level was significantly correlated with the WBC counts of (144,011 ± 14,313) were significantly higher than after treated (40,916 ± 7285, P < 0.001) (Fig. 2B).

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CAP patients, (before treatment: $Y=0.123X+90.886$, $R=0.593$, $P_{0.001}$; after treatment: $Y=0.103X+61.718$, $R=0.396$, $P_{0.01}$) (Fig. 3A and B), as well as the neutrophil count (before treatment: $Y=11.497X+6576.199$, $R=0.587$, $P_{0.001}$; after treatment: $Y=6.26X+3589.479$, $R=0.334$, $P_{0.05}$) (Fig. 3A and B), but not with the number of lymphocyte and monocyte (data not shown).

4. Discussion

CAP had ever been an important cause of mortality until the emergence of effective antibiotics. Among possible etiological factors, bacteria have been the most common causes of CAP, while bacterial infection may weaken the patient’s defense system together with various accompanied symptoms, such as fever (>38.5 °C), purulent expectoration, pleuritic chest pain or leukocytosis (WBC count of >10,000/mm³). As showed in Fig. 1A, the WBC counts of CAP patients were well over 10,000/mm³, which was obviously higher than that of control subjects. A consistent finding has been reported in a previous study [23]. Furthermore, in our study, the WBC and neutrophils counts of CAP patients were reduced after being treated with antibiotics (Table 1), which clearly indicated that CAP patients were fully recovered after an antibiotic treatment despite of what antibiotics were used, since WBC counts and MMP-9 level were not significantly different between various antibiotic (data not shown).

MMPs have been known to be involved in degradation of extracellular matrix. Among these MMPs, MMP-2 and MMP-9 play an important role in pathological pulmonary processes [24–26]. Recently, Hartog et al. [19] demonstrated a significant increased of MMP-8 and MMP-9 in plasma and in mini-BAL fluids of patients with hospital-acquired pneumonia compared with control subjects. Torii et al. [14] have discovered a higher concentration of MMP-9 and MMP-2 in BAL fluids of patients with adult respiratory distress syndrome compared with healthy subjects. Ricou et al. [27] also had the same observation for MMP-9. Mautino et al. [28] reported that MMP-9 content in BAL fluids from untreated asthmatics was increased compared with control subjects. Those studies were consistence with our results that MMP-9 activity and level were significantly higher in CAP patients than control subjects (Fig. 2B and C) and suggested that MMP-9 may play an important role in various pulmonary inflammatory diseases.

The major sources of MMP-9 are inflammatory cells such as monocytes/macrophages, neutrophil and eosinophils [29]. It has been reported that there was a significant correlation between the percentage of neutrophils and the concentrations of MMP-9 in sputum of asthma and chronic patient [30], as well as in several lung diseases [16,27]. On the other hand, Starr et al. [31] reported that Mannheimia haemolytica pneumonia products stimulate MMP-9 production and release by bovine monocytes, macrophages and neutrophils with an in vitro study. In our present study, the MMP-9 level and WBC counts appeared to
decrease quickly in patients who had a quick recovery and there was a correlation between WBC counts and the MMP-9 level. Therefore, we believed that MMP-9 release was related to the extent of the process of CAP. It further suggested that inflammatory cells, including neutrophils, possibly have involved in the pathogenesis of CAP.

In conclusion, our studies showed that counts of WBC and neutrophils were higher in CAP patients than in normal subjects and reduced after being treated with antibiotics and the activity and level of MMP-9 have the same trend. We also found that MMP-9 level was correlated with the WBC counts as well as with the number of neutrophils. Our studies suggested that MMP-9 plays an important role in the pathogenesis of CAP, which could be related with neutrophils. These findings may be helpful in understanding the role of plasma MMP-9 in the pathogenesis of CAP. Further studies with a larger number of patients should be performed to assess the prognostic values of MMPs for an antibiotic treatment of CAP patients.

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References


