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EFFECT OF INTERMITTENT NORMOBARIC HYPOXIA ON THE MORPHOLOGICAL CHANGES IN THE RESPIRATORY PART OF LUNGS IN DIFFERENT SEASONS OF THE YEAR

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Abstract

The purpose of this work was to study and compare the effect of intermittent normobaric hypoxia on the morphological and biochemical indices of the functional state and structure of the respiratory part of the lungs in the spring and autumn. The experimental rats were daily exposed to hypoxic gas mixture (12% oxygen in nitrogen) in intermittent mode: 15 minutes deoxygenation / 15 minutes reoxygenation for 2 hours. The duration of the experiment was 28 days. The histological preparations were made by the standard methods. The slides were photographed using a digital camera on the microscope «Nikon» (Japan). The morphometric analysis was performed on digital images with using the computer program "Image J". In the lung tissue was determined the concentration of total hydroxyproline and lipids. The results of our studies showed the seasonal differences in the effect of intermittent normobaric hypoxia on the morpho-functional state of respiratory part of the lungs. It can be related to the features of its activity in the spring and autumn. In spring, during a period of lesser activity of respiratory part of the lung, prolonged exposure to intermittent normobaric hypoxia slightly increases its functional activity. But in autumn, during a period of greater activity, it decreases. Thus, the use of intermittent normobaric hypoxia makes it possible to smooth seasonal differences in respiratory part of the lung activity.

Key words: **intermittent normobaric hypoxia, lung, morphometry.**

INTRODUCTION

Lung diseases occupy the third place in the structure of total mortality in the world. During the human life, lungs are exposed to various unfavorable factors, such as: air pollution from industrial emissions and exhaust fumes, smoking, drug abuse etc. The seasonality of exacerbations is most pronounced for many chronic lung diseases especially in the transitional seasons of the year – in spring and autumn. This is due not only to the specific etiology and pathogenesis of these diseases or seasonal changes in climatic and other environmental factors (temperature, humidity, air saturation of pollutants), but also features of seasonal biorhythms of the human body, including respiratory organs. In this case, the respiratory parts of the lungs suffer most.

One of the effective non-pharmacological methods for treatment and prevention of lungs diseases is the method of intermittent normobaric hypoxia (INH). Currently, the sanogenic level of INH is increasingly used in clinical practice for treatment and prevention of a number diseases of the cardiovascular, respiratory, endocrine, digestive and immune systems, as well as to increase the human nonspecific resistance and adaptive capacity [1-3].

The literature data about the effect of INH on lungs are incomplete and ambiguous. This may be due to different conditions of the experiments: the use of normo- or hypobaric hypoxia, the level of oxygen partial pressure, the seasonality of the experiments, the total duration of exposure to hypoxic gas mixtures, the ratio of periods of deoxygenation and reoxygenation etc. [4, 5].

The aim of our work was to investigate and compare the effect of intermittent normobaric hypoxia on the functional state and structure of the lungs respiratory part in spring and autumn.

MATERIAL AND METHODS

The study was carried out on 48 male rats of the Wistar line. The animals were taken from the vivarium nursery of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine. The age of rats at the end of the experiment was 4 months, weight 270 ± 10 g.

Rats were held in unified conditions and the standard diet. The animals were divided into 4 groups: I and III – control rats in spring (March-April) and autumn (October-November), respectively; II and IV – experimental rats in the same seasons of the year, respectively. Experimental rats were daily exposed effects of hypoxic gas mixture. The rats were placed in a sealed chamber for hypoxic session. A hypoxic gas mixture (12% oxygen in nitrogen) was supplied into this chamber from the membrane gas separator element in the intermittent mode: 15 min deoxygenation / 15 min reoxygenation for 2 hours. The rats were in cages and breathed atmospheric air at all the remaining time of day (22 hours). The total duration of the experiment was 28 days.

The rats were removed from the experiment by decapitation under ether narcosis. All research protocols corresponded to the provisions of the Council of Europe Convention on Bioethics (1997), the Helsinki Declaration of the World Medical Association (1996), the European Convention for the Protection of Vertebrates, which are used for experimental and other scientific purposes (Strasbourg, 1985), the general ethical principles of animal experiments, adopted by the First National Congress of Ukraine on Bioethics (2001), as well as a committee with biomedical ethics of the A. A. Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine.

Histological preparations of lung tissue were prepared according to a standard procedure: fixed in Buen's liquid, dehydrated in spirits of increasing concentration and dioxane and poured into paraffin. The obtained preparations were used for morphological and morphometric studies. The sections were stained with Bemer's hematoxylin and eosin, and for the detection of connective tissue elements – by the Van Gyzon and Mason method [6]. Microscopic preparations were photographed on a microscope "Nicon" (Japan) using a digital camera. The morphometry of the preparations digital images was performed using the computer program "Image J".

The next parameters of the lung tissue histological sections were measured: the mean diameter of the alveolar lumen, the depth and area of the alveolus, the width of the entrance to the alveolus, the thickness of the interalveolar septum, the diameter of the respiratory bronchioles, alveolar courses and sacs. The ratio of the alveolar entrance width to its depth and the ratio of the respiratory bronchioles diameter, alveolar courses and sacs to the double depth of the alveolus were determined [7]. The concentration of total hydroxyproline in the lung tissue was determined photometrically by oxidation of hydroxyproline with chloramine T. The lipids concentration in lung tissue was determined by the phosphor-valeric method using a standard set of reagents from the firm «Filisit Diagnostika».

Statistical processing was carried out using variation statistics methods using the computer program Statistica 6.0. The normal distribution of digital arrays was verified using the Pearson criterion. When the distribution was normal, the Student's t-test was used to estimate the difference in the reliability of the difference between the control and experimental groups. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The lungs respiratory part (RPL) is represented by respiratory bronchioles (RB), alveolar courses (AC), alveolar sacs (AS) and alveolus. Because of the difficulty in identifying structural differences between AC and AS in histological sections, as well as the differences between peripheral bronchioles and AC, they are generally considered to be one group [7].

Morphological differences in RPL between control rats at different seasons of the year are revealed. It is established that in autumn animals had a significantly larger cross-sectional area and alveolar depth (by 13 %), a smaller thickness of the interalveolar septum (by 18 %), a smaller ratio of the alveolar entrance width to its depth (by 16 %) and the ratio of the RB, AC and AS diameter to the double depth of the alveolus (by 14 %) compared with rats in spring (Table 1).

Such differences may indicate a greater total area of the alveolar surface, airiness of the alveolus, functional activity of the rats RPL in the autumn. These lungs morphological and functional differences should be taken into account when conducting experimental studies in different seasons of the year.

The lumens of RB, AC and AS were free, and desquamation of the alveolar epithelium was not observed in both experimental groups of rats. The differences in RPL structure are also noted in animals exposed to INH in different seasons of the year. Experimental rats had a significantly smaller width of the entrance to the alveolus (by 9 %), a smaller ratio of the width of the entrance to the alveolus to its depth (by 12 %) compared to the control rats in spring. At the same time, the dimensions of the alveolus had only a slight tendency to increase (Table 1).

Table 1

Morphometric indices of the state of the lungs respiratory part of control and experimental groups (M ± m, n = 12)

Index	Spring		Autumn	
	Control	Hypoxia	Control	Hypoxia
Mean diameter of alveolus lumen, µm	23,6±0,63	23,7±0,59	24,3±0,59	22,7±0,49
Depth of alveolus, µm	21,7±0,13	22,5±0,61	24,5±0,56**	22,9±0,74
Width of the entrance to the alveolus, µm	14,6±0,21	13,3±0,21*	13,7±0,29	13,8±0,52
Cross-sectional area of alveolus, µm^2	671,5±18,3	689,3±20,7	758,0±22,8**	652,6±15,2*
Diameter of lumen of respiratory bronchioles, alveolar courses and alveolar sacs, µm	72,5±2,38	72,2±2,15	70,7±1,91	62,3±3,11*
Thickness of interalveolar septum, µm	5,1±0,24	4,3±0,16*	4,2±0,25**	3,9±0,17
The ratio of the width of the entrance to the alveolus to its depth	0,67±0,02	0,59±0,02*	0,56±0,03**	0,60±0,03
The ratio of the diameter of the respiratory bronchioles, alveolar courses and alveolar sacs to the double depth of the alveolus	1,67±0,07	1,6±0,05	1,44±0,06**	1,36±0,06

Note: here and in Table 2 * $p < 0.05$ - significant differences in comparison with the control

** $p < 0.05$ - significant differences compared to the control in the spring period

The mean of diameter, depth and area of alveolus in the lungs of experimental rats decreased respectively by 3 %, 7 % and 14 % ($p<0.05$) after effect of INH in autumn. These animals had a significantly smaller diameter of RB, AC and AS at 12 % (Table 1, Fig.). The changes in these indicators may reflect a decrease in the alveolar surface, which has a significant effect on the state of the lungs gas exchange function.

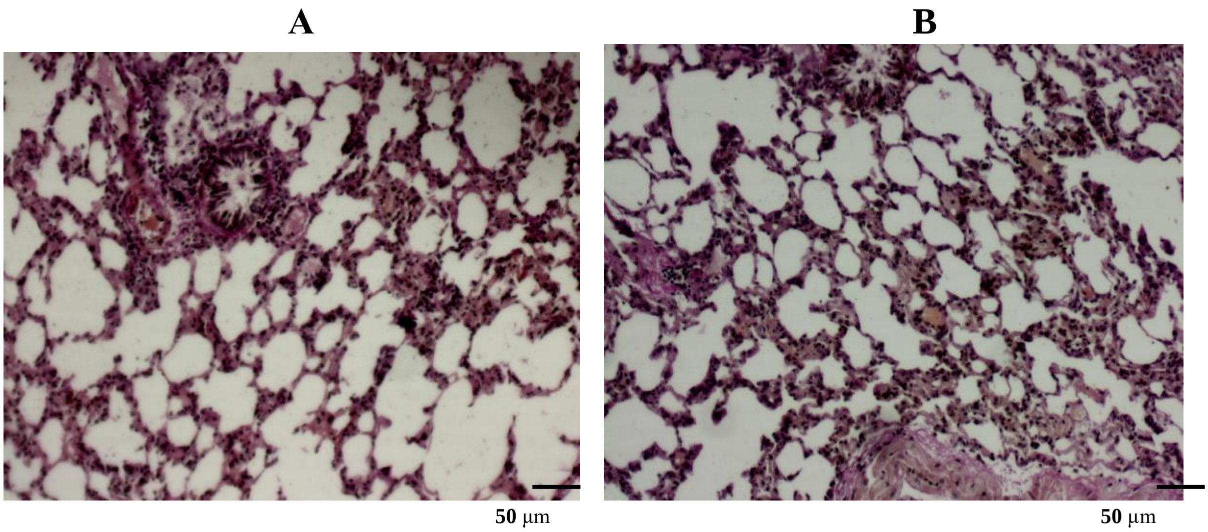

Fig. Microphotograph of the respiratory part of the lung in control rats (A) and exposed to INH (B) in the autumn. Van Gieson color. Increase 200.

The interalveolar septum consisted of epithelial layers of alveolus, subepithelial basal membranes, a network of capillaries, as well as elastic, reticular and collagen fibers [8]. The thickness of interalveolar septum decreased by 16 % after INH exposure in spring and by 7 % – in the autumn, compared to the control (Table 1). This, perhaps, is associated with a decrease in thickness of connective tissue fibers in the interalveolar septum, which leads to an increase in rate of gas exchange and lungs ventilation [7].

The results of the connective tissue specific staining showed no significant differences in the color intensity and the collagen fibers amount between the control and experimental rats. The greatest amount of collagen fibers was located around the RB and blood vessels. There were significantly fewer in the interalveolar septum (Fig.).

Collagen is known to be the main structural component of connective tissue. Its characteristic feature is a high content of hydroxyproline (12-14 %). This allowed to consider hydroxyproline as a specific marker of collagen. Its concentration can be judged by the rate of collagen catabolism. The determination of the concentration of hydroxyproline is often used to assess the state of connective tissue in various organs [9]. In our studies, the concentration of hydroxyproline, regardless of the season of INH exposure, remained at the level of control values (Table 2).

Table 2

The concentration of hydroxyproline and lipids in the lung tissue of control and experimental groups (M ± m, n = 12)

Index	Spring		Autumn	
	Control	Hypoxia	Control	Hypoxia
Concentration of hydroxyproline in the lungs, μg / mg	4,03±0,39	4,15±0,46	4,97±0,66	5,06±0,53
Concentration of lipids in the lungs, mg / g	20,05±0,50	22,6±0,24*	21,61±3,95	13,62±0,99*

A number of studies have shown an increase in the expression of collagen and growth factor in lung tissue after effect of hypobaric and normobaric hypoxia [10-12]. Falanga V. et al. showed that the total collagen concentration, which was determined by the content of hydroxyproline, increased only under conditions of hypobaric hypoxia. But after effect of normobaric hypoxia it remained unchanged [13]. Apparently, the differences in content of oxygen and atmospheric pressure magnitude caused the peculiarities of the lung connective tissue reaction to hypoxic action.

One of the important characteristic of lung tissue is the relatively high lipid content, compared to other organs. Lipids, phospholipids, which are synthesized in lung tissue, are used mainly for the synthesis of surfactants [8, 14]. Surfactants, in the form of a bi-cellular layer, are located in the alveolus at the boundary with the air and regulate the surface tension with a change in their volume. It was revealed that after the influence of INH in spring the concentration of lipids in the lungs tissue increased by 13 % ($p < 0.05$). The lipids concentration in the lungs tissue of the experimental rats, on the contrary, was significantly less (by 37 %) in autumn, compared to the control rats (Table 2). The results of our study are consistent with prior studies. Adaptive changes in rats RPL after effect of hypoxia were shown by E.V. Rozova [4]. The major changes lay in the fact that the number of surfactant-synthesizing structures increased – lamellar bodies (by 40 %) and free surfactants (by 75 %).

CONCLUSIONS

Analysis of the results of our studies suggests that the seasonal differences in the effect of INH on the morphological and functional state of the lungs respiratory part may be associated with the peculiarities of its physiological activity in spring and autumn. In spring, when the total area of the alveolar surface and the airiness of the alveolus are less, the prolonged exposure to intermittent normobaric hypoxia slightly increases the gas exchange function of the lungs, but in autumn it acts oppositely. Thus, the use of intermittent normobaric hypoxia makes it possible to smooth seasonal differences in lung activity. However, the physiological significance of this effect requires further study and refinement.

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