Evaluation of the protease-antiprotease balance disorder in smoking and non-smoking patients treated for acute chronic obstructive pulmonary disease (COPD) at different clinical stages of the illness

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Abstract

Introduction: *The purpose of this paper is to present the level of protease-antiprotease balance disorder with patients hospitalised for acute COPD depending on the stage of the condition in relation to smokers and non-smokers.*

Materials and Methods: *The research was carried out on 61 patients with COPD. They consisted of 27 women and 34 men; 32 smokers and 30 non-smokers, age 18-82 (average 59.9±12 years). All patients underwent: measurement of concentrations: alfa-1-antiproteinase (α_1-PI) and elastase (EN) (ELISA Immuno Diagnostic) in serum.*

Results: *A negative Spearman correlation between the concentration of elastase and the concentration of 1-antiprotease in the group of patients with severe and serious COPD and in the smokers' group; respectively (r=–0.845 and p=0.001); (r=–0.529 and p=0.014) and (r=–0.506 and p=0.003). Statistically significant difference of elastase concentrations between the severe COPD group (by GOLD) and the remaining groups with p<0.05. Statistically significant difference of EN and α_1-PI concentrations between the groups of smokers and non-smokers; with p<0.0001 and p=0.027.*

Conclusions: *Patients with COPD develop a severe protease-antiprotease balance disorder to the advantage of proteases. The balance disorder increases together with an increase in the level of COPD. Smoking increases the protease-antiprotease balance disorder and constitutes a significant risk factor for the intensification of irreversible obstructive changes within the respiratory system with patients with COPD. Giving up smoking causes a decrease in the adverse changes in the balance and is the most significant factor that improves the prognosis for patients with COPD.*

Key words: *elastase, alfa-1-antiproteinase, COPD, protease-antiprotease balance.*

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Introduction

The era of research into protease-antiprotease balance disorder in the pathophysiology of lung disease goes back to 1963, when Laurell and Erickson described the set of symptoms related to inborn deficiency of alfa-1-antitripsine as the main cause of emphysema [1]. Besides emphysema this disorder is a recognized element of diseases like bronchiectasis, ARDS, cystic fibrosis and finally COPD [2].

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Various internal and external factors cause the imbalance, change the structure of the lung tissue and accelerate the progression of lung diseases [3].

The protease with the most significant function is elastase of proteolitic neutrophil granulocytes (NE) and among antiproteases it is alfa-1-antiproteinase (α_1-PI), former name alpha-1-antitrypsin.

Elastase (molecular weight 33 kDA) belongs to the proteolitic enzymes from the serine proteases group. It is synthetized in bone marrow and stored in neutrophilic granulocytes [4] and also in macrophages, monocytes and fibroblasts. It hydrolyzes proteins of connective tissue (e.g. collagen, elastin, fibronectin and proteoglycanes). The main group of diseases with pathogenesis characterized by increased elastase activity are those diseases in which the leading feature of the pathogenesis is an inflammatory condition. One such disease is undoubtedly COPD. The activity of elastase is significantly increased by nicotine. Nicotine passed into bone marrow induces the expression of the elastase gene, causing the increase of elastase activity in cells [8]. Chemotactic factors released in the inflammatory reaction lead to degranulation, which causes the release of elastase into the surroundings, where it is neutralized by inhibitors in blood and in body fluids, remaining with them in a dynamic balance.

The natural defence of tissues against the activity of elastase is its neutralization and consequently a reduction of elastase influence on lung tissue and reduced production of chemotactic factors. The main inhibitor of elastase is alfa-1-antiproteinase, former name alpha-1-antitrypsin [5]. It is synthesized by neutrophiles and monocytes. It inactivates elastase through an irreversible bond with methionine [6]. A reduced activity of α_1-PI may be caused by various factors eg. innate deficiency of α_1-PI (it is the most common disorder in the Caucasian race), factors that increase its synthesis or excessive anti-proteolitic activity. A decrease of α_1-PI is also observed as a result of many factors: endogenic (superoxide radicals) and egzogenic (bacteria toxins, tobacco smoke and environment pollution). The mechanism of tobacco smoke activity is based on the oxidation of the methionine active centre and, indirectly, on the activation of phagocytes to produce antioxidants. Therefore the activity of tobacco smoke is the most significant factor leading to emphysema with people with correct α_1-PI activity.

The purpose of this paper is to present the level of protease-antiprotease balance disorder with patients hospitalized for acute COPD depending on the stage of the condition in relation to smokers and non-smokers.

Materials and Methods

The research was carried out on 61 patients with COPD hospitalized in the Department of Allergology and Internal Diseases in Bydgoszcz due to aggravation of the disease. They consisted of 27 women and 34 men; 32 smokers and 30 non--smokers who had stopped smoking at least one year before; age 18-82 (average 59.9±12 years). Tests were performed in the morning, in a fasting state; none of the patients had taken any bronchodilative drugs for at least 12 hours before the test. COPD was diagnosed on the basis of an examination of the detailed medical history of each patient, clinical symptoms, spirometric tests; test in rest and a diastolic test after the application of short acting beta 2-sympathomimetic. The characteristic feature was no increase of FEV_1 or its increase after beta 2-sympathomimetic of below 12% when compared to appropriate values.

All patients underwent:

1. Spirometric test with Lung Test 1000 according to previously stated assumptions.
2. Measurement of concentrations: alfa-1-antiproteinase (α_1-PI) with the ELISA technique by the firm ImmunoDiagnostic (the test is based on the bondage of α_1-PI with Polyclonal Rabbit Antibodies on the surface of thrombocytes, after which sheep anti-α_1-PI antibodies linked to peroxydase are added to the newly formed complex and then follows induction with its substrate tetramethylbenzidine (TMB). Due to the chemical reaction in a test- tube the solution changed its color from blue to yellow and the intensity of the color was directly proportional to the concentration of α_1-PI. The concentrations were then calculated from appropriate calibration curves for absorption with a 450 nm wave length.
3. Elastase (EN) test with the ELISA technique by the firm ImmunoDiagnostic (the procedure of the test was identical with the one for α_1-PI concentration).

The statistical analyses of the data were performed by Statistica 5.0 software package. Averages and standard deviations were calculated. The significance of differences was shown by U Mann-Whitney test. The correlation analysis was performed through the Spearman's nonparametric correlation test.

Results

On the bases of the spirometric test and in compliance with the GOLD 2003 report, the patients were divided into 6 groups (table 1):

1. Severe COPD: (FEV_1=26.8±4.3% independent value) 11 patients (4 F and 7 M aged 57.8±8.74);
2. Serious COPD: (FEV_1=42.29±4.86% independent value) 21 patients (9 F and 12 M aged 61.9±16.3);
3. Medium COPD: (FEV_1=64.86±6.1% independent value) 24 patients (12 F and 12 M aged 58.8±11.4);
4. Light COPD: (FEV_1=77.5±7.0% independent value) 6 patients (3 F and 3 M aged 610.2±7.33);
5. Smokers with COPD: 32 patients (14 F and 18 M aged 60.0±14.4);

Table 1. Average values of anti-protease and elastase concentrations were as follows

Mean ±SD	Antiprotease [mg/dl]	Elastase [ng/ml]
total	168.4±120.2	1098.4±465.2
group 1	137.2±79.4	1393.2±335.4
group 2	152.1±109.4	1055.2±514.3
group 3	191.6±143.5	1022.7±456.3
group 4	151.5±91.6	1002.9±335.8
smokers' group	123.1±48.5	1382.4±362.9
non-smokers' group	213.2±362.9	790.7±344.7

6. Non-smokers with COPD: 30 patients (13 F and 17 M aged 59.57±10.77).

Outcome

1. A negative Spearman correlation between the concentration of elastase and the concentration of 1-antiprotease in the group of patients with severe and serious COPD and in the smokers' group; respectively (r=–0.845 and p=0,001); (r=–0.529 and p=0.014) and (r=–0.506 and p=0.003).
2. Statistically significant difference of elastase concentrations between the severe COPD group (by GOLD) and the remaining groups with $p<0.05$.
3. Statistically significant difference of EN and α_1-PI concentrations between the groups of smokers and non-smokers; with $p<0.0001$ and $p=0.027$.

Discussion

The research measured the concentration of elastase (NE) and alfa-1-antiproteinase (α_1-PI) in the blood serum of smoking and non-smoking patients hospitalized due to exacerbation of COPD, depending on the level of obturation of the bronchial tree. It was proven that in these cases there was a significant disorder of this balance to the advantage of protease (Elastase) and that the level of this disorder increases with the level of severity of COPD – the research showed a significant statistical difference of elastase concentration between the group with severe COPD (according to GOLD) and the remaining groups of $p<0.05$; the statistical significance of this difference increased while comparing groups with greater differences in the level of obturation of the bronchial tree. A very high (correlation coefficient =–0.845) negative Spearman correlation was observed between the concentration of elastase and the concentration of 1-antiproteinase in the group of patients with severe COPD and a slightly smaller one (correlation coefficient =–0.529) in the group of patients with serious COPD.

A separate issue is the disorder in the balance of NE and α_1-PI with smoking and non-smoking patients with COPD. A statistically significant difference in the concentration of NE and α_1-PI was observed between smoking and non-smoking patients; respectively with $p<0.0001$ and $p=0.027$ and a negative Spearman correlation between the concentration of NE and the concentration of α_1-PI in the group of smokers; respectively (r=–0.506 and p=0.003). According to numerous scientific papers the protease-anti-protease balance plays a fundamental role in the pathogenesis of obstructive pulmonary diseases. As is commonly known, COPD is characterized by chronic inflammation process, in which the most significant role is played by neutrophilic granulocytes and macrophages [9-11]. Their granules contain a large amount of NE, which released in the inflammatory reaction leads to many structural changes in the respiratory system [3]. Suzuki et al proved that the application of NE spray to guinea pigs caused damage to the epithelium in their respiratory systems within 20 minutes [12]. During in vitro tests, NE reduced the frequency of the movement of cilia in the respiratory system [13], which then reduced the cilia clearance, which is the characteristic feature of COPD. Application of NE to the respiratory system of a hamster led to hyperplasia of its salivary glands [15]. According to some researchers, NE is a much more sensitive marker of the inflammation process than acute phase proteins [4, 12] and the estimation of NE level in the serum has not only a diagnostic value but prognostic value as well. With healthy people NE remains in a dynamic balance with α_1-PI [17]. It (α_1-PI) inactivates NE through irreversible binding of its active centre with methionine [6]. The reduced activity of α_1-PI may be caused by its innate deficiency [21] or by external factors, among which tobacco smoke is the most significant. It acts multi-directionally. On one hand it inactivates α_1-PI through the oxidation of methionine at the active centre and indirectly it activates phagocytes to produce oxidants (this way it is the main factor that leads to emphysema with people with correct activity of α_1-PI) [8, 17]. On the other hand, through its absorption to the bone marrow it induces the expression of NE gene and leads to a higher activity of NE in cells [8]. With the appearance of the inflammation process NE is released to the extracellural space where it is inactivated by α_1-PI in milliseconds. The increase of NE is proportional to the intensity of the inflammation process. In a massive inflammatory condition the release of NE is so high that the function of the defense mechanisms may not be sufficient. Moreover, α_1-PI is very sensitive to the activity of oxidants and proteolytic enzymes [17] due to its easily accessible active centre that contains aminoacid – methionine which is sensitive to oxidants [19]. In pathological conditions the activity of oxidants and proteolytic enzymes can be so high that the duration time of elastase extracellural activity can be extended 2000 times,

which enables the start of tissue destruction. This very moment (defined as protease-antiprotease balance disorder) is a key moment for the initiation of an uncontrolled destruction of tissues by NE and for the formation of permanent damage to various organs [20], including the occurrence of COPD.

Conclusions

1. Patients with COPD develop a severe protease- anti-protease balance disorder to the advantage of proteases.
2. The balance disorder increases together with an increase in the level of COPD.
3. Smoking increases the protease- anti-protease balance disorder and constitutes a significant risk factor for the intensification of irreversible obstructive changes within the respiratory system with patients with COPD.
4. Giving up smoking causes a decrease in the adverse changes in the balance and is the most significant factor that improves the prognosis for patients with COPD.

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