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Prolidase Activity Dysregulation and its Correlation with Oxidative–Antioxidative Status in Chronic Obstructive Pulmonary Disease

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Background: Chronic obstructive pulmonary disease (COPD) is a consequence of an underlying chronic inflammatory disorder of the airways that is usually progressive and causes dysregulation in the metabolism of collagen. Prolidase has an important role in the recycling of proline for collagen synthesis and cell growth. *Objective*: We measured and compared prolidase activity in healthy individuals with COPD patients to find out that whether its activity might reflect disturbances of collagen metabolism in the patients. We also investigated oxidative–antioxidative status and its relationship with prolidase activity in this disease. *Methods*: Thirty voluntary patients with COPD and 30 healthy control subjects with similar age range and sex were included into the study. Plasma prolidase activities, total antioxidant capacity (TAC) and lipid peroxidation (LPO) levels were measured in the patient and control groups. *Results*: Plasma prolidase activity and TAC levels were significantly lower, and LPO levels were significantly higher in the patients than those in the control subjects ($P<0.05$, $P<0.001$, and $P<0.001$, respectively). Significant correlations were detected between plasma prolidase activity and TAC and LPO levels in the patients group ($r=0.679$, $P<0.001$; $r=-426$, $P<0.05$, respectively). *Conclusions*: The results suggest that oxidative–antioxidative balance and collagen turnover are altered by the development of COPD in human lungs, and prolidase activity may reflect disturbances of collagen metabolism in this pulmonary disease. Monitoring of plasma prolidase activity and oxidative–antioxidative balance may be useful in evaluating fibrotic processes and oxidative damage in the chronic inflammatory lung disease in human. J. Clin. Lab. Anal. 25:8–13, 2011. © 2011 Wiley-Liss, Inc.

Key words: COPD; lipid peroxidation; oxidative stress; prolidase; total antioxidant capacity

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive condition characterized by poorly reversible airflow limitation that is associated with an abnormal inflammatory response of the lung. Persons with COPD have difficulty in breathing because they develop smaller air passageways and have partially destroyed alveoli that are not fully reversible. It is most often due to tobacco smoking but can be due to other airborne irritants such as coal dust, asbestos, or solvents. Microscopically, there is infiltration of the airway walls with inflammatory cells, particularly neutrophils. Inflammation is followed by scarring and remodelling that thickens the walls, resulting in narrowing of the

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small airway. Further progression leads to metaplasia (abnormal change in the tissue) and fibrosis (further thickening and scarring) of the lower airway. One of the consequences of this kind of inflammatory diseases is dysregulation in the metabolism of collagen and its interaction with cell surface integrin receptors. Although extracellular metalloproteinases initiate the breakdown of collagen in tissues, the final step of its degradation is mediated by prolidase (1–4).

Prolidase (E.C. 3.4.13.9) is a cytosolic Mn(II)-activated metalloproteinase that specifically hydrolyzes imidodipeptides and imidotripeptides with C-terminal proline or hydroxyproline, releases these two amino acids for collagen re-synthesis and cell growth. This enzyme has two forms such as prolidase I (M_W: 105,000) and prolidase II (M_W: 151,000) but only prolidase I has been found in human plasma (2,3). Prolidase deficiency is a rare autosomal recessive disease characterized by chronic ulcerative dermatitis, splenomegaly, mental retardation, frequent infections, and massive urinary excretion of iminodipeptides (4,5). Since 1968, more than 50 cases of prolidase deficiency have been described (6,7). The reasons for this deficiency still remain a mystery as sited by Kurien et al. (8). It is thought that an abnormal response in the chemotactic arm of the immune system may be responsible for the high incidence of infection seen in prolidase deficiency. However, it is unclear whether this is a consequence of another problem, such as interference with the filtering function of the spleen, or whether this is a primary abnormality of the immune system. Splenomegaly is very common in patients with prolidase deficiency.

The enzyme apparently contributes to the conservation of iminoacids from endogenous and exogenous protein sources, mainly collagen II and its activity determines the rate of collagen turnover. Its activity is generally determined by photometric methods based on the measurement of proline levels produced by prolidase. However, thin-layer chromatography, paper electrophoresis, amino acid analyzer, spectrometry, isotachophoresis, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, mass spectrometry, fluorometry, high-voltage electrophoresis, and capillary electrophoresis all have been used for the assessment of prolidase activity as cited by Kurien et al. (8). The plasma prolidase activities have been found elevated in conditions that are characterized by chronic inflammation of the tissue and/or by the accumulation or increased turnover of collagen. In several studies focused on many different diseases such as chronic uremia (9,10), chronic liver disease (11–13), type 2 diabetes mellitus (14), cardiac hypertrophy (15), and osteoarthritis (16) prolidase activity have been evaluated. However, to the best of our knowledge, there are no studies in the medical literature focused on prolidase activity in COPD. Our goal, therefore, in this study was to determine plasma prolidase activity in COPD patients and compare them to healthy individuals and to find out whether its activity may reflect disturbances of collagen metabolism in this pulmonary disease. We also evaluated oxidative–antioxidative status and its relationship with prolidase activity in these patients, and tried to sort out whether there is a correlation between them.

MATERIALS AND METHODS

Subjects

The study was conducted on 30 patients with COPD who applied to the outpatients department of the Chest Diseases Clinic, Harran University-Research Hospital, and on 30 healthy volunteers. The male-to-female ratio, age, number of years smoking in pack years, and number of cigarettes per day were similar between two groups. Smokers had a minimum 10 pack-year smoking history for 20–30 years in both groups. All patients' routine hematological and biochemical parameters were determined. COPD was diagnosed on the basis of history, physical examination, and spirometric data, i.e. forced expiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC) (expressed as percent predicted for both) according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (17). None of the patients had any illness but moderate–severe COPD. All the subjects were fully informed about the study and asked for their consent.

Ethical Issues

This work was approved by the Institutional Review Board and performed according to the National and International regulations under the supervision of a bioethics consultant.

Samples

Fasting blood samples were drawn into heparinized-tubes and centrifuged at 3,000 rpm for 10 min to separate the plasma. The samples were stored at −80°C until analysis.

Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Company (Steinheim, Germany) unless otherwise stated.

Determination of Prolidase Activity

Prolidase activity was determined by a photometric method based on the measurement of proline levels

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produced by prolidase (18). Plasma samples (100 μl) were mixed with 100 μl of serum physiological. A total of 25 μl of the mixture was preincubated with 75 μl of the preincubation solution (50 mmol/l Tris HCl buffer pH 7.0 containing 1 mmol/l GSH, 50 mmol/l $MnCl_2$) at 37°C for 30 min. The reaction mixture containing 144 mmol/l gly-pro, pH 7.8 (100 μl) was incubated with 100 μl of preincubated sample at 37°C for 5 min. To stop the incubation reaction, 1 ml glacial acetic acid was added. After adding 300 μl Tris HCl buffer, pH 7.8 and 1 ml of ninhydrin solution (3 g/dl ninhydrin was melted in 0.5 mol/l orthophosphoric acid) the mixture was incubated at 90°C for 20 min and cooled with ice and subsequently its absorbance was measured at a wavelength of 515 nm for determining proline level as proposed by Myara (11–13). This method is a modification of Chinard's method (14). Intra- and interassay CVs of the assay were lower than 10%.

Determination of Lipid Peroxidation

Plasma lipid peroxidation (LPO) was evaluated by the fluorimetric method based on the reaction between MDA and thiobarbutiric acid (TBA) (19). Briefly, 50 μl of plasma were added to 1 ml of 10 mmol/l diethylthiobarbutiric acid (DETBA) reagent in phosphate buffer (0.1 mol/l, pH 3). The mixture was mixed for 5 sec and incubated for 60 min at 95°C. Samples were placed in ice for 5 min and then added 5 ml of butanol. The mixture was shaken for 1 min to extract the DETBA-MDA adduct then centrifuged at $1{,}500 \times g$ for 10 min at 4°C. Fluorescence of the butanol extract was measured at excitation wavelength of 539 and emission wavelength of 553. 1,1,3,3-Tetraethoxypropane was used as a standard solution and the values were presented as μmol/l.

Determination of the Total Antioxidant Capacity

Plasma total antioxidant capacity (TAC) levels were determined by using a novel automated measurement method, developed by Erel (20). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in the Reagent 1, is mixed with hydrogen peroxide, which is present in the Reagent 2. The reaction sequentially produces potent radicals such as brown colored dianisidinyl radical cation. According to this method, the antioxidative capacity of the sample is measured toward the known free radicals solution. The assay has excellent precision values lower than 3%. The results are expressed as mmol Trolox Equiv./l.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc., Chicago, IL) was used for all statistical analyses. Parametric statistical methods were used to analyze the data. The student's *t*-tests were used for pairwise comparisons. Correlations were examined using Pearson rank correlation coefficients (r) and values for corrected for ties. The 2-tailed significance values were used. A P value of 0.05 or less was considered to be significant.

RESULTS

The social and demographic data (age, sex, etc.) of the patients and their controls showed homogeneity, and there were no significant differences between the groups ($P > 0.05$). The general characteristics, FEV1 (% predicted), and FEV1/FVC (%) values of the patients are shown in Table 1.

In this study, we found that there were significant differences between COPD patients and healthy controls with respect to prolidase activity. Plasma prolidase activity levels were significantly lower in the patients with COPD than those of the control subjects ($P < 0.05$). Plasma TAC levels were also significantly lower in the patients with COPD than those of the control subjects ($P < 0.001$). However, plasma LPO levels were significantly higher in the patients with COPD than those of the control subjects ($P < 0.001$). The results are summarized in Table 2.

Prolidase activity was negatively correlated with LPO levels ($P < 0.05$, $r = -0.426$), while positively correlated with TAC levels ($P < 0.001$, $r = 0.679$). There was also a significant negative correlation between TAC and LPO

TABLE 1. Demographic Characteristics of the Patients with COPD and Controls

Parameters	COPD	Control	*P*
Case	30	30	>0.05
Age (years)	59.29±7.2	56.76±8.1	>0.05
Female gender	8/30	7/30	>0.05
Smokers	26/30	24/30	>0.05
FEV1, % predicted	42.90±17.4	87.65±12.1	<0.001
FEV1/FVC(%)	61.30±11.9	91.50±9.2	<0.001

Values are expressed as mean±SD.

TABLE 2. Prolidase Activity, TAC, and LPO Levels of the Patients with COPD and Controls

	COPD ($n = 30$)	Control ($n = 30$)	*P*
Prolidase (U/l)	30.8±4.9	39.5±9.1	<0.05
TAC (mmol Trolox Eq./l)	1.58±0.11	1.68±0.19	<0.001
LPO (μmol/l)	72±11	35±7	<0.001

Values are expressed as mean±SD; TAC, total antioxidant capacity; LPO, lipid peroxidation.

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TABLE 3. Correlations of Prolidase Activity with TAC and LPO Levels, and TAC Levels with LPO Levels in Patients' Group

		TAC	LPO
Prolidase	$r =$	0.679	−0.426
	$P <$	0.001	0.05
TAC	$r =$		−0.534
	$P <$		0.01

levels ($P < 0.01$, $r = -0.534$). The results are summarized in Table 3.

DISCUSSION

COPD is a slowly progressive disease of the airways that is characterized by a gradual loss of lung function. In general, the term COPD includes chronic bronchitis, chronic obstructive bronchitis, or emphysema, or combinations of these conditions (1). In chronic diseases such as COPD, the active inflammatory response is induced with neutrophilic infiltration. These neutrophils, macrophages, and/or monocytes produce oxygen-free radicals that can cause lipid–protein oxidation and also oxidative DNA damage to the adjacent cells. The DNA damage provoked by reactive oxygen species (ROS) can have very harmful consequences, leading to gene modifications that are potentially carcinogenic. COPD has been associated with generation of ROS and increased nitric oxide levels, which lead to oxidative stress in the lung. Increased oxidative stress has an important role in the pathogenesis of COPD (21–27). Normally, the level of oxidative stress is regulated by endogenous antioxidant systems including enzymatic antioxidants such as superoxide dismutase, which degrades superoxide anion ($O_2^{\cdot -}$), and catalase and glutathione peroxidase (GSH-Px), both of which detoxify hydrogen peroxide (H_2O_2) and nonenzymatic antioxidants such as the GSH (glutathione) redox system, mucin, bilirubin, ceruloplasmin, transferrin, and albumin (25,26). There are also some exogenous antioxidants such as vitamins A (β-carotene), C (ascorbic acid), and E (α-tocopherol), which protect against ROS-mediated cellular damage through their free radical scavenging properties (25,27). In particular, vitamin C is the major antioxidant present in the airway surface liquid of the lung, and it may protect against endogenous agents as well as against exogenous agents such as aeroallergens, cigarette smoke, and environmental air pollutants (28–30).

Since several oxidants and antioxidants are likely to be involved in the pathogenesis of the inflammatory process in COPD, we tried to investigate TAC to see how far antioxidative status is affected by this condition. The alteration in antioxidant defense might be an increase or a decrease depending on whether the changes are due to a defense response (increase) or neutralization by oxidants (decrease), whereas if the reserves are sufficient, there might be no change. We found that TAC levels are decreased in the patients with COPD. It is probable that there is an oxidative injury that consumes antioxidants. We detected very high LPO in these patients, which confirm the presence of oxidative stress. In healthy conditions, when ROS production is low, LPO is inhibited by the combined activities of various antioxidants present in the plasma. However, in the event of excessive ROS production, as hypothesized in our study by the increase in LPO, this protection may be inadequate as a defense mechanism of the organism against the ongoing oxidative burden. We also determined a significant negative correlation between LPO and TAC levels in COPD patients. While LPO indicating oxidative stress increased, TAC indicating antioxidative defense decreased. The increased levels of pro-oxidative factors yield in severe oxidative stress can modulate many processes in the pulmonary epithelium (31–34). It has been reported that chronic inflammation causes thickening of the walls, which progress further to fibrosis of the lower airway and consequently dysregulation in the metabolism of collagen. It is also known that prolidase releases carboxy-terminal proline or hydroxyproline from oligopeptides and has a main role in the collagen metabolism (2,3,11,12).

As it is reported in the literature by Black et al. (35) small airways are the major site of airflow obstruction in COPD, which is attributed to loss of elastin in alveoli and fibrosis in small airways. Previously we showed significantly decreased prolidase levels in bronchial asthmatic children by using a modified version of Chinard's method as we stated in the "Material and methods" section (36). Confirming this data, in this study, we demonstrated that there was a significant lower plasma prolidase activity in the patients with COPD compared to the healthy controls, which may be interpreted as an evidence for decreased collagen recycling. Although we used an optimized version of the modified Chinard's method, by which the protein-precipitating step of modified Chinard's method (Myara method) has been eliminated and we expected to detect higher prolidase activities, we found lower enzyme activity in the patients than in the controls. Also, we found significant correlations between prolidase activity and TAC and LPO levels. To the best of our knowledge, this is the first study investigating prolidase activity and its relationship with oxidative–antioxidative status in the patients with COPD. Therefore, the cause of decreased prolidase activity is not known. There are conflicting data about prolidase activity in various diseases.

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Although some suggested that prolidase activity decreased in several conditions such as chronic uremia (9,10) and type 2 diabetes mellitus (14), however, in chronic liver diseases increased prolidase activity has been reported (13). Considering our data here, it may be possible to postulate that chronic inflammation in COPD causes tissue and cellular injury including damaged protein turnover such as collagen and/or oxidatively stressful events occurring in COPD, which may damage protein turnover at the cellular level including collagen re-synthesis.

In the light of these data, it is possible to conclude that COPD creates oxidative stress in the lung, which causes oxidative tissue and cellular damage. Decreased prolidase activity and its correlations with TAC and LPO suggested that this injury occurs not only at the cellular level but also at the protein level. It can also be concluded from the study that, plasma prolidase activity may reflect disturbances in tissue collagen metabolism and turnover in lung and it may therefore serve as a marker of the disease particularly for the progression of fibrotic process in the airways. Further studies, however, should be undertaken in order to clarify the ethiopathogenetic mechanisms underlying the observed alterations and to compare them between the moderate and severe and very severe patient population, thereby to find out that whether these changes reflect the progression of the disease.

REFERENCES

1. Braido F, Riccio AM, Guerra L, et al. Clara cell 16 protein in COPD sputum: A marker of small airways damage? Respir Med 2007;101:2119–2124.
2. Oono T, Yasutomi H, Ohhashi T, et al. Characterization of fibroblast-derived prolidase. The presence of two forms of prolidase. J Dermatol Sci 1990;1:319–324.
3. Cosson C, Myara I, Miech G, et al. Only prolidase I activity is present in human plasma. Int J Biochem 1992;24:427–432.
4. Powell GF, Rasco MA, Maniscalco RM. A prolidase deficiency in man with iminopeptiduria. Metabolism 1974;23:505–513.
5. Kodama H, Umemura S, Shimomura M, et al. Studies on a patient with iminopeptiduria. I. Identification of urinary iminopeptides. Physiol Chem Phys 1976;8:463–473.
6. Bissonnette R, Friedmann D, Giroux JM, et al. Prolidase deficiency: A multisystemic hereditary disorder. J Am Acad Dermatol 1993;29:818–821.
7. Lopes I, Marques L, Neves E, et al. Prolidase deficiency with hyperimmunoglobulin E: a case report. Pediatr Allergy Immunol 2002;13:140–142.
8. Kurien BT, Patel NC, Porter AC, et al. Prolidase deficiency and the biochemical assays used in its diagnosis. Anal Biochem 2006;349:165–175.
9. Gejyo F, Kishore BK, Arakawa M. Prolidase and prolinase activities in the erythrocytes of patients with chronic uremia. Nephron 1983;35:58–61.
10. Evrenkaya TR, Atasoyu EM, Kara M, et al. The role of prolidase activity in the diagnosis of uremic bone disease. Ren Fail 2006;28:271–274.
11. Myara I, Marcon P, Lemonnier A, et al. Determination of prolinase activity in plasma. Application to liver disease and its relation with prolidase activity. Clin Biochem 1985;18:220–223.
12. Myara I, Cosson C, Moatti N, Lemonnier A. Human kidney prolidase—Purification, preincubation properties and immunological reactivity. Int J Biochem 1994;26:207–214.
13. Brosset B, Myara I, Fabre M, Lemonnier A. Plasma prolidase and prolinase activity in alcoholic liver disease. Clin Chim Acta 1988;175:291–295.
14. Erbagci AB, Araz M, Erbagci A, et al. Serum prolidase activity as a marker of osteoporosis in type 2 diabetes mellitus. Clin Chem 2002;35:263–268.
15. Demirbag R, Yildiz A, Gur M, et al. Serum prolidase activity in patients with hypertension and its relation with left ventricular hypertrophy. Clin Biochem 2007;40:1020–1025.
16. Altindag O, Erel O, Aksoy N, et al. Karaoglanoglu M: Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatol Int 2007;27:339–344.
17. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD). Available from: http://www.goldcopd.org, 2006.
18. Ozcan O, Gultepe M, Ipcioglu OM, et al. Optimization of the photometric enzyme activity assay for evaluating real activity of prolidase. Turk J Biochem 2007;32:12–16.
19. Jain SK, Levine SN, Duett J, Hollier B. Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. Metabolism 1990;39:971–975.
20. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112–119.
21. Ceylan E, Aksoy N, Gencer M, et al. Evaluation of oxidative-antioxidative status and the L-arginine-nitric oxide pathway in asthmatic patients. Respir Med 2005;99:871–876.
22. Ceylan E, Kocyigit A, Gencer M, et al. Increased DNA damage in patients with chronic obstructive pulmonary disease who had once smoked or been exposed to biomass. Respir Med 2006;100:1270–1276.
23. Vibhuti A, Arif E, Deepak D, et al. Correlation of oxidative status with BMI and lung function in COPD. Clin Biochem 2007;40:958–963.
24. Ceylan E, Gencer M, Uzer E, Celik H. Measurement of the total antioxidant potential in chronic obstructive pulmonary diseases with a novel automated method. Saudi Med J 2007; 28:1339–1343.
25. Vural H, Aksoy N, Ceylan E, et al. Leukocyte oxidant and antioxidant status in asthmatic patients. Arch Med Res 2005;36:502–506.
26. Picado C, Deulofen R, Lleonart R, et al. Dietary micronutrientsand their relationship with bronchial asthma severity. Allergy 2001;56:43–49.
27. Aksoy N, Vural H, Sabuncu T, Aksoy S. Effects of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. Cell Biochem Funct 2003;21:121–125.
28. Delman CJ, Bast A. Oxygen radicals in lung pathology. Free Radic Biol Med 1990;9:381–400.
29. Cluzel M, Damon M, Chanez P, et al. Enhanced alveolar cell luminol-dependent acemiluminescence in asthma. J Allergy Clin Immunol 1987;80:195–201.
30. Hatch GE. Asthma, inhaled oxidants, and dietary antioxidants. Am J Clin Nutr 1995;61:625–630.
31. Gencer M, Ceylan E, Aksoy N, Uzun K. Association of serum reactive oxygen metabolite levels with different histopathological types of lung cancer. Respiration 2006;73:520–524.
32. Yang P, He XQ, Peng L, et al. The role of oxidative stres in hormesis induced by sodium arsenite in human embryo lung fibroblast (HELF) cellular proliferation model. J Toxỳcol Env Health 2007;70:976–983.

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33. Confalonieri M, Chetta A. Oxidative stress during exercise: Further proof that being lean is detrimental for chronic obstructive pulmonary disease patients. Respiration 2006;73:757–761.
34. Aytacoglu BN, Calikoglu M, Tamer L, et al. Alcohol-induced lung damage and increased oxidative stress. Respiration 2006;73: 100–104.
35. Black PN, Ching PS, Beaumont B, et al. Changes in elastic fibres in the small airways and alveoli in COPD. Eur Respir J 2008;31:913–914.
36. Cakmak A, Zeyrek D, Atas A, Celik H, Aksoy N, Erel O. Serum prolidase activity and oxidative status in patients with bronchial asthma. J Clin Lab Anal 2009;23:132–138.

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