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FEATURES OF BALT IN RATS WITH EXPERIMENTAL CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ADMINISTRATION OF THIOMETRISOL

Abstract. *Background. Cigarette smoke is a major etiological factor associated with the development of chronic obstructive pulmonary disease (COPD). Despite the importance and growing prevalence of COPD, little progress has been made in developing effective drug therapy. The development and progression of COPD is characterized by chronic progressive inflammation. An objective of the research: to establish the morphological parameters of immunocompetent cells in the composition of BALT rats with experimental chronic obstructive pulmonary disease and the administration of thiometrisol.*
An object of this study: 60 adult rats of Wistar line, using immunohistochemical method by determining the CD4, CD8, CD20, CD67 (Thermo Scientific, USA) and administration of thiometrisol. As a result of our research, it was revealed that BALT consists of lymphoepithelial lymphoid nodules. Lymphoid nodules were a highly organized structure of lymphocytes and antigen-presenting cells. CD20+ cells formed lymphoid nodules, sometimes with a germinal center, which are surrounded by a perifollycular area consisting of T cells of predominantly CD4+ origin. CD67+ cells are located between the T cells for transport and the presentation of antigens from the lumen of the bronchi to the T cells. Lymphocytes enter the BALT through high endothelial venules (HEV), which are present in the lymphoid nodules. Sometimes there were other lymphoid accumulations, mainly in the lung parenchyma, which did not meet all the criteria for BALT. These lymphoid structures were not in contact with the bronchial epithelium and were more in contact with the lumen of the alveoli than with the bronchial or bronchiolar lumen. They were predominantly CD20+ cells with a weakly pronounced germinal center, surrounded mainly by CD4+ T cells, but also diffusely located CD8+ T cells. These lymphoid accumulations were more often observed in animals after exposure to tobacco smoke, which was the trigger factor for their development Thus, the study showed that in experimental animals there are violations of the cellular, humoral and nonspecific parts of the immune system. An increase in the content of B-lymphocytes (CD20 + cells), the main lymphocyte phenotypes (CD4 + and CD8 + cells) provide an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines. Conclusion. The results confirm the need for adequate treatment of patients with COPD for the elimination of chronic inflammation; they justify the use of thiometrisol in complex therapy. Its use can successfully supplement standard therapy and improve the state of the local immune system of the lungs.
Keywords: *COPD, rats, BALT, immunohistochemical method, thiometrisol.*

Introduction. In recent years, there has been an increase in the number of chronic respiratory diseases, a special place among which is chronic obstructive pulmonary disease (COPD), which is a significant medical and social problem. According to the World Health Organization, about 220 million people in the world today suffer from this disease, which in 2020 will take the third place in the world in terms of mortality [1]. In Ukraine, the current epidemiological situation is characterized by the incidence of COPD at the level of 7% of the population of Ukraine, or about 3 million people.

The development and progression of COPD is characterized by an incompletely reversible airway obstruction, accompanied by chronic progressive inflammation, mucociliary dysfunction, structural changes with airflow restriction, as well as a systemic component. One of the insufficiently

studied phenomena in the study of morphological changes in chronic nonspecific lung diseases is the reaction of connective tissue elements, such as microvessels and immunocompetent cells [2, 3, 4].

Cigarette smoke is a major etiological factor associated with the development of chronic obstructive pulmonary disease (COPD). Despite the importance and growing prevalence of COPD, little progress has been made in developing effective drug therapy [5]. For the treatment strategy to work, it is necessary to slow down and inhibit the inflammatory and destructive processes underlying this disease. A morpholine-based chemical compound 2(5-(4-pyridyl)-4(2-methoxyphenyl)-1,2,4-triazol-3-ylthio)acetate (hereinafter abbreviated as thiometrisol) is an original active substance, with cytoprotective, antioxidant, anti-inflammatory properties. On the basis of deep scientific research, its effectiveness has been proven due to the presence of anti-inflammatory, antioxidant, anti-hypoxic, and immunocorrective action in it [6].

Obviously, the morphofunctional characteristics of the influence of thiometzole on the local immune system of the lungs remain relevant and not fully studied.

An objective of the research: to establish the morphological parameters of immunocompetent cells in the composition of BALT rats with experimental chronic obstructive pulmonary disease and the administration of thiometrisol.

Material and methods. The work was performed on 60 adult rats of Wistar line, divided into 3 groups: 1 - 20 intact animals; 2 - experimental group, 20 animals with experimental chronic obstructive pulmonary disease; 3 - 20 animals to whom, on the background of a modeled chronic nonspecific lung disease, thiometrasol was administered. To simulate an experimental chronic obstructive pulmonary disease, the world-famous model (Geraghty P. et al., 2014) will be applied, according to which experimental animals are exposed to tobacco smoke in a specially designed chamber for 4:00 a day, 5 days a week for 2 months with a total solids concentration of 80 mg / m3. For the correction of chronic non-specific lung disease, thiometrasol was used - a chemical compound based on morpholinium 2 (5- (4-pyridyl) -4 (2-methoxyphenyl) -1,2,4-triazol-3-ylthio) acetate (intraperitoneally in the amount of 25 mg per kg of animal body weight 2 times a day for 5 days).

The animals were taken out of the experiment according to the prescribed time limit. For an immunohistochemical study, material from the bronchi and lungs was first fixed in a 10% solution of neutral buffered formalin for 10–12 hours and compacted into paraffin. Slices made 4-6 µm thick were mounted on adhesives SuperFrost Plus slides (Menzel Glaser, Germany). In our study, CD4, CD8, CD20, CD67 (Thermo Scientific, USA) were used as primary antibodies. The next stage of immunohistochemistry was performed using the latest generation Quanto imaging system (Thermo Scientific, USA). Secondary antibodies with a high content of horseradish peroxidase molecules were applied in sections and incubated in humid chambers for 10 minutes with washing in TRIS-buffer solution between each stage for 5 minutes. The reaction was identified by applying a DAB chromogen under microscope control. pas for 20 seconds to 1 minute, with specific structures showing dark brown color. Morphological study of the obtained sections was performed using a Primo Star light microscope (Zeiss, Germany) with a documentation system Using computer morphometric analysis among elements of lymphoid tissue associated with the trachea and bronchi, the average number of immunocompetent cells was noted: CD4+, CD8+, CD20+, CD67+ cells.

A quantitative analysis of the results of a morphometric study and statistical processing of morphometric data will be carried out according to generally accepted statistical methods and using Microsoft Office Excel and Statistica 6.1. The significance of differences between the values of independent micrometric values is determined by Student's criterion

Results. Protection of the mucous membrane of the respiratory tract is carried out by the mucous barrier with the epithelium, which provides mucociliary clearance, intraepithelial lymphocytes, and lymphoid tissue associated with the mucous membrane of the bronchi. As a result of our research, it was revealed that BALT consists of lymphoepithelial lymphoid nodules. Lymphoid nodules were a highly organized structure of lymphocytes and antigen-presenting cells. CD20+ cells formed lymphoid nodules, sometimes with a germinal center, which are surrounded by a

perifollycular area consisting of T cells of predominantly CD4+ origin. CD67+ cells are located between the T cells for transport and the presentation of antigens from the lumen of the bronchi to the T cells. Lymphocytes enter the BALT through high endothelial venules (HEV), which are present in the lymphoid nodules. Sometimes there were other lymphoid accumulations, mainly in the lung parenchyma, which did not meet all the criteria for BALT. These lymphoid structures were not in contact with the bronchial epithelium and were more in contact with the lumen of the alveoli than with the bronchial or bronchiolar lumen. They were predominantly CD20+ cells with a weakly pronounced germinal center, surrounded mainly by CD4+ T cells, but also diffusely located CD8+ T cells. These lymphoid accumulations were more often observed in animals after exposure to tobacco smoke, which was the trigger factor for their development (tab. 1).

Table

Dynamics of the quantitative composition of immunocompetent cells in the composition of BALT of rats

Group	Type of immunocompetent cells			
	$CD4^+$	$CD8^+$	$CD20^+$	$CD67^+$
I	35,48±0,95	25,90±0,50	19,15±0,16	11,22±0,33
II	45,60±0,71*	36,24±1,24***	38,25±2,91***	25,37±2,13***
III	37,86±2,51	27,19±1,96**	20,56±0,61	14,94±1,98***

I - intact group; II - animals with experimental chronic obstructive pulmonary disease; III - animals with experimental chronic obstructive pulmonary disease and administration of thiometrisol.

** -p <0.05; ** - p <0.01; *** - p <0.001 (in comparison with intact animals).*

Discussion. Thus, the study showed that in experimental animals there are violations of the cellular, humoral and nonspecific parts of the immune system. An increase in the content of B-lymphocytes (CD20 + cells), the main lymphocyte phenotypes (CD4 + and CD8 + cells) provokes an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines. All this creates conditions for the formation of chronic immune deficiency, which is a marker of an adverse course of COPD [2, 5].

Conclusion. The results confirm the need for adequate treatment of patients with COPD for the elimination of chronic inflammation; they justify the use of thiometrisol in complex therapy. Its can use successfully supplement standard therapy and improve the state of the local immune system of the lungs.

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