JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008, 59, Suppl 6, 145–152
www.jpp.krakow.pl

B. BRAJER[1], H. BATURA-GABRYEL[1], A. NOWICKA[1],
B. KUZNAR-KAMINSKA[1], A. SZCZEPANIK[2]

CONCENTRATION OF MATRIX METALLOPROTEINASE-9 IN SERUM OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND A DEGREE OF AIRWAY OBSTRUCTION AND DISEASE PROGRESSION

[1]Department of Pulmonary Diseases and [2]Department of Cardiology,
Poznan University of Medical Sciences, Poznan, Poland

Chronic obstructive pulmonary disease (COPD) is chronic inflammation leading to irreversible airway obstruction. Previous studies showed increased metalloproteinases (MMP) level, especially MMP-9, as a sign of local inflammation. Up-to-date, only a few studies estimated the MMP-9 serum concentration in COPD with respect to correlation with systemic inflammation. The aim of the present study was to estimate the MMP-9 serum concentration in COPD and to evaluate the correlation between MMP-9 and a degree of airway obstruction in COPD. Twenty three COPD patients and 23 healthy controls were enrolled. In both groups spirometry was performed. MMP-9 concentration in sera taken from both groups was studied using ELISA. We found that COPD patients had increased serum MMP-9 concentration compared with the control group (P=0.0005). In the COPD group, the MMP-9 levels were negatively correlated with FEV1 (P=0.01) and FEV1/FVC (P=0.0002). In conclusion, the results suggest that MMP-9 plays an important role in systemic inflammation in COPD. Higher MMP-9 serum concentration is connected with higher airway obstruction and disease progression.

Key words: *airway obstruction, chronic obstructive pulmonary disease, serum metalloproteinases-9*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of respiratory morbidity and mortality worldwide. The increased morbidity in COPD is

explained by increasing smoking habits, especially in developing countries and across different groups within these countries. This disease is characterized by cough, sputum production, dyspnea, and impaired gas exchange and airway limitation. COPD is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to its severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases (1).

COPD is a chronic inflammatory process which includes chronic bronchitis with fibrosis and obstruction of small airways, emphysema with enlargement of airspace and destruction of lung parenchyma, loss of lung elasticity, and closure of small airways. The following belong to the extrapulmonary effects of this disease: loss of muscles mass and their dysfunction, osteoporosis, sleep disturbances, depression (1).

A lot of studies focus on the nature of this inflammatory process and its description (2 - 4). One of the main hypotheses concerning the pathogenesis of emphysema, a key cause of morbidity and mortality in COPD, is the protease-antiprotease imbalance. It may be induced by cigarette smoking. This theory proposes that the antiprotease protects the normal lung from locally elaborated proteases and a reduction in pulmonary antiproteases leads to parenchymal destruction (5).

Previous studies have shown an important role of increased elastolytic activity released from neutrophils in emphysema. Recent studies have demonstrated that matrix metalloproteinases (MMP) play a central role in the lung remodeling in COPD (6-8). In those studies, MMP-9 concentration in sputum and BAL (bronchoalveolar lavage) was measured as a sign of local inflammation in COPD patients. Up-to-date, only a few studies have estimated the MMP-9 serum concentration in COPD patients with respect to the correlation with systemic inflammatory process.

The aim of the present study was to find out whether the concentration of MMP-9 increases in COPD (current or previous smokers) patient sera, as a sign of systemic inflammatory process and to look for the relationship between MMP-9 serum concentration and airway obstruction.

MATERIAL AND METHODS

Study subjects

All subjects studied gave written informed consent. The study was approved by a local Ethical Committee.

Twenty three COPD patients (17 male and 6 female) were enrolled into the study from patients treated in the Department of Pulmonary Diseases in Poznan University of Medical Sciences, Poland. The COPD patients with any other coexisting pulmonary diseases, such as bronchiectases,

tuberculosis, asthma, and lung cancer were excluded. COPD was defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2007) criteria. COPD was diagnosed as a post-bronchodilator (400 μg of salbutamol) FEV1<80% of predicted value and the FEV1/FVC ratio lower than 70%. The average age in the COPD group was 59.6 ±9.4 yr.

Age-matched 23 subjects (17 male and 6 female) of a control group were selected from healthy volunteers. The average age in this group was 55.2 ±9.1 yr. None of the subjects had ever suffered from the pulmonary diseases, neoplasm, and other chronic conditions. All pulmonary lung function tests in this group were within the normal predictive range

All the subjects in both estimated groups were former or current smokers. The former smoker was defined as one who had smoked 100 cigarettes in their lifetime and had quit smoking before the preceding study year. The amount of cigarette smoking was assessed using Brinkman's index (the number of cigarettes per day x the numbers of years).

Lung function testing

The pre and post-bronchodilator forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio were determined using a Jaeger spirometer and performed by a respiratory physician. Values were expressed as percentages of the predicted normal values for age, sex, and height according to the European Community for Steel and Coal guidelines (9, 10).

MMP - 9 measurement

Blood samples for the MMP-9 measurements were centrifuged, plasma was removed and used for the assay. The total MMP-9 concentration (pro and active MMP-9 protein) in the serum samples was measured using the Quantikine human MMP-9 Immunoassay (ELISA; R & D System Inc., MN). The minimum detectable amount of MMP-9 was typically less than 0.156 $ng{\cdot}ml^{-1}$. All assays were performed strictly following the manufacturer's recommendation.

Statistical analysis

The results were expressed as means ±SD. The groups were compared by analyzing the age, gender, and pack-years. The differences between the examined groups were analyzed with a Mann-Whitney U test. Correlations between spirometric parameters and MMP-9 concentration were evaluated using Spearman's correlation coefficient. All statistical analyses were performed using Statistical 7.0 Package for Windows. $P<0.05$ was considered statistically significant.

RESULTS

The clinical characteristics of the COPD patients and control subjects are given in *Table 1*. No difference in subjects' age was observed between the examined groups (P=0.1). There were significant differences between spirometric parameters (FEV1, FVC, and FEV1/FVC) between the COPD and control groups, which were worse in COPD patients (P<0.001).

The serum MMP-9 concentration was significantly higher in the COPD patients than that in the control group (COPD: 248.2 ±101.0 $ng{\cdot}ml^{-1}$ (max-min 439.9-82.2 $ng{\cdot}ml^{-1}$); control subjects: 150.1 ±56.9 $ng{\cdot}ml^{-1}$ (max-min 238.8-43.2 $ng{\cdot}ml^{-1}$); P=0.0005; *Fig. 1*). The MMP-9 concentration change depended on the

Table 1. Clinical characteristics of the COPD patients and control subjects.

	COPD	Control
Subjects (n)	23	23
Age (yr)	59.6 ±9.4	55.2 ±9.1
Males/Females	17/6	17/6
Current smoker	23	23
FEV1/FVC ratio	56.9 ±10.7	Normal predicted value
FVC %pred	56.3 ±17.7	
FEV1 %pred	43.2 ±16.1	

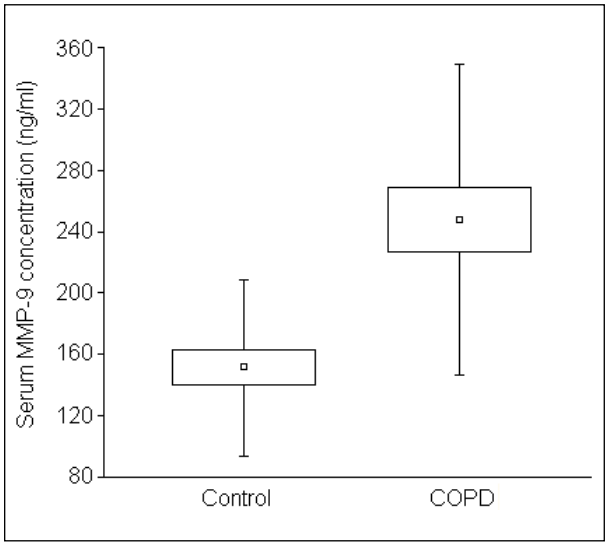

Fig. 1. Serum MMP-9 concentration in COPD patients and control subjects. There was a significant difference between the two groups; Mann-Whitney U test; P=0.0005.

degree of airway obstruction, expressed as FEV1 %pred, FVC %pred, and the FEV1/FVC ratio. The MMP-9 concentration correlated negatively with FEV1 (P=0.01; r= -0.51) and with FEV1/FVC ratio (P=0.0002; r= -0.46).

DISCUSSION

According to the last definition modification (GOLD 2007), COPD is a disease with some significant pulmonary (chronic bronchitis/bronchiolitis and/or emphysema) and extrapulmonary effects that may contribute to the severity in individual patients (1). The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Chronic local and systemic inflammation is a reason for remodeling of lung tissue and extrapulmonary systemic change.

MMPs are a family of zinc- and calcium-dependent enzymes that are capable of degrading all components of the extracellular matrix (ECM) and over twenty enzymes of this family have been described. In the MMP family, MMP-9 is a major elastolytic MMP, responsible for tissue remodeling and repair through the degradation of basement membrane type IV collagen and other matrix proteins. It is released by macrophages which are derived from circulating monocytes and from neutrophils (11, 12).

MMPs are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs), of which four members have been identified. TIMP-1 has been described as an MMP-9 activity inhibitor by binding to its precursors and active form (13, 14). The previous studies have shown increased sputum MMP-9 and TIMP-1 concentrations and also that alveolar macrophages trigger larger amounts of MMP-9 with greater enzymatic activity in COPD patients (15) Another research has shown positive correlation between MMP-9/TIMP-1 ratio and airway obstruction assessed by FEV1 measurement (16).

Beeh *et al.* (12) found that sputum concentration of MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio were higher in COPD patients than in control subjects. MMP-9 concentration correlated negatively with the severity of airway obstruction (FEV1%FVC). These data underline the significance of protease/antiprotease imbalance for the pathogenesis of COPD. Culpitt *et al* (17) observed that sputum from COPD patients contained increased levels of MMP-9 compared with non-smokers, non-symptomatic cigarette smokers, and asthmatics. Vignola *et al.* (16) found that HRCT scan abnormalities were associated with the sputum MMP-9/TIMP-1 ratio in COPD patients. The authors suggested that sputum levels of these markers reflect the extent of structural changes of the airways.

Kang *et al.* (18) revealed that lung parenchymal MMP-9 concentration and the molar ratio of MMP-9 to TIMP-1 were correlated with the amount of cigarette smoked. The MMP-9 concentration correlated inversely with FEV1. Their study showed that MMP-9 expression in human lung parenchyma is associated with cigarette smoking and with the obstruction of airflow, suggesting that MMP-9 may play a role in the pathogenesis of the cigarette-induced obstruction of airflow known, as a characteristic of COPD.

Segura-Valdez *et al.* (19) examined the expression of MMP-2 and -9 in lung tissue of COPD patients and control subjects. In their study, an immunohistochemical analysis of COPD lungs showed a markedly increased expression of both MMP-2 MMP-9. They found that neutrophils are the main cells showing a positive signal for MMP-9. Their findings suggest that there is an upregulation of MMP-2 and MMP-9 in lung tissue, which may contribute to the pathogenesis of COPD.

Only in a few studies have the authors examined the serum MMP-9 concentration, which may be a useful marker of systemic inflammation in COPD. Extrapulmonary manifestations of the disease consist of skeletal muscle

dysfunction, muscle wasting, osteoporosis, and atherosclerosis, and their associated complications (20-23). Higashimoto *et al.* (24) found that serum MMP-9 concentration do not differ among the three groups: COPD patients, asthmatics, and control subjects. In that study, concentration of circulating tissue inhibitor of metalloproteinases-1 (TIMP-1) was significantly higher in stable COPD patients than in control and asthmatic subjects and this concentration was significantly correlated with the FEV1/FVC % in COPD patients. Cataldo *et al.* (25) measured the release of MMP-9 and TIMP-1 from peripheral blood granulocytes in healthy subjects, asthmatics, and COPD patients. They did not find any difference in the release of these molecules within the three groups. They concluded that circulating granulocytes from COPD patients and asthmatics do not display an abnormal secretion of MMP-9. Aldonyte *et al.* (26), on the other hand, investigated the release of pro-inflammatory molecules, containing MMP-9, from blood monocytes isolated from healthy and COPD subjects. The authors revealed that the release of MMP-9 was 2.5-fold higher from COPD patient monocytes than from healthy controls.

Our present study demonstrates that serum concentration of MMP-9 was significantly higher in COPD patients than in control subjects. The result shows that MMP-9 may play a key role not only in local, but also in systemic inflammatory process in COPD. In COPD group, serum MMP-9 concentration correlated negatively with airway obstruction (FEV1 %pred and the FEV1/FVC ratio).

Mao *et al.* (27) examined the influence of all-trans retinoic acid (ATRA) on the balance of MMP-9 and TIMP-1 in patients with emphysema. The authors observed that plasma MMP-9 levels were higher in patients with emphysema compared with age-matched, non-smoking control subjects. A high baseline level of circulating MMP-9 in patients with emphysema and their down-regulation in response to ATRA suggest that the plasma MMP-9 might act as a disease related marker of pulmonary protease activity. The results of the present study correspond to those data.

In conclusion, the present study confirms that MMP-9 may play an important role in the systemic inflammatory process in COPD. The serum concentration of MMP-9 changes depending on the degree of airway obstruction and disease progression.

Acknowledgments: This work was supported a grant from Poznan University of Medical in Poznan, Poland.

Conflicts of Interest: No conflicts of interest were declared with relation to this work.

REFERENCES

1. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD 2007).

2. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 2003; 22: 672-688.
3. Mroz RM, Noparlik J, Chyczewska E, Braszko JJ, Holownia A. Molecular basis of chronic inflammation in lung diseases: new therapeutic approach. *J Physiol Pharmacol* 2007; 58 Suppl 5: 453-460.
4. Mroz RM, Holownia A, Chyczewska E et al. Cytoplasm-nuclear trafficking of CREB and Creb phosphorylation at SER133 during therapy of chronic obstructive pulmonary disease. *J Physiol Pharmacol* 2007; 58 Suppl 5: 437-444.
5. Belvisi MG, Bottomley KM. The role of matrix metalloproteinases (MMPs) in the pathophysiology of chronic obstructive pulmonary disease (COPD): the therapeutic role for inhibitors of MMPs? *Inflamm Res* 2003; 52: 95-100.
6. Vignola AM, Paganin F, Capieu L *et al*. Airway remodeling assesses by sputum and high-resolution computed tomography in asthma and COPD. *Eur Respir J* 2004; 24: 910-917.
7. Vernooy JHJ, Liendeman JHN, Jacobs JA, Hanemaaier R, Wouters EFM. Increased activity of matrix metalloproteinase-8 and matrix metalloproteinse-9 in induced sputum from patients with COPD. *Chest* 2004; 126: 1802-1810.
8. Montano M, Beccerril C, Ruiz V, Ramos C, Sansores RH, Gonzalez-Avilaa G. Matrix metalloproteinases activity in COPD associated with wood smoke. *Chest* 2004; 125: 466-472.
9. Quanjer PH. Standarized lung function testing. Report of Working Party for the European Community for Steel and Coal. *Bull Eur Physiopath Respir* 1983; 19: 22-27.
10. Sherril DL, Lebowitz MD, Knudson RJ, Burrows B. Continuous longitudinal regression equations for pulmonary function measures. *Eur Respir J* 1992; 5: 452-462.
11. Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 1991; 5: 2145-2154.
12. Shapiro SD. Matrix metalloproteinases degradation of extracellular matrix; Biological consequences. *Curr Opin Cell Biol* 1998; 10: 602-608.
13. Cataldo D, Munaut C, Noel A *et al*. MMP-2 and MMP-9 linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 2000; 123: 259-267.
14. Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* 2003; 97: 634-639.
15. Russel RE, Culpitt SV, DeMatos C *et al*. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1990; 159: 596-602.
16. Vignola AM, Riccobono L, Mirabella A *et al*. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airway obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998; 158: 1945-1950.
17. Culpitt SV, Rogers DF, Travers SL, Barnes PJ, Donnelly LE. Sputum matrix metalloproteinases: comparison between chronic obstructive pulmonary disease and asthma. *Respir Med* 2005; 99: 703-710.
18. Kang MJ, Oh JM, Lee JCh *et al*. Lung matrix metalloproteinase-9 correlates with cigarette smoking and obstruction of airflow. *J Korean Med Sci* 2003; 18: 821-827.
19. Seruga-Valdez L, Pardo A, Gaxiola M *et al*. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchyma cell health in COPD. *Chest* 2000; 117: 684-694.
20. Man SFP, Sin DD. Effects of corticosteroids on systemic inflammation in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005; 2: 78-82.
21. Soriano JB, Visick GT, Muellerova H, Payvandi N, Hansell AL. Patterns of comorbidities in newly diagnosed COPD and asthma in primary care. *Chest* 2005; 128: 2099-2107.

22. Agusti AG. Systemic effects of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005; 2: 367-370.
23. Sin DD, Anthonisen NR, Soriano JB, Agusti AG. Mortality in COPD: role of comorbidities. *Eur Respir J* 2006; 28: 1245-1257.
24. Higashimoto Y, Yamagata Y, Iwata T *et al*. Increased serum concentrations of tissue inhibitor of metalloproteinase-1 in COPD patients. *Eur Respir J* 2005; 25: 885-890.
25. Cataldo D, Munaut C, Noel A *et al*. Matrix metalloproteinases and TIMP-1 production by peripheral blood granulocytes from COPD patients and asthmatics. *Allergy* 2001; 56: 145-151.
26. Aldonyte R, Jansson L, Piitulainen E, Janciauskiene S. Circulating monocytes from healthy individuals and COPD patients. *Respir Res* 2003; 4: 11.
27. Mao JT, Tashkin DP, Belloni PN, Baileyhealy I, Baratelli F, Roth MD. All-trans retinoic acid modulates the balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with emphysema. *Chest* 2003; 124: 1724-1732.

Received: June 23, 2008
Accepted: Seprember 9, 2008

Author's address: B. Brajer, Department of Pulmonary Diseases, Poznan University of Medical Sciences, Szamarzewskiego 84 St., 60-569 Poznań, Poland; phone/fax: +48 61 8427061; e-mail: bebab@wp.pl