14.00.00 Medical science

14.00.00 Медицинские науки

UDC 616.24:577.121.7

The Protein Carbonyl Derivatives in Blood of Patients With Chronic Obstructive Pulmonary Disease

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ABSTRACT. The protein carbonyl derivatives in plasma and erythrocyte of patients with chronic obstructive pulmonary disease (COPD) were studied. There were 2 groups: 16 patients with COPD and 17 healthy persons as control subjects. There were statistically significant decreasing of protein carbonyl derivatives in plasma and erythrocytes of patients with COPD. For explaining the decline of carbonyl derivatives in blood of COPD patients the hypothesis was put forward.

Keywords: chronic obstructive pulmonary disease; protein carbonyl; plasma; erythrocytes.

INTRODUCTION. Chronic obstructive pulmonary disease (COPD) is a heterogenous syndrome characterised by irreversible progressive airflow limitation. Risk factors of COPD include a1-antitrypsin deficiency, smoking, air pollution, social-economic status and lower birth weight. COPD is obviously disease of imbalance of proteins – oxi/antioxidant, protease/antiprotease, apoptosis/proliferation, acetylases/deacetylases, that can no longer perform their proper function to keep the homeostasis in the new environmental settings of the oxidative stress [1].

The major target for reactive oxygen species (ROS) are proteins. Proteins can scavenge the majority (50–75 %) of ROS. Oxidative damage of proteins is induced either directly by ROS or indirectly by reaction of secondary by-products of oxidative stress and can occur via different mechanisms, leading to peptide backbone cleavage, cross-linking, and/or modification of the side chain of amino acids [2]. Oxidative damage of proteins has wide range of metabolic impairment and downstream functional consequences. The purpose of our research was designed to define the content of carbonyl content in blood plasma and erythrocyte proteins in patients with COPD.

MATERIALS AND METHODS. There were 3 groups of persons who taking part in our investigation: 1 group included 12 patients with COPD of bronchial type, 2 group – 13 patients with mixed type of COPD and 3 group – 17 healthy persons as control subjects. Control subjects were healthy volunteers without any medication. All patients were on hospitalization and inspection. The syndrome of bronchial obstruction has been revealed at 100 percent of patients at receipt in a hospital. Respiratory insufficiency (RI) was established in reason of a syndrome of a short wind in a rest condition and at the insignificant physical loading representing walking on 100 meters by slow rate (speed of movement is not higher than 5 steps in one minute). RI of I degrees is diagnosed at 25 % of

patients and RI of II degrees - at 75 % of patients. Verification of the diagnosis was carried out on the basis of the complex of the standard criteria. At 100 percent of patients the habit to smoking tobacco is revealed. The index of smoking person has made > 200 at 67 % of patients of 1-st group and at 73 % of the patients including in 2-nd group. At 45 % of patients are marked professional harm (the experience of underground work on collieries over 10 years, work on woodworking enterprises, cement works), atmospheric pollution (inhabitants of industrial cities Temirtau, Karaganda, Balkhash have made 79 % surveyed). Basic clinical displays of COPD were cough with sputum and a short wind. Cough was marked during all day, less often only at night. The quantity of sputum was small, outside of aggravations its character was mucous. At 54 % of patients elimination of sputum was occuring after long cough. All patients carried out cytologic research of sputum which found out presence of alveolar epithelial cells, elastic fibres, siderophages. A plenty of the leukocytes submitted basically neutrophiles was found out in 54 % of patients of 1 group and 61 % of patients of 2 groups during an aggravation. It was interpretated as the evidence of pyo-inflammatory process in mucous of bronchial tree.

At research of blood (the general analysis) at 67 % of patients with COPD of bronchical type in a stage of an aggravation it was observed neutriphil leucocytosis with band-neutrophile shift and increase of erythrocyte sedimentation (ES) in limits from 15 up to 39 mm /hour. At mixed type of COPD in a stage of aggravation the erythrocyte sedimentation raised from 14 till 27 mm/hour. At stable current of COPD considerable changes of the maintenance of leukocytes in peripheral blood was not marked.

At patients with RI II degrees with development of hypoxemia were observed: polycytemia syndrome with increasing of hematocrite > 45% at females and > 50% at males, increase of number of erythrocytes (from 5,5 up to $6,3x10^{12}$/L), a high level of hemoglobin (from 175 up to 190 g/L), low ES (within the limits of 1-2 mm / hour).

Electrocardiography has revealed at 30 % of patients signs of hypertrophy of the right chambers of heart. Function of external breath was investigated. The volume of the forced exhalation in the first second (FEV_1) has been reduced at 100 percent of patients. The pharmacological test carried out for an estimation of convertibility of obstructive infringements of ventilation. Reference value of FEV_1 compared to the same parameter through 30-45 minutes after inhalation of sympatomimetic drug (400 mkg) or holinolitic one (80 mkg), or combinations of broncholitics with the different mechanisms of effect. Gain of FEV_1 made less than 10 % that was regarded as irreversibility of bronchial obstruction. Peak-flowmetry was carried out for same purpose. The gain of peak flow of an exhalation less than on 10 % was marked at patients of 1-st group and less than on 15 % at patients of 2-nd group.

The medical ethics committee of the Medical University (Karaganda) approved the study. All patients and healthy subjects have received the full information on probable inconveniences and complications at the blood sampling before giving their consent to participate.

Blood collected from the brachial vein (3 ml/sample) was drawn into vacutainer tubes containing heparin. The count of erythrocytes was detected by using Mindray BC-3200 Hematology Analyzer. The plasma and erythrocytes obtained following the protocol of C.L. Hawkins et al [3]. The plasma and erythrocyte used within 1-2 hours of collection. We measured the content of protein carbonyl derivates following the protocol of Levine R., et al. [4].

Triplicate aliquots of plasma or hemolysates (0.8 mL) were added with 0.2 mL of 10 % trichloroacetic acid. The samples were centrifuged and 1 mL of either 2M HCl or 10 mM 2.4-dinitrophenylhydrazine (DNPH) in 2M HCl were added to the precipitates and incubated at 37oC for 90 min. After the samples were centrifuged (8,000g, 10 min) and the DNPH excess was removed with ethanol-ethyl acetate 1:1 (v/v). The protein was then dissolved by addition of 6M of guanidine hydrochloride. Quantification was performed using a spectrophotometer PD - 303 UV APEL (Japan) at an absorbance of 370 nm. Concentration of protein carbonyl derivates calculated using the extinction coefficient at 370 nm = 22,000 mol-1cm-1. Carbonyl content values were given in nmol/g hemoglobin and nmol/ml plasma. Comparisons of protein carbonyl derivates content between patients and controls were performed using non-parametric Mann-Whitney U-test (for independent variables).

DISCUSSION

The similar trend of protein carbonyl content was observed in erythrocytes and plasma in patients with COPD. As compared to control ones in erythrocytes of patients there were statistically significant decreasing of carbonyls by 2-2.5 times ($p< 0.05$). There was difference between content of carbonyls in erythrocytes of patients with bronchical type and mixed type of COPD. The degree of carbonyl reduction was more significant ($p< 0.001$) in plasma of patients as compared to control

subjects. There was no difference in carbonyl contents in plasma of patients with bronchial type and mixed type of COPD.

The carbonyl content was lower both in plasma and erythrocytes of patients with COPD. We suppose the arising of aberrant proteins in plasma of patients with severe COPD. It may be connected with the generation of aberrant protein isoforms and with formation of aberrant proteins in circulation. In first case the main reason of production of aberrant proteins may be limitation or defects in the molecular chaperone systems. This hypothesis is confirmed with data demonstrate participation of chaperons in COPD initiation and progression [5].

In second case the main reason of formation of aberrant proteins may be decreasing of carbonylation sites by previous oxidation of proteins. It was shown oxidation products forming from direct ROS oxidation of amine acid side chains in proteins, formation of advanced glycation endproducts, and formation of adducts with lipid peroxidation products were simultaneously recognized and assigned to specific sites in proteins [6,7]. So, individual proteins in human plasma have different amount of carbonylation sites. Aberration of protein may be connected with limiting of carbonylation sites by previous oxidation in circulation at patients with severe COPD.

The rapid carbonylation of mistranslated or otherwise aberrant proteins points to an important physiological role of carbonylation in protein quality control [8]. The decreasing of carbonylation of aberrant proteins prevent damaged proteins enter the degradation pathway. Under severe oxidative stress, the decrease in the proteolytic degradation and accumulation of aberrant proteins may contribute in COPD progression.

In our opinion an albumin is the best candidate for oxidative modification. In plasma albumin displays an important antioxidant activity and susceptible to different oxidative modifications, especially carbonylation. Albumin exists in both reduced and oxidized forms, and the percentage of oxidized albumin increases in several diseases [9]. Oxidation of albumin impairs its function and impact on antioxidant potential.

In erythrocytes of patients with COPD the diminution of proteins with low capability to react with DNPH can be also connected with previous formation of oxidized proteins (mainly hemoglobin). We suppose that oxidation of proteins of erythrocytes of patients with COPD can take place not only in circulation, but during maturation of red cells. The antioxidant function of erythrocytes is scavenging activity towards reactive oxygen and nitrogen species. According to modern hypothesis [10], the oxidatively modified red cell can contribute to the formation of an oxidative microenvironment by providing a pro-oxidant signal to vascular cells. The oxidatively modified red cell increases its aggregability and adhesiveness to the endothelium and to other blood cells, thus contributing to vascular damage. Erythrocytes can also protect towards cigarette smoke–induced oxidation total human plasma proteins and albumin [11]. The oxidatively modified red cells do not perform this function with high efficiency.

There is difference in concentrations of carbonyl products in erythrocytes of patients with bronchical type and mixed type of COPD. We suppose the impact of different degree of oxidative stress.

The further challenge will be to expand analyzing of oxidized proteins (AOPP, glycation endproducts, etc) in blood of patients at different stages and types of COPD and to estimate their impact in progression of COPD.

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УДК 616.24:577.121.7

Карбониловые производные белков в крови больных с хронической обструктивной болезнью легких

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Аннотация. Изучено содержание карбониловых производных белков в плзаме и эритроцитах больных с хронической обструктивной болезнью легких (ХОБЛ). Были сформированы 2 группы: 16 больных с ХОБЛ и 17 практически здоровых людей в качестве контроля. Обнаружено статистически значимое снижение карбониловых производных белков в плазме крови и эритроцитах больных с ХОБЛ. Для объяснения снижения карбониловых производных белков в крови больных с ХОБЛ предложена гипотеза.

Ключевые слова: хроническая обструктивная болезнь легких; карбониловые производные белков; плазма; эритроциты.