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SERUM BIOMARKERS OF EXTRACELLULAR MATRIX METABOLISM AS PREDICTORS OF LUNG FUNCTION DECLINE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ARTERIAL HYPERTENSION

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Abstract

The aim of the study was to evaluate possible correlations between serum glycosaminoglycans: chondroitin-6-sulfates, chondroitin-4-sulfates/dermatan sulfates and heparansulfates/keratan sulfates, matrix metalloprotease-9, serum and aldosterone with deterioration of respiratory function in patients with chronic obstructive pulmonary disease combined with arterial hypertension.

The level of glycosaminoglycans was determined in blood serum of 74 patients with arterial hypertension and chronic obstructive pulmonary disease of II and III stages in accordance with spirometric classification GOLD 2014, and the levels of aldosterone and matrix metalloprotease-9 – by enzyme-linked immunosorbent assay.

Patients with chronic obstructive pulmonary disease combined with arterial hypertension showed significantly higher levels of chondroitin-6-sulfates than arterial hypertension and control groups, while the level of heparansulfates/keratan sulfates appeared to be significantly lower ($p<0.05$). Levels of aldosterone and matrix metalloprotease-9 in blood serum of study groups were significantly higher than the indicators of control group (67.1±3.2 and 12.01±1.3 versus 43.1±1.2 pg/ml and 4.2±0.6 ng/ml, $p<0.05$). Pulmonary disease progression was associated with the increase of chondroitin-6-sulfates and decrease of heparansulfates/keratan sulfates ($p<0.05$). The deterioration of lung function was positively related to serum chondroitin-6-sulfates, aldosterone and matrix metalloprotease-9, and negatively – to heparansulfates/keratan sulfates ($p<0.001$). Matrix metalloprotease-9 level showed a positive correlation with chondroitin-6-sulfates ($r=0.5$; $p=0.000$) and a negative correlation with heparansulfates/keratan sulfates ($r=-0.805$; $p=0.000$), while aldosterone level negatively correlated with heparansulfates/keratan sulfates ($r=-0.558$; $p=0.000$).

Thus, chronic obstructive pulmonary disease is characterized by altered serum glycosaminoglycan content, possibly mediated by aldosterone and matrix metalloprotease-9, and is strongly associated with lung function decline.

Keywords: *extracellular matrix, glycosaminoglycans, matrix metalloprotease-9, aldosterone, chronic obstructive pulmonary disease, arterial hypertension.*

Introduction

Chronic obstructive pulmonary disease and arterial hypertension are major public health epidemy with increasing prevalence. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), patients with this pathology are at higher risk for the development of cardiovascular diseases, which is a result of general inflammatory process [*GOLD, 2014*]. It is known that extracellular matrix of airway walls undergoes significant changes in patients with obstructive disease [*Kranenburg A et al., 2006; Annoni R et al., 2012*]. Thickening of the walls and narrowing of the lumen of respiratory tracts occurs in small bronchi

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of smokers with pulmonary disease, which is the main reason for bronchoobstruction [*Hogg J et al., 2004; Sturton G et al., 2008; McDonough J et al., 2011*]. Extracellular matrix is a complex structured network of secreted macromolecules and proteolytic enzymes that provide the interaction between cells and intercellular substances. Glycosaminoglycans are the main components of extracellular matrix. In humans, seven types of glycosaminoglycans have been identified: chondroitin sulfate A, dermatan sulfate, chondroitin sulfate C, heparin, heparan sulfate, hyaluronic acid and keratan sulfate. In the human lungs, keratan sulfate was found on the apical surface of ciliated epithelial cells, chondroitin sulfate and dermatan sulfate – in epithelial and submucosa gland cells, and heparan sulfate – tracheal tissue sections [*Monzon M et al., 2006*]. However, the role of glycosaminoglycans in the development and progression of pulmonary dysfunction in obstructive pulmonary disease, as well as in its combination with arterial hypertension remains unclear.

It is established, that matrix metalloprotease-9, which takes part in degradation of alveolar wall, is involved in progression of chronic obstructive disease [*Brajer B et al., 2008; Linder R et al., 2013*]. It is considered to play a crucial role in the pathogenesis of tissue damage and fibrosis, stimulating the production of various collagens and proteins of extracellular matrix [*Shapiro S, 1998; Brajer B et al., 2008*], whereas, pro-fibrotic hormone aldosterone directly and adversely influences vascular remodeling via promoting inflammation, immune cell activation, contributes to fibrotic processes, and possibly lung fibrosis [*Zhao L et al., 1998*]. The involvement of metalloprotease-9 and aldosterone in the regulation of extracellular matrix degradation remains obscure and needs further studies. It is hypothesized, that serum glycosaminoglycans may reflect changes of extracellular matrix in lungs and matrix metalloprotease-9 and aldosterone – modulate these changes and explain lung dysfunction in chronic obstructive pulmonary disease with arterial hypertension. Present study is aimed to investigate the role of matrix metalloprotease-9 and aldosterone in changing content of serum glycosaminoglycans as components of extracellular matrix in patients with obstructive pulmonary disease combined with arterial hypertension.

Material and methods

Study included 74 patients with chronic obstructive pulmonary disease combined with essential arterial hypertension. According to the spirometric criteria they were divided into II groups: II and III groups were consisted of patients with chronic obstructive pulmonary disease of II and III stages. The exclusion criteria were oncological diseases, tuberculosis, respiratory tract infections, strokes and myocardial infarctions in anamnesis. The diagnosis of pulmonary disease of mentioned groups was established on the basis of typical symptoms by modified dyspnea scale suggested by Medical Research Council (mMRC, 2011), Chronic obstructive pulmonary disease Assessment Test and spirometry according to GOLD guidelines [*GOLD, 2014*]. Arterial hypertension was diagnosed according to European Association of Hypertension European Society of Cardiology (ESH/ESC) criteria in parameters of arterial pressure over 140/90 *mmHg* [*Mancia G et al., 2013*].

The reference group (I group) included 26 patients with isolated arterial hypertension of II stage, and the control group was consisted of 20 healthy volunteers adjusted for age and sex. The spirometric investigation included the determination of forced expiratory volume per 1 second, lung capacity, forced vital capacity, the ratio of forced expiratory volume and lung capacity, forced expiratory flows between 25%, 50% and 75% of vital capacity, and peak expiratory flow rate. Isolation and fractionation of serum glycosaminoglycans was performed by means of resorcin method [*Patent UA 29198 MPC (2006) G 01N 33/48, 2007*]. First fraction comprised chondroitin-6-sulfates, the second one – chondroitin-4-sulfates/dermatan sulfates and the third one – heparansulfates/keratan sulfates. Serum aldosterone concentrations were measured by enzyme-linked immunosorbent assay, using antibody-coated microwell plate kit for *in vitro* diagnostics (DRG International Inc., USA), according to the manufacture instructions. Quantitation of immunoreactive matrix metalloprotease-9 was carried out by ELISA kit (eBioscience, Austria). ELISA was performed on 96-well microwell plate using standard protocols.

Statistical analysis of obtained data was performed using Microsoft Excel “Statistica 6”. Standard methods excluding mean values and standard

deviations (Me±SD) were used to describe the groups. Criteria x2 was used for the analysis of qualitative signs. The significance of differences in the groups was estimated by Mann-Whitney (chem hishym senc er grvum te che) criteria, Spearman correlation analysis was used aimed to reveal the links between studied values. During the statistical analysis the differences were considered as significant at $p<0.05$.

Results

Table 2 shows the laboratory data of the patients and control group. Patients with essential hypertension and arterial hypertension combined with chronic obstructive pulmonary disease showed significant higher levels of chondroitin-6-sulfates on 8% and 24% respectively in comparison with the control group ($p<0.05$). The level of chondroitin-4-sulfates/dermatan sulfates and heparansulfates/

Table 1

Patients with arterial hypertension combined with chronic obstructive pulmonary disease of different stages of bronchoobstruction

Parameters		Groups			
		Control	I	II	III
N		20	26	38	36
Age (years)		60.2±5.6	65.1±7.0	62.7±7.3	67.4±6.2
Male/Female		13/7	16/10	22/16	22/14
Smoking experience (packs/years)		--	--	38.0±16.7	42.7±21.0
Body mass index (kg/m^2)		20.4±4.0	23.4±3.8	25.6±4.2	23±4.8
Forced expiratory volume (*l*)		3.15±0.11	2.81±0.07	2.0±0.06[1, 3]	1.2±0.04[1, 2, 3]
Forced vital capacity (*l*)		4.10±0.7	3.71±0.61	2.97±0.2[1, 3]	2.47±0.1[1, 2, 3]
Lung capacity (*l*)		4.2±1.05	3.8±0.02	3.2±0.03[1, 3]	2.6±0.01[1, 2, 3]
Forced expiratory flow (*l/min)*	25%	5.2±0.25	4.87±0.17	3.0±0.3[1, 3]	1.9±0.1[1, 2, 3]
	50%	4.5±0.2	4.3±0.2	1.7±0.35[1, 3]	1.28±0.2[1, 2, 3]
	75%	2.11±0.2	2.01±0.2	1.0±0.19[1, 3]	0.6±0.11[1, 2, 3]
Peak expiratory flow (*l/min*)		4.01±0.12	3.77±0.1	3.2±0.08[1, 3]	2.4±0.04[1, 2, 3]

Notes: [1] – values are significantly different from the control group ($p<0.05$), [2]– values are significantly different from the patients of I group, [3] – values are significantly different from the patients of II group.

Table 2

Fractions of glycosaminoglycans, levels of aldosterone and matrix metalloprotease-9 in the sera of study group patients

Parameters	Groups			
	Control	I	II	III
N	20	38	36	26
Total glycosaminoglycans (*g/l*)	0.11±0.007	0.101±0.004	0.108±0.002	0.115±0.003
Chondroitin-6-sulfates (*g/l*)	0.055±0.001	0.061±0.001[1]	0.068±0.001[1, 3]	0.076±0.002[1, 2, 3]
Chondroitin-4-sulfates/dermatan sulfates (*g/l*)	0.035±0.002	0.021±0.001[1]	0.022±0.002[1]	0.026±0.003[1]
Heparansulfates/keratan sulfates (*g/l*)	0.025±0.007	0.019±0.001[1]	0.018±0.002[1,	0.013±0.001[1, 2, 3]
Aldosterone (*pg/ml*)	43.1±1.2	56.2±2.21[1]	65.1±3.2[1, 3]	68.4±4.2[1, 3]
Matrix metalloprotease-9 (*ng/ml*)	4.2±0.6	9.3±0.71[1]	10.06±1.1[1]	13.8±1.4[1, 2, 3]

Notes: *[1] – values are significantly different from the control group ($p<0.05$), [2]– values are significantly different from I group, [3] – values are significantly different from III group.*

keratan sulfates were significant lower in the study groups compared to the control (p<0.05). Serum aldosterone levels of the hypertensive patients with or without pulmonary disease were significantly higher than that of the control (67.1±3.2 and 56.2±2.21 versus 43.1±1.2 *pg/ml*, p=0.0001). The level of matrix metalloprotease-9 in hypertensive patients with or without pulmonary disease was also significantly higher in all study groups (12.01±1.3 and 9.3±0.71versus 4.2±0.6 *ng /ml*, p=0.0001). An increase of chondroitin-6-sulfate levels in blood serum on 11% and 18.8% was noted in patients of II and III groups in comparison with I group (p<0.05). However, there was no significant difference between levels of chondroitin-4-sulfates/dermatan sulfates in patients of II and III groups in comparison with I group (0.022±0.002 and 0.024±0.002 *g/l* versus 0.021± 0.001) (p>0.05). Patients of III group had significantly lower serum levels of heparansulfates/keratan sulfates than the patients of I group (p<0.05). Serum aldosterone levels were higher in patients with arterial hypertension and obstructive disease than in patients with isolated arterial hypertension (67.1±3.2 versus 56.2±2.21 *pg/ml*, p=0.023), and the level of matrix metalloprotease-9 was also significantly higher (12.01±1.3 versus 9.3±0.71 *ng / ml*, p=0.0001). While analyzing the progression of bronchoobstruction, the patients of III group had higher serum chondroitin-6-sulfates on 11% and lower levels of heparansulfates/keratan sulfates on 27% compared to the patients of II group (p<0.05), while levels of serum chondroitin-4-sulfates/dermatan sulfates showed a tendency to increase (p>0.05). Serum matrix metalloprotease-9 level in patients of III group was significantly higher than in patients of II group (13.8±1.4 versus 10.06±1.1 *ng/ml*, p=0.0001). Differences between serum aldosterone levels weren't found, nevertheless, there was a tendency to its increase (p>0.05).

Results of Spearman's correlation analysis between total glycosaminoglycans, chondroitin-6-sulfates, chondroitin-4-sulfates/dermatan sulfates, heparansulfates/keratan sulfates and spirometric data in patients with arterial hypertension and obstructive disease are presented in table 3. A significant (p<0.05) but weak direct correlation was observed between serum total glycosaminoglycans with forced expiratory volume per second (r=0.471; p=0.012), forced vital capacity (r=0.527; p=0.023) and forced expiratory flow of 25% (r=0.312; p=0.035). On the other hand, chondroitin-6-sulfates strongly inversely correlated with all studied spirometric parameters (p<0.001). Finally, very strong correlation was found between heparansulfates/keratan sulfates and parameters of respiratory function (p<0.01).

Significant negative correlations of matrix metalloprotease-9 with forced expiratory volume per second (r=-0.677; p=0.001), forced vital capacity (r=-0.327; p=0.003), lung capacity (r=-0.383; p=0.005), forced expiratory flows of 25% (r=-0.570; p=0.001), 50% (r=-0.840; p=0.001) and

Table 3

Results of correlation analysis of glycosaminoglycan fractions and spirometry data in study group patients

Parameters		Total glycosaminoglycans		Chondroitin-6-sulfates		Chondroitin-4-sulfates//dermatan sulfates		Heparansulfates/keratan sulfates	
		r	p	r	p	r	p	r	p
Forced expiratory volume (*l*)		0.471	0.012*	-0.421	0.004**	0.085	0.251	0.880	0.001**
Forced vital capacity (*l*)		0.527	0.023*	-0.441	0.000**	0.200	0.321	0.758	0.001**
Lung capacity (*l*)		0.2413	0.057	-0.602	0.003**	0.142	0.457	0.630	0.002**
Forced expiratory flow (*l/min*)	25%	0.035*	-0.542	0.000**	0.028	0.320	0.940	0.000**	0,000**
	50%	0.147	-0.824	0.001**	0.006	0.451	0.510	0.000**	0,000**
	75%	0.053	-0.811	0.001**	0.008	0.232	0.510	0.005**	0,005**
Peak expiratory flow (*l/min*)		0.244	0.064	-0.347	0.004**	0.008	0.877	0.754	0.001**

Notes: ** – p<0.05; ** p<0.01.*

75% (r=-0.793; p=0.000), as well as peak expiratory flow (r=-0.840; p=0.010). Serum aldosterone level showed weak negative correlation with forced expiratory volume per second (r=-0.317; p=0.034), lung capacity (r=-0.315; p=0.022) and peak expiratory flow (r=-0.64; p=0.002) (Table 4).

Significant negative correlation was found between the level of serum aldosterone with chondroitin-4-sulfates/dermatan sulfates (r=-0.495; p=0.00001) (Fig. 1) and the level of heparansulfates/keratan sulfates (r=-0.55; p=0.0001) (Fig. 2) during the correlation analysis of factors affecting the metabolism of extracellular matrix and its components. Moreover, matrix metalloprotease-9 showed a significant positive correlation with levels of chondroitin-6-sulfate (r=0.5; p=0.000) (Fig. 3) and a negative correlation with heparansulfates/keratan sulfates (r=-0.805; p=0.000) (Fig. 4).

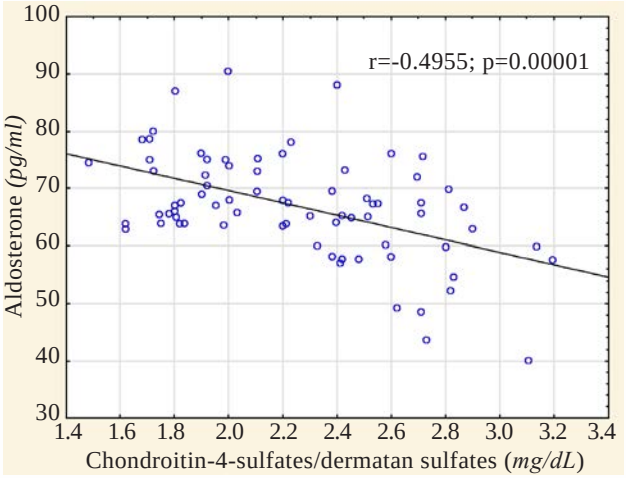

Figure 1. Negative correlation between serum aldosterone and chondroitin-4-sulfates/dermatan sulfates in patients with chronic obstructive pulmonary disease combined with arterial hypertension

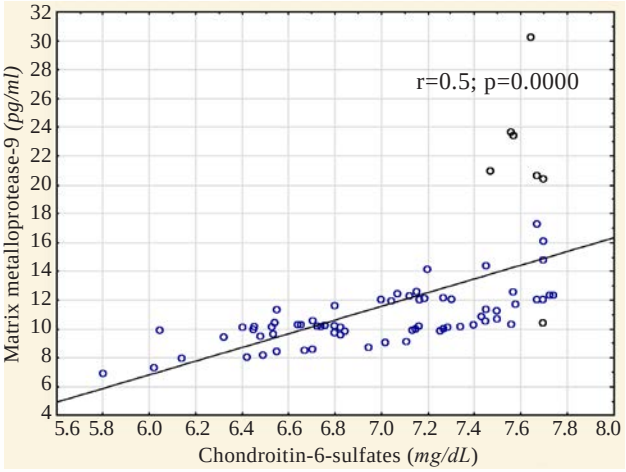

Figure 3. Positive correlation between serum matrix metalloprotease-9 and chondroitin-6-sulfates in patients with chronic obstructive pulmonary disease combined with arterial hypertension

Discussion

The content of extracellular matrix has an important role in determining airway structure and is a three-dimensional comprised of various interconnected and intercalated macromolecules, among which are the glycosaminoglycans. Glycosaminoglycans are either an integral part of extracellular matrix or they are located directly on the cellular membrane, where they can function as protein receptors or activators. There are two main types of glycosaminoglycans: the non-sulphated – hyaluronic acid and the sulphated – heparansulfate and heparin, chondroitin sulfate, dermatan sulfate and keratan sulfate. In lungs, glycosaminoglycans support the structure of interstitium, subepithelial

Table 4

Results of correlation analysis of serum aldosterone, matrix metalloprotease-9 levels and spirometry data in study group patients

Spirometry parameters		Aldosterone		Matrix metalloprotease-9	
		r	p	r	p
Forced expiratory volume (*l*)		-0.317	0.034*	-0.677	0.001**
Forced vital capacity (*l*)		-0.210	0.012	-0.327	0.003**
Vital capacity (*l*)		-0.315	0.022*	-0.383	0.005**
Forced expiratory flow (*l/min*)	25%	0.067	-0.570	0.001**	0,001**
	50%	0.242	-0.840	0.001**	0,001**
	75%	0.483	-0.793	0.000**	0,000**
Peak expiratory flow (*l/min*)		-0.64	0.002*	-0.344	0.010**

*Notes: * – p<0.05; ** – p<0.01.*

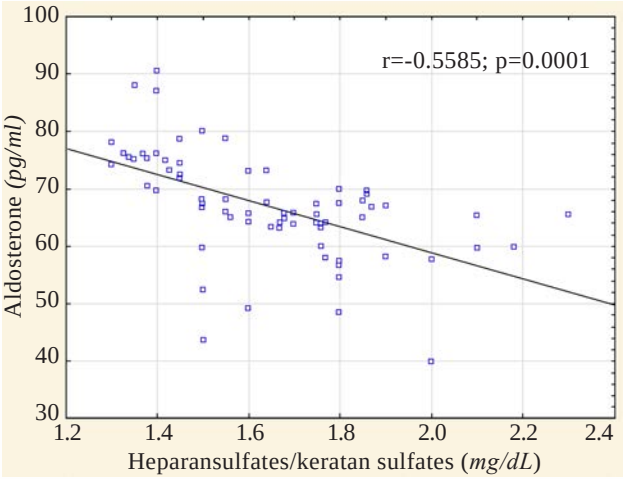

FIGURE 2. *Negative correlation between serum aldosterone and heparansulfates/keratan sulfates in patients with chronic obstructive pulmonary disease combined with arterial hypertension*

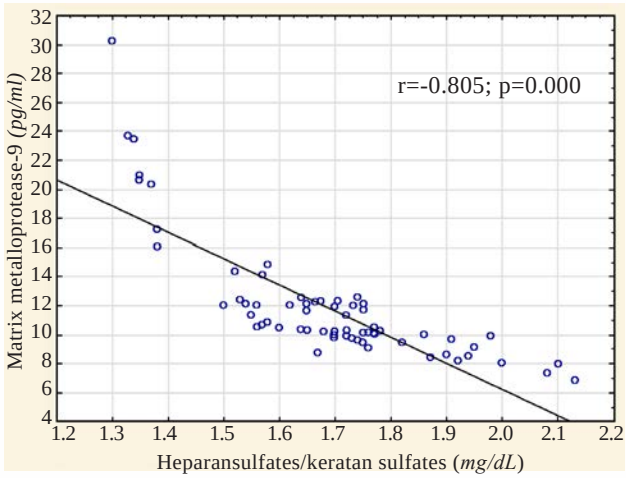

FIGURE 4. *Negative correlation between serum matrix metalloprotease-9 and heparansulfates/keratan sulfates in patients with chronic obstructive pulmonary disease combined with arterial hypertension*

tissue and bronchial walls, and are secreted in the airway secretions. Besides maintaining lung tissue structure, glycosaminoglycans also play an important role in lung function, as they regulate hydration and water homeostasis, modulate the inflammatory response and influence lung tissue reparation and bronchi remodeling. Interestingly, depending on their size and/or degree of sulphation, and their immobilization or solubilization in extracellular matrix, specific glycosaminoglycans in the lungs either support normal lung physiology, or they are related to lung pathology [*Papakonstantinou E, Karakiulakis G, 2009*].

Recent studies have shown that glycosaminoglycans play a significant role in inflammatory and non-inflammatory lung diseases, exhibiting spatio-temporally distinct effects on epithelial or mesenchymal cell types [*Laurent G et al., 2007*]. Changing extracellular matrix turnover is a hallmark of several pulmonary diseases, including idiopathic pulmonary arterial hypertension, pulmonary fibrosis, asthma or chronic obstructive pulmonary disease, which underlines the importance of matrix homeostasis for proper lung function [*Papakonstantinou E, Karakiulakis G, 2009*].

Expression of glycosaminoglycans in the lungs was studied in patients with idiopathic pulmonary arterial hypertension, *in vivo* and *in vitro* the expression and localization of glycosaminoglycans metabolizing enzymes were also analyzed [*Papakonstantinou E et al., 2008*]. The authors found that in idiopathic pulmonary arterial hypertension lung tissues exhibit a significantly increased content of non-sulphated glycosaminoglycans: hyaluronic acid, while the content of the sulphated glycosaminoglycans – heparansulfates, dermatan sulfates or chondroitin sulfates was decreased, indicating an increased ratio of non-sulphated glycosaminoglycans to sulphated ones. It has been reported, that extracellular matrix is also altered in the airway walls of patients with chronic obstructive pulmonary disease [*Kranenburg A et al., 2006; Annoni R et al., 2012*]. It is established, that abnormal and exaggerated deposition of extracellular matrix is involved in the pathogenesis of obstructive disease [*Chen L et al., 2013*]. Alterations of main components of extracellular matrix are widespread in all lung compartments of patients with obstructive disease, and authors suggest that they might contribute to persistent airflow obstruction [*Annoni R et al., 2012*].

Therefore, it is hypothesized, that the measurement of serum glycosaminoglycan levels in chronic obstructive pulmonary disease may testify of alterations in synthesis and degradation of extracellular matrix in lungs, reflecting in the progression of pulmonary diseases. The leading causes of morbidity and mortality in patients with mild-to-moderate bronchoobstruction are cardiovascular diseases. In the Lung Health Study of nearly 6000 smokers, whose forced expiratory volume per second was in the range of 55% and 90%, cardiovascular diseases were the leading

cause of hospitalization and the second leading cause of mortality, accounting for a quarter of all deaths [*Anthonisen N et al., 1994*].

In the present study, the decrease of the levels of chondroitin-4-sulfates/dermatan sulfates and heparansulfates/keratan sulfates was found in the serum of patients of all 3 groups, whereas the levels of chondroitin-6-sulfates were significantly higher ($p<0.05$). These components of extracellular matrix may reflect the extent of structural changes of the airways in obstructive disease, since glycosaminoglycans are main constituents of extracellular matrix of the lungs. Significant differences were found between II and III groups (Table 2) during the progression of bronchial obstruction, which manifested as an increase of chondroitin-6-sulfate levels (0.068±0.001 versus 0.076±0.002 g/l, $p<0.05$) and reduction of serum heparansulfate/keratan sulfate levels (0.018±0.002 versus 0.013±0.001, $p<0.05$).

Both increased chondroitin-6-sulfates and decreased heparansulfates/keratan sulfates were strongly associated with lung dysfunction ($p<0.001$). It is known, that heparansulfates are the main components of sulphated glycosaminoglycans within the lung parenchyma, the synthesis processes of which might be impaired in chronic persistent inflammation [*Handel T et al., 2005*]. They are also involved in a variety of biological processes, including cell-matrix interactions and activation of chemokines, enzymes and growth factors [*Kreuger J et al., 2002; Whitelock J, Iozzo R, 2005*], and exerts anti-inflammatory effects. Administration of heparansulfates graded the development of lethal toxic shock in mice, induced by staphylococcal enterotoxin B due to suppressing the production of TNF-α and IL-6, and attenuating inflammatory tissue injury. Moreover, heparin has been shown to protect against exercise-induced bronchoconstriction [*Garrigo J et al., 1996*]. The authors suggested that this effect might be due to prevention of mediator release rather than a direct effect on smooth muscle. Furthermore, heparin may be capable of modulating the extent of remodelling of the airway wall, by modulating the actions of a range of proteins, including proteins of extracellular matrix, growth factors and certain enzymes, and by inhibiting the proliferation of lung fibroblasts and airway smooth muscle cells [*Tyrell D et al., 1995*]. Additionally, heparin is released in the airways physiologically as a homeostatic mechanism to limit the extent of the cellular adhesion and diapedesis. Thus, the release of heparin provides a plausible homeostatic mechanism to limit tissue damage and remodelling following an inflammatory insult to the mucosal surface [*Page C, 1997*]. Therefore, the decrease of serum heparansulfate in patients with chronic obstructive pulmonary disease, found in the present study, might serve as an unfavorable prognostic factor for the disease progression. Chondroitin sulfates are key regulators for the activation and degradation of proteins of extracellular matrix. The function of chondroitin sulfate depends on the location of its sulfate groups, which determine its structure and binding characteristics. It has been shown that chondroitin sulfate activates enzymes degrading extracellular matrix, such as matrix metalloproteases, which are widely involved in various pulmonary diseases [*Greenlee K et al., 2007*]. Chondroitin sulfates are involved in the degradation of aggrecan – chondroitin sulfate-rich proteoglycan together with matrix metalloprotease-2 [*Iida J et al., 2007*], matrix metalloprotease-13 and ADAMTS4 [*Miwa H et al., 2006*]. Thus, increased serum chondroitin-6-sulfates might also cooperate with matrix metalloprotease-9 in pathophysiology of extracellular matrix turnover in chronic obstructive pulmonary disease.

Segura-Valdez L. and co-authors (2000) examined the expression of matrix metalloprotease-2 and -9 in lung tissue of patients with chronic obstructive disease and healthy individuals. In their study, an immunohistochemical analysis of pulmonary disease showed a markedly increased expression of both matrix metalloprotease-2 and -9 in lung tissues. They found that neutrophils are the main cells showing a positive signal for matrix metalloprotease-9 and its concentration of sputum was found to be higher in patients with obstructive disease compared to the control group and correlated negatively with the severity of airways obstruction [*Brajer B et al., 2008*]. In another study it is noted, that matrix metalloprostease-9 level is increased in sputum of patients with obstructive pulmonary disease compared with non-smokers, non-symptomatic cigarette smokers, and asthmatics [*Culpitt S et al., 2005*]. There is evidence that

changes in the lungs of patients with chronic obstructive disease were associated with matrix metalloprotease-9/tissue inhibitor of metalloproteinase-1 ratio in sputum during computerized tomography [*Vignola A et al., 1998*]. The authors suggested that sputum levels of these markers reflect the extent of structural changes of the airways. All these data underline the significance of metalloprotease-9 involvement in extracellular matrix turnover in lung tissues during obstructive pulmonary disease. However, the data about the relationship of chondroitin sulfates with matrix metalloprotease-9 are absent in the literature. Meanwhile, the involvement of glycosaminoglycans in lung function is apparently mediated by specific enzymes responsible for the synthesis or degradation of glycosaminoglycans. Taking into account the latter, serum metalloprotease-9 level and its involvement in extracellular matrix turnover was estimated by studying the relations of matrix metalloprotease-9 with serum glycosaminoglycans in patients with obstructive disease combined with hypertension. As the results showed, serum metalloprotease-9 level was significantly higher in patients of II and III groups compared to I and control groups ($p<0.05$). Increased serum metalloprotease-9 significantly correlated with decreased lung function and disease progression, which is consistent with the results of several studies [*Brajer B et al., 2008; Higashimoto Yu et al., 2009; Linder R et al., 2013*]. Moreover, a significant positive correlation of matrix metalloprotease-9 with chondroitin-6-sulfates ($r=0.5$; $p=0.0000$) and significant negative correlation with heparansulfates/keratan sulfates ($r=-0.805$; $p=0.0000$). Therefore, matrix metalloprotease-9, responsible for the degradation of extracellular matrix, might influence its content through mediating chondroitin-6-sulfates and heparansulfates/keratan sulfates in lung parenchyma. Thus, matrix metalloprotease-9 might be a potentially novel way of affecting the content of extracellular matrix of lungs in chronic obstructive pulmonary disease. On the other hand, fibrotic processes in lung tissue come along with the degradation of extracellular matrix.

Profibrotic agent aldosterone is involved in extracellular matrix turnover [*MacFadyen R et al., 1997; Zannad F et al., 2000*] and is associated with lung fibrosis [*Zhao L et al., 1998*]. There is experimental evidence that the lung epithelium is a physiological target tissue for aldosterone [*Illek B et al., 1990; Hirasawa G et al., 1997*], and that spironolactone might ameliorate pulmonary fibrosis [*Zhao L et al., 1998*]. High serum aldosterone levels have been reported in patients with lung obstruction in hypoxemia [*Farber M et al., 1984*]. A positive effect of aldosterone antagonist has been shown on lung diffusion in chronic heart failure [*Agostoni P et al., 2005*]. Based on the abovementioned, a hypothesis of the influence of aldosterone on lung function was evaluated in patients of I and II groups. Serum aldosterone level appeared to be higher in patients of I and II groups compared to patients of III and control groups ($p<0.05$). Aldosterone level had significantly negative correlation with forced expiratory volume per second, lung capacity and peak expiratory flow ($p<0.05$). Meanwhile, serum aldosterone concentrations showed significantly strong correlation with serum heparansulfate/keratan sulfate levels ($r=-0.558$; $p=0.000$). This might be of interest since it is known, that heparins are able to inhibit the secretion of aldosterone. Possibly, decreased serum heparansulfates in lung obstruction and hypertension contribute to increased serum aldosterone concentration, and lead to its deleterious effects.

Relations between the level of chondroitin-4-sulfate/dermatan sulfates and progression of lung obstruction were not found. Their decrease might be explained by reduced antioxidative potential in lung obstruction, since chondroitin sulfates have been reported to have antioxidant activity by blocking NF-kappaB and caspase activation [*Campo G et al., 2008; Iovu M et al., 2008*]. Whereas dermatan sulfates are known to function as a docking molecule for a range of human pathogenic microorganisms. It has been shown that dermatan sulfates mediate the adhesion of variety of microorganisms to the host's extracellular matrix [*Srinoulprasert Y et al., 2006; Tonnaer E et al., 2006*]. Thus, the relationships of glycosaminoglycans, aldosterone and matrix metalloprotease-9 remain interesting for further investigations, and there is no doubt, that serum levels of glycosaminoglycans in patients with chronic obstructive pulmonary disease combined with arterial hypertension reflect altered content of extracellular matrix in the progression of the disease.

Conclusion

Recent studies have clearly indicated the great significance of glycosaminoglycans in the pathophysiology of respiratory diseases. The fact that the same molecule depending on the degree of sulphation may exert both positive, as in case of heparin and heparansulfates, and negative effects, as the case of chondroitin-6-sulfates, is of particular interest. Observed increased ratio of very low-sulphated glycosaminoglycans to sulphated ones in serum of patients significantly related to lung dysfunction, suggests that there is an upregulation of glycosaminoglycans in lung tissue, associated with increased serum matrix metalloprotease-9 and aldosterone that contribute to the pathogenesis of chronic obstructive disease. This allows using chondroitin-6-sulfates, heparansulfates and matrix metalloprotease-9 as putative therapeutic targets for the treatment of chronic obstructive pulmonary disease. The use of modern technologies in glycochemistry and glycobiology provides wide possibilities for creating new pathogenic approach in chronic obstructive pulmonary disease.

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