THE INFLUENCE OF SPIRONOLACTONE ON EXTRACELLULAR MATRIX METABOLISM IN PATIENTS WITH ARTERIAL HYPERTENSION AND CONCOMITANT CHRONIC OBSTRUCTIVE PULMONARY DISEASE

DELEVSKAYA V.Y.

Kharkiv National Medical University, the Ukraine

Резюме.
Цель – изучение влияния блокады альдостероновых рецепторов на метаболизм экстрацеллюлярного матрикса у больных артериальной гипертензией в сочетании с хроническим обструктивным заболеванием легких.
Материалы и методы. В исследовании приняли участие 80 больных артериальной гипертензией в сочетании с хроническим обструктивным заболеванием легких II-III степени бронхообструкции. Все пациенты были разделены на 2 группы. Первая группа (41 больной) получала базисную терапию, вторая группа (39 пациентов) дополнительно получала спиронолактон в дозе 50 мг один раз в день в течение 3 месяцев. Уровень гликозаминогликанов в сыворотке крови пациентов измеряли резорциновым методом, ММП-9 – иммуноферментным методом. Лабораторное исследование проводили всем пациентам до и после лечения.
Результаты. Включение спиронолактона в базисную терапию пациентов с артериальной гипертензией и хроническим обструктивным заболеванием легких приводило к снижению уровня хондроитин-6-сульфатов и повышению уровня гепарансульфатов/кератансульфатов, что сопровождалось снижением уровня ММП 9 в сыворотке крови ($p<0,05$). Побочных эффектов при применении спиронолактона у исследованных пациентов не наблюдалось.
Заключение. Добавление спиронолактона в базисную схему лечения хронической сердечной недостаточности у больных с артериальной гипертензией и хроническим обструктивным заболеванием легких приводит к положительным изменениям метаболизма экстрацеллюлярного матрикса и снижению активности процессов деградации в соединительной ткани, что может объяснять положительное влияние спиронолактона на сердечно-сосудистую систему и предупреждение прогрессирования сердечной недостаточности.
Ключевые слова: спиронолактон, гликозаминогликаны, матриксная металлопротеаза-9, хроническое обструктивное заболевание легких, артериальная гипертензия.

Abstract.
Objectives. To study the effect of aldosterone receptors blockade on extracellular matrix metabolism in patients with arterial hypertension combined with chronic obstructive pulmonary disease.
Material and methods. A total of 80 patients with arterial hypertension combined with chronic obstructive pulmonary disease of II-III degree of airways obstruction were included in the study. All patients were divided into 2 groups. The first group (41 patients) received basic therapy and the second group (39 patients) additionally received spironolactone in a daily dose of 50 mg during 3 months. Thw level of glycosaminoglycanes in the blood serum of patients was measured by resorcin method and MMP-9 – by enzyme-linked immunosorbent assay. All patients underwent laboratory investigation before and after the treatment.
Results. The obtained results have shown, that the addition of spironolactone to the basic treatment of patients with arterial hypertension and chronic obstructive pulmonary disease led to the decreased level of serum chondroitin-6-sulphates and the increased level of heparansulfates/keratansulfates, which was accompanied by the decrease of MMP-9 level in the blood serum ($p<0,05$). No adverse effects while using spironolactone were registered in the studied patients.
Conclusions. The addition of spironolactone to the basic treatment of heart failure in the patients with arterial hypertension and chronic obstructive pulmonary disease results in positive changes of ECM metabolism and the reduction in the activity of connective tissue degradation processes, that might contribute to the positive effect of spironolactone on cardiovascular system and progression of heart failure.
Key words: spironolactone, glycosaminoglycanes, matrix metalloprotease-9, chronic obstructive pulmonary disease, arterial hypertension.

Arterial hypertension (AH) and chronic obstructive pulmonary disease (COPD) often coexist together and overburden the course of each disease. Thus, COPD was reported to adversely impact on prognosis of heart failure patients, being an independent predictor of mortality and hospitalization [1, 2]. Pathophysiology of heart failure in this cohort of patients remains to be determined. In recent years the attention of researchers has been focused on the role of extracellular matrix (ECM) metabolism in the progression of heart failure [3]. The effect of both AH and COPD on extracellular matrix metabolism up to date is a poorly understood issue.

It is known that ECM is altered in the airway walls of patients with COPD [4]. The ECM is a complex structured network of secreted macromolecules and matrix metalloproteases that provide the basis of cell-cell and cell-matrix interactions. In the lung ECM components fundamentally influence the structure and function of the airways. Glycosaminoglycanes (GAGs) are one of the main representatives of ECM. GAGs due to the differences in the structure have been differentiated into chondroitin sulfates, dermatan sulfates, heparin, heparan sulfates, hyaluronic acid and keratan sulfates.

The role of GAGs in the development and progression of respiratory and heart failure is widely discussed. Thus, changes of specific GAGs levels with accumulation of their different fractions were reported in patients with AH by Cullman et al. Besides, excessive ECM turnover is considered to contribute to congestive heart failure [3].

On the other hand, it is well known, that ECM metabolism is strongly modulated by specific enzymes – matrix metalloproteases. Thus, alveolar wall degrading enzyme matrix metalloprotease-9 (MMP-9) has been found to be involved in COPD progression. It is considered to play a crucial role in the pathogenesis of tissue damage and fibrosis, stimulating the production of various collagens and ECM proteins. Moreover, MMP-9 is found to be associated with ishemic heart disease, acute coronary syndrome and AH [7, 8, 9]. The fact of strong relationships between MMP-9 and GAGs induces the search for therapeutic regulation of ECM degradation processes and activity of MMP-9 in order to reduce high level of mortality in patients with concomitant lung and heart pathologies.

In recent years aldosterone antagonists are prescribed to the patients with heart failure, and variety of studies have shown that these drugs reduce the risk of hospitalization and death from cardiovascular diseases [3, 10]. Hence the aim of the present study is to investigate the role of spironolactone on ECM turnover and MMP-9 activity as they are strongly involved in the pathogenesis of COPD and AH.

Material and methods

80 patients with II stage of AH and COPD of the 2nd - the 3rd degree of bronchial obstruction (GOLD 2-3) according to GOLD criteria were recruited in the study.

The diagnosis of COPD was made on the basis of typical symptoms, modified Medical Research Council (mMRC) dyspnea scale, COPD Assessment Tool (CAT) and spirometry according to GOLD guidelines (GOLD 2014) [11]. Diagnosis of AH was made according to ESH/ESC criteria that consider the values of blood pressure over 140/90 mmHg as hypertension [12].

The criteria of exclusion from the study are: oncological diseases, tuberculosis, respiratory tract infections, strokes and myocardial infarctions in anamnesis.

The first group consisted of 41 patients with AH and GOLD 2-3 (24 males and 17 females, their mean age was 64,31±1,74) treated with standard therapy of COPD and AH with using β2-agonists, corticosteroids, anticholinergics, ACE inhibitors and diuretics. The second group consisted of 39 patients (23 males and 16 females, their mean age was 61,42±2,5) with AH and GOLD 2-3, who in addition to standard therapy received spironolactone in a dose of 50 mg per day for 3 months.

All the patients underwent general clinical and biochemical investigations and spirometry. Isolation and fractionation of serum GAGs was performed by means of resorcin method [13]. The first fraction included chondroitin-6-sulphates, the second one – chondroitin-4-sulphates/dermatansulfates and the third one – heparansulfates/keratansulfates. Serum glycoproteins were measured by a modified Steinberg-Dotsenko method [14]. The investigation of serum chondroitinsulfates was performed by Nemeth-Csoka method in Sluczkij L. I. modification [15].

Serum MMP-9 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) method, using antibody-coated microwell plate kit for in vitro diagnosis (eBioscience, Vienna, Austria) according to the manufacture instructions.

Statistical analysis was performed by the software Statistica for Windows 10.0. Data are presented as mean values and standard error of mean (M±m). The comparison of data was performed by Mann-Whitney criterion. Valid values were considered at the level of $p<0,05$.

Results

The investigation of parameters of ECM metabolism in patients with AH and COPD showed some changes after the basic therapy. Thus, patients with AH and GOLD 2 demonsrated decrease of glycoproteins level by 8,1% and chondroitin-6-sulfates level – by 2,8% ($p<0,05$), indicating the slight reduction of inflammation in the connective tissue after traditional drug therapy (Table 1). Whereas no statitically significant differences in the levels of MMP-9, total GAGs, chondroitinsulfates, chondroitin-4-sulfates / dermatansulfates and heparansulfates / keratansulfates were observed.

While analyzing the data of patients with AH and GOLD 3, who received basic therapy, we observed only the decrease of glycoproteins by 4,1% ($p<0,05$), while serum levels of different GAGs fractions remained unchanged (Table 2).

According to our previous study [16] the altered fractional GAGs composition and high levels of serum MMP-9 indicate a significant ECM degradation with connective tissue turnover and intensification of systemic inflammation in patients with AH and COPD, associated with significant worsening of their structural and functional echocardiographic characteristics and increased risk of cardiovascular events.

Since basic therapy of AH and GOLD 3 does not affect the altered GAGs composition and intensity of ECM degradation and does not improve life prognosis of the studied patients, there is a necessity of the study of the additional therapy.

Thus, different results were obtained in spironolactone-treated patients with AH and GOLD 2. Comparing to the data before treatment the patients demonstrated a significant decrease of glycoproteins by 26,6% and chondroitinsulfates by 28,5% ($p<0,05$). They also showed an improvement in the altered fractional composition of GAGs, characterized by decreased serum chondroitin-6-sulfates (by 8,4%) and increased chondroitin-4-sulfates / dermatansulfates (by 9%) and heparansulfates / keratansulfates (by 16,6%) ($p<0,05$).

Since there is evidence that MMP-9 promotes ECM reorganization, it was important to analyze its dynamics after the treatment with aldosterone antagonist. Thus, it was found that serum MMP-9 concentration decreased by 18,4% ($p<0,05$).

MMP-9 is known to play an important role in cardiovascular and lung pathology. Thus, Tayebjee M. H., Jasmin W. S., Yabluchanskiy A. et al. showed that patients with AH had higher

Table 1 – Dynamics of serum MMP-9 and ECM metabolism biomarkers in patients with AH combined with GOLD 2 after basic therapy with and without spironolactone, (M±m)

Parameter	Basic therapy (n=20)		Basic therapy + spironolactone (n=19)	
	before treatmnt	after treatment	before treatmnt	after treatment
Total GAGs (g/l)	0,111±0,001	0,111±0,001	0,112±0,001	0,107±0,001
Chondoitin-6-sulfates (g/l)	0,070±0,001	0,068±0,001*	0,071±0,001	0,065±0,001*
Chondoitin-4-sulfates/ dermatan-sulfates (g/l)	0,023±0,001	0,023±0,001	0,022±0,001	0,024±0,001*
Heparansulfates/ keratansulfates (g/l)	0,013±0,001	0,019±0,001	0,018±0,001	0,021±0,001*
Chondoitinsulfates (g/l)	0,29±0,01	0,26±0,01	0,28±0,01	0,20±0,01*
Glycoproteins (g/l)	0,61±0,01	0,56±0,01*	0,60±0,01	0,44±0,01*
MMP-9 (ng/ml)	12,25±0,82	12,01±0,67	10,40±0,51	8,48±0,45*

Note: * - p <0,05.

Table 2 – Dynamics of serum MMP-9 and ECM metabolism biomarkers in patients with AH combined with GOLD 3 after basic therapy with and without spironolactone, (M±m)

Parameter	Basic therapy (n=21)		Basic therapy + spironolactone (n=20)	
	before treatmnt	after treatment	before treatmnt	after treatment
Total GAGs (g/l)	0,111±0,001	0,110±0,001	0,112±0,001	0,111±0,001
Chondoitin-6-sulfates (g/l)	0,073±0,001	0,072±0,001	0,074±0,001	0,068±0,001*
Chondoitin-4-sulfates/ dermatan-sulfates (g/l)	0,022±0,001	0,021±0,001	0,021±0,001	0,022±0,001
Heparansulfates/ keratansulfates (g/l)	0,016±0,001	0,017±0,001	0,017±0,001	0,020±0,001*
Chondoitinsulfates (g/l)	0,22±0,01	0,23±0,01	0,19±0,01	0,20±0,01
Glycoproteins (g/l)	0,72±0,01	0,69±0,01*	0,74±0,01	0,63±0,01*
MMP-9 (ng/ml)	13,34±0,74	13,10±0,62	14,60±0,78	12,62±0,65*

Note: * - p <0,05.

levels of MMP-9 comparing with those with normal blood pressure [7, 9, 17]. Zervoudaki A. et al. reported a relationship between the level of MMP-9 and MMP-9 gene in patients with AH [18]. Moreover, the expression of serum MMP-9 was found to be increased in patients with acute coronary syndrome [19]. Study of Kalela A. et al. in patients with coronary atherosclerosis revealed that serum concentrations of MMP-9 were gradually increased according to the number of affected arteries and remained statistically significant after adjusting for age, sex and diabetes [20]. Furthermore, Blankenberg S. et al. according to the study, which included 1127 individuals with coronary artery atherosclerosis, suggested the use of MMP-9 as a new additional predictor of cardiovascular mortality [21]. The results of this study showed that average concentration of MMP-9 in the coronary sinus of patients who died of cardiovascular events was significantly higher than that of patients without fatal cardiovascular events. Apart from that, the study of Zhong-Xuan Ye. et al., which included 91 patients with ischemic heart disease with confirmed coronary atherosclerosis and further revascularization showed that the initial level of serum MMP-9 was an independent predictor of adverse cardiovascular events after revascularization and even more significant than C-reactive protein [22].

Thus, the evidence of MMP-9 as a marker of vascular and coagulation hemostasis disorders, leading to the increased risk of cardiovascular complications [8], suggests the necessity of MMP-9 decrease in patients with high cardiovascular risk.

No surprise, that Onal I. K. and colleagues even chose for the subject of their research the dynamics of MMP-9 as a criterion for efficacy of antihypertensive therapy in patients with AH [23]. Therefore, we consider the observed in the present study decrease of MMP-9 by addition of spironolactone as a favorable change in ECM turnover.

Up to date, the ability of spironolactone to modulate the activity of matrix metalloproteasis has been reported only concerning MMP-2 [24]. Taking into account the studies of foreign researchers, who mentioned the ability of aldosterone to activate MMP-9, the decrease of MMP-9 in patients with AH and COPD by aldosterone antagonist spironolactone is pathogenetically explained [25].

The addition of spironolactone to the basic therapy in patients with AH and GOLD 2 compared with basically-treated patients showed lower levels of glycoproteins by 21,1%, chondroitinsulfates – by 23% chondroitin-6-sulfates – by 4,4%, MMP-9 – by 29,3% and higher levels of heparansulfates / keratansulfates by 10,5% (p<0,05). Spironolactone-indused restoring of the balance of GAGs fractional composition, associated with inhibition of MMP-9, contributes to the prevention of further ECM degradation and excessive growth of connective tissue in the inflammatory foci and hence possibly lead to the improvement of significant structural and functional changes in myocardium and broncho-pulmonary tree inherent to both AH and COPD.

Spironolactone-treated patients with AH and GOLD 3 comparing with the data before

the treatment revealed a significant decrease of glycoproteins by 14,8%, chondroitin-6-sulfates by 7,4%, MMP-9 by 13,5% and increase of heparansulfates / keratansulfates by 17,6% ($p<0,05$).

Comparing with basically-treated patients spironolactone-treated patients demonstrated lower levels of glycoproteins by 8,6%, chondroitinsulfates – by 13,8%, chondroitin-6-sulfates – by 4,1% and increase of heparansulfates / keratansulfates by 17,6% ($p<0,05$).

Thus, aldosterone blockade exhibited a beneficial effect on connective tissue metabolism in patients with AH and COPD, as evidenced by the redistribution of GAGs fractional composition with the decrease of nonsulfated GAGs and increase of highsulfated ones.

Moreover, patients with AH and GOLD 3, who received spironolactone, comparing with those who received basic treatment, showed a significant decrease of MMP-9 level – by 3,6% ($p<0,05$), that might have a positive effect on reducing the risk of cardiovascular complications.

Conclusions

Therefore, the obtained results confirm the cardioprotective properties of spironolactone. The present study lightened the evidence of beneficial effects of spironolactone on ECM metabolism and may explain its ability to improve survival prognosis and reduce hospitalizations in heart failure patients. Prescribed even in small doses, spironolactone is also beneficial for pharmacoeconomic criteria, simple dosing and good tolerability.

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Сведения об авторах:
Делевская В.Ю. – аспирант кафедры внутренней медицины №2, клинической иммунологии и аллергологии Харьковского национального медицинского университета, Украина.

Адрес для корреспонденции: Украина, 61018, г. Харьков, ул. Балакирева, д. 23, кв. 10. E-mail: valentinka_1987@ukr.net – Делевская Валентина Юрьевна.