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Morphology of lymph nodes and lymphocytes of peripheral blood during acute hypobaric hypoxia

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The article provides information about the research work carried out to study the characteristic features of morphofunctional changes occurring in the mesenteric lymph nodes and lymphocytes of peripheral blood during acute barocamera hypoxia. The study was conducted on adult male white rats with a mass of 180-200 grams. Healthy animals included in control group were not intervened, respectively acute hypoxia model was established on the II group of animals. 2 and 5 days after the experiment, the mesenteric lymph nodes were taken from the peritoneal cavity, blood from the tail vein of the animals. Mesenteric lymph nodes and peripheral blood indicators were studied using histological and morphometric methods of examination. Conducted studies have shown that in acute hypoxia, sufficient structural-functional changes of systemic character occur in lymphoid organs. At an early stage of the experiment, these changes are of a nonspecific «stress» nature, transient neutrophil leukocytosis, eosinopenia and the formation of temporary lymphopenia lead to the development of involutive-cell dystrophy, thereby initiating the first phase – mobilization phase, characterized by a decrease in adaptation intensity.

Keywords: *Acute hypobaric hypoxia, mesenterica lymph node, lymphocyte, follicle, structure*

INTRODUCTION

Hypoxia is one of the main irritant factors affecting the human body, causing the development of various pathologies, especially a number of changes in the immune system (Берова, 2007; Nikolsky, 2012). At the same time, the development of hypoxia plays a role in the formation of compensatory-adaptive reactions aimed at restoring the normal supply of tissues with oxygen. At this time, a number of biochemical reactions aimed at weakening the oxygen starvation of cells are active, and in the formation of these reactions, along with the organs of the cardiovascular and respiratory system, blood and lymphatic systems also have great importance (Alieva, 2011; Antipov, 2012; Volkov, 2015).

Regulating the interaction of organs and systems, blood is the main indicator of the internal environment of the organism, reacts immediately to oxygen deficiency. According to the authors, due to the high content of catecholamines, thyroid and corticosteroid hormones in the blood, erythrocytes from the bone marrow and blood vessels pass into the general blood circulation, resulting in the development of polycythemia (erythrocytosis), which increases the oxygen capacity of the blood. This leads to the development of long-term compensatory-adaptive reactions in the body during hypoxia of various origins (acute hypoxia, and repeated moderate-intensity hypoxia) (Khaibullina et al., 2012; Ivanov, 2014)

Leukocytes (white blood cells), especially lymphocytes, are more sensitive to the hypoxia (Kiseleva et al., 2012). Lymphocytes are not only involved in inflammation or immune response, but they also form an important system, providing homeostasis in organs, adaptation to the pathological conditions and new environments (Ivanova et al., 2014).

Despite the fact that numerous research studies have been devoted to the study of the effect of hypoxia on the immune system (Charles et al., 2019; Willard-Mack, 2006; Helbling et al., 2017),

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it is of great interest to study the morphological state of lymphoid organs (mesenteric lymph nodes and peripheral blood lymphocytes), their role in the course and development of hypoxic pathology, as well as in the formation of pathogenetic mechanisms, taking into account the high potential capabilities of immune system organs.

The aim of the study was to study the characteristic features of morpho-functional changes occurring in mesenteric lymph nodes and lymphocytes of peripheral blood in conditions of acute hypobaric hypoxia.

MATERIALS AND METHODS

To study the effect of acute hypobaric hypoxia, the experiment was conducted on 40 white rats weighing 180-200 grams. Animals are divided into 2 groups – control and experience groups. The animals included in the control group were not intervened, and the second group of experimental animals was experi¬mented in the daytime (about 10-15). To this end, they were put into the barocamera for 2 hours and created a model of acute hypoxia, 5 times a week with a break of 1 hour, 2 times a day and 2 hours every other day. In the barocamera, the tempera¬ture was 19-20ºC, atmospheric pressure was equal to the pressure 2000-3000 m above the sea level, the particles of natron lime ($Ca(OH)_2$ 81%+NaOH 3,4%+H_2O 15,6%) were used to absorb the CO_2 generated during respiration. The animals removed from the barocamera were provided with water and food and kept under control in standard vivarium conditions. On the 2nd and 5th day of the experiment, intraperitoneal anesthesia was performed by introducing 2.0-2.5% thiopental-sodium solution (100 mg/kg) into the peritoneal cavity of animals. Preparations for histological and morphometric examination were taken from the mesenteric lymph nodes and peripheral blood of decapitated animals.

Mesenteric lymph nodes and peripheral blood parameters were studied using histological and morphometric methods.

The sections of the mesenteric lymph nodes are stained with hematoxylin-eosin and covered with encrusted glass through the Canadian balm. Microscopic examination was performed under x20 and x40 magnification. Microphotography of the structural elements of the lymph nodes was performed by a digital camera of the microscope «Olympus BX-41», and morphometric parameters were calculated by Microsoft Excel computer program.

Lymphocytes of peripheral blood were studied by a binocular microscope (XSZ-107BN). The cells stained by the Romanovsky-Gimza method (Azur eosin stain) were examined in standard swabs. The leukocytic formula of the blood was calculated, leukocytes were counted, as well as morphometric analysis of lymphocytes was performed on the stained blood. The obtained morphometric indicators are (StatSoft. Inc.) calculated by the Statistica 10 computer program; statistical processing on W – Wilcoxon test (paired samples) was carried out with the control group.

Animal research was carried out in Pharmacology and Experimental Surgery departments, and the Electron Microscopy Laboratory of the Scientific Research Center of Azerbaijan Medical University. The design of the experiment was approved by the ethical committee (Protocol No. 31 of the Ethics Rules Commission and Bioethics Committee under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008.).

RESULTS AND DISCUSSION

In preparations stained with hemotoxin-eosin, each lymph node is covered with the capsules. The capsule is made by numerous collagen fibers, and the smooth muscle fibers in the hilus area are also found. The capsule sends dense connective tissue septa into the lymph node to form its stroma. Beneath the capsule, there is a small subcapsular sinus, whose walls are covered by endothelial cells and including the fine-grained network formed by reticular fibers and macrophages. The subcapsular sinus is penetrated by the numerous lymph vessels, and microscopically the parenchyma of the lymph nodes appears as a dense network of the lymphatic sinuses (Fig. 1).

The lymph node consists of the cortical and medullary substance, the paracortical zone located at the border of the cortical and medullary substance. The cortical substance is made up of follicles. Numerous lymphocytes in the center of the follicles, rich in blood vessels and forming the no-

dules, attract attention. The parenchyma of the medullary substance of the lymph node consists of clusters of lymphoid tissue and medullary trabeculae formed by reticular fibers. In the paracortical zone of the lymph node, venules covered with cuboidal endotheliocytes are visualized.

Fig. 1. Control group. Normal histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

Small lymphocytes in the cortical substance, the large lymphocytes in the medullary substance, and, medium-sized lymphocytes in the paracortical zone predominate. Histological study of lymphocytes in the peripheral blood of experimental animals included in the control group showed that the nucleus of lymphocytes repeats the shape of cells, they have a rounded shape, the size of which is within the norm (Fig. 2).

The number of lymphocytes in white blood cells of animals is 67.60±1.04%, and the number of neutrophils is 26.00±1.09% (table).

2 days after the experiment, the rats included in the II experimental group macroscopically were relatively immobile, no changes in their weight were observed. In experimental animals, weak changes in the structure of the lymph nodes are observed, morphological changes in the structure of peripheral blood lymphocytes are not detected, the nucleus of lymphocytes is round, and the size has not changed. In histological preparations, the border between the cortical and medullary substances of the mesenteric lymph nodes appears weak, the paracortical zone is not visualized, the number of lymphoid cells is reduced, in some areas lymphocytes are destructed.

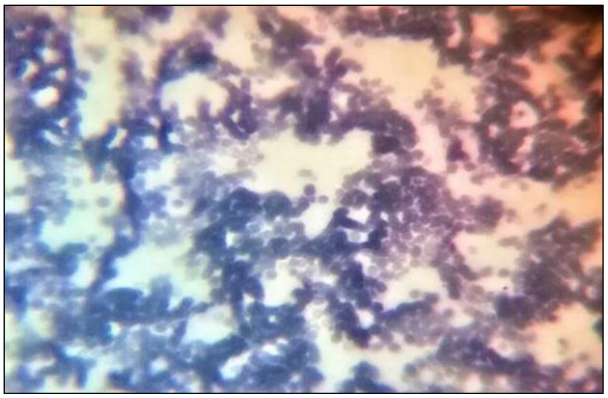

Fig. 2. Control group. Normal histological picture of peripheral blood lymphocytes. Stain: Romanovsky-Gimza x20.

In particular, the increase in the number of degranulated cells in the sinuses of the medullary substance attracts attention. Under the influence of hypoxia, there is an increase in the activity and number of macrophages in the lymph nodes of the mesenteric lymph nodes, mainly in the cavities of the sinuses. At the same time, eosinophils with a crystal structure are found in the microscope, most of which are located in the cortex. The plethoric capillaries in the microcirculatory bed are detected (Fig. 3).

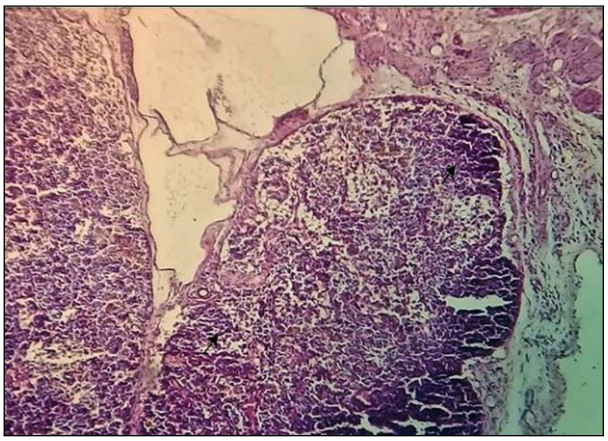

Fig. 3. 2nd day of hypoxia. Histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

Table. Parameters of leukocytic formula in peripheral b ood in norm and hypoxia *(M±m), (min-max)*

Leucocytes (L)		Control group (n)	Duration of experiment (days)	
			2nd day	5th day
Neutrophils (N)	Stab neutrophils	23.10±1.05	23.70±1.05	24.20±1.06
	Min-max	20.50-29.6	20.05-30.30	20.90-30.90
	%n	–	2.3	4.5
	%2nd day	–	–	2,1
	Segmented neutrophils	2.90±0.24	2,6±0,24	2.80±0.24
	Min-max	1.5-4.0	1.2-3.7	1.4-3.9
	%n	–	-9.6	-5.5
	%2nd day	–	–	4.6
Eosinophils		2.80±0.21	2.90±0.21	2.90±0.21
Min-max		1.9-4.4	1.99-4.49	1.93-4.43
%n		–	2.8	1.1
%2nd day		–	–	-1,7
Basophils		0.40±0.06	0.40±0.05	0.40±0.07
Min-max		0.2-0.7	0.23-0.72	0.18-0.78
%n		–	6.8	1.3
%2nd day		–	–	-5.2
Monocytes		3.20±0.33	3.20±0.33	3.20±0.33
Min-max		1.5-4.6	1.50-4.61	1.57-4.70
%n		–	0.2	2.4
%2nd day		–	–	2.3
Lymphocytes		67.60±1.04	67.20±1.03	66.6±1.01
Min-max		62.40-73.15	62.09-72.73	61.58-71.88
%n		–	-0.6	-1.5
%2nd day		–	–	-0.9
L/N		(67.60±1.04)/(26.00±1.09)	(67.20±1.03)/(26.30±1.08)	(66.60±1.01)/(26.90±1.07)

Note: n – control group; L – Lymphocytes*; N –* neutrophils*; M±m: M – average indicator of variation, m-standard error, p<0,01.*

In histological preparations, the nuclei of peripheral blood cells have a different form, the size of which has increased (Fig. 4).

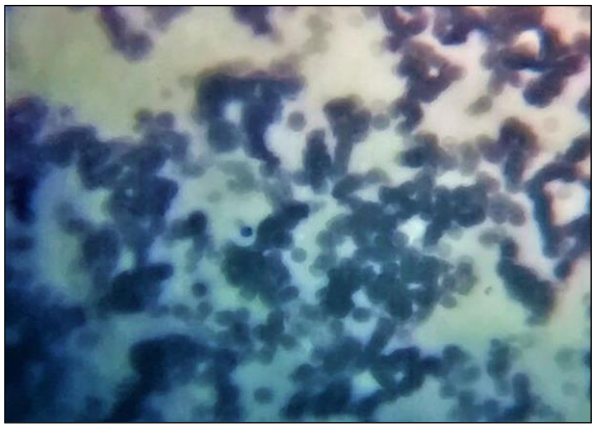

Fig. 4. 2nd day of hypoxia. Histological picture of peripheral blood lymphocytes. Stain: Romanovsky-Gimza x20.

At the same time, the number of individual populations of leukocytes in the blood, especially lymphocytes and neutrophils, was different. Thus, in the first days of the study, a decrease in the number of lymphocytes (67.20±1.03%) and a slight increase in the number of neutrophils (26.30±1.08%) was observed (tab.1). This is explained by the distribution of cell elements between lymphoid organs, circulating blood and bone marrow, as well as the transition to connective tissue.

On the 5th day of the acute hypoxia model, the examinations showed that the experimental animals were immobilized, reduced their weight, and increased their heart rate. In histological preparations, acute dystrophic and destructive changes develop in the cytoarchitectonics of morphofunctional zones of lymph nodes, as well as lymphocytes of peripheral blood. Lymph nodes grow in volume, the capsule is stretched, the parenchyma is hardened. It is impossible to distinguish the border between the cortical and medullary substances of the mesenteric lymph nodes, as well as the paracortical zone. The number and size of lymphoid nodules in the cortex of lymph nodes are reduced, and lymphocytolysis is observed (Fig. 5).

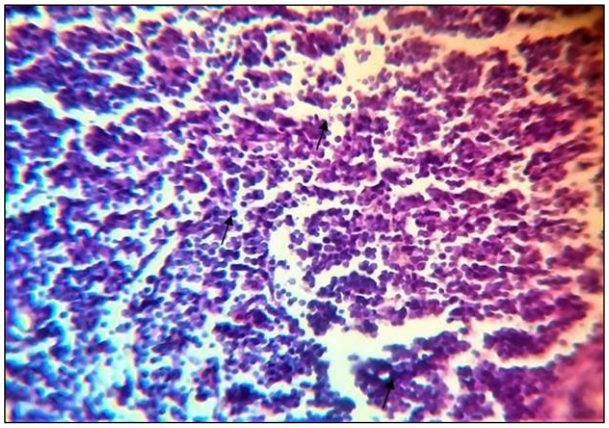

Fig. 5. 5th day of hypoxia. Histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

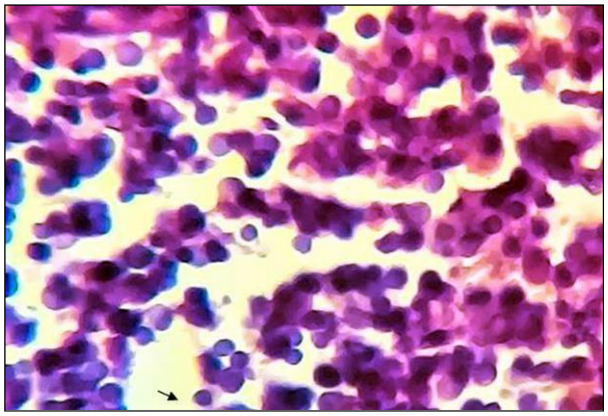

Fig. 6. 5th day of hypoxia. Histological picture of peripheral blood lymphocytes.
Stain: Romanovsky-Gimza x40.

In some areas, especially in the nuclei of lymphocytes of the medullary substance, pyknotic changes occur. Under the influence of acute hypoxia, macrophages are destroyed, destruction processes of lymphocytes are intensified, formation and increasing of the number of eosinophil granulocytes are noted. Cell changes caused by the effects of hypoxia were similar for all zones of the lymph nodes, resulting in the destruction of its reticular stroma.

At an early stage of acute hypoxia, morphological differences in the structure of lymphocytes are observed (Fig. 6). In a single volume of blood, the proliferation of lymphocytes, a decrease in large-sized lymphocytes, an increase in the number of small-sized lymphocytes and neutrophils is detected. Thus, the number of lymphocytes was 66.6±1.01%, and the number of neutrophils increased by 3.3% compared to the control group and by 2.3% compared to the second day of the experiment. This is due to an increase in the number of stab neutrophils and a decrease in the number of segmented neutrophils. In general, the number of leukocytes in the blood of animals has increased compared to the control group, which is explained by an increase in the function of neutrophils. In connection with hypoxia developed in cells, neutrophils migrate to connective tissue, regulating the activation and performance of their functions in the stromal macrophages.

The cause of changes in blood composition is the general mobilization of the body against hypoxia and stimulation of the functional activity of lymphocytes in the blood under the influence of hypoxia. On the 5th day of the experiment, the study of morphometric parameters of peripheral blood showed weak neutrophil leukocytosis and lymphocytopenia.

Thus, on the 2nd and 5th days of acute hypoxia, the quantitative indicators of lymphocytes in peripheral blood are reduced compared to the control group and are the lowest (table)

CONCLUSION

Thus, the analysis of the results of histological and morphometric studies shows that during acute hypoxia, sufficient morphofunctional changes occur in the lymphoid organs of a systemic character. These changes in the lymphoid organs are of a nonspecific "stress" nature, transient neutrophil leukocytosis, eosinopenia and the formation of transient lymphopenia lead to the development of involutive-cell dystrophy, thereby initiating the first phase – mobilization phase, characterized by a decrease in adaptation intensity. Changes in the number and morphological structure of leukocytic cells in the course of the experiment are a general mobilization of the body's protection against physical, chemical and biological factors, as well as stress factors, and can be considered an indispensable investigation to evaluate the nonspecific adaptive reaction.

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Kəskin hipobarik hıpoksiya zamanı müsariqə limfa düyünlərinin və periferik qanın limfositlərinin morfologiyası

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Məqalədə kəskin barokamera hipoksiyası zamanı müsariqə limfa düyünlərində və periferik qanın limfositlərində baş verən struktur-funksional dəyişikliklərin səciyyəvi xüsusiyyətlərinin öyrənilməsi məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir. Tədqiqat 2 qrup üzrə ayrılmış kütləsi 180-200 qram olan yetkin erkək ağ siçovullar üzərində aparılmışdır. I kontrol qrupuna daxil edilən sağlam təçrübə heyvanlarına müdaxilə edilməmiş, II qrup üzərində kəskin hipoksiya modeli yaradılmışdır. Eksperimentdən 2 və 5 gün sonra heyvanların periton boşluğundan müsariqə limfa düyünləri, quyruq venasından qan götürülmüşdür. Müsariqə limfa düyünləri və periferik qanın göstəriciləri histoloji və morfometrik müayinə metodlarından istifadə etməklə öyrənilmişdir. Aparılmış tədqiqatlar göstərmişdir ki, kəskin hipoksiya zamanı limfoid orqanlarda sistemli xarakter daşıyan kifayət dərəcədə struktur-funksional dəyişikliklər baş verir. Limfoid orqanlarda baş verən bu dəyişikliklər qeyri-spesifik «stress» xarakteri daşıyır, keçici neytrofil leykositoz, eozinopeniya və müvəqqəti limfopeniyanın meydana gəlməsi involyütiv-hüceyrə distrofi-

yasının inkişafına səbəb olur, bununla da uyğunlaşma intensivliyinin azalması ilə xarakterizə olunan ilk faza – səfərbərlik fazası başlanır.

Açar sözlər: *Kəskin hipobarik hipoksiya, müsariqə limfa düyünü, limfosit, follikul, struktur*

Морфология лимфоцитов брыжеечных лимфоузлов и периферической крови при острой гипобарической гипоксии

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В статье представлена информация об исследовательской работе, проведенной с целью изучения характерных особенностей структурно-функциональных изменений в брыжеечных лимфоузлах и лимфоцитах периферической крови при острой барокамерной гипоксии. Исследование проводилось у взрослых белых крыс-самцов весом 180-200 грамм, разделенных на две группы. Здоровые подопытные животные, входящие в I группу контроля, не подвергались вмешательству, у II группы подопытных животных была создана соответствующая модель острой гипоксии. Через 2-е и 5-ро суток после эксперимента у подопытных животных была взята кровь из хвостовой вены и из брыжеечных лимфатических узлов в брюшной полости. Показатели крови из брыжеечных лимфатических узлов и периферической крови изучались с использованием методов гистологического и морфометрического обследования. Проведенные исследования показали, что при острой гипоксии в лимфоидных органах в колоссальной степени происходят структурно-функциональные изменения, имеющие системный характер. На ранней стадии эксперимента эти изменения носят неспецифический «стрессовый» характер, а появление переходного нейтрофильного лейкоцитоза, эозинопении и временной лимфопении становится причиной развития инволютивно-клеточной дистрофии, что в свою очередь дает начало первой фазе – фазе мобилизации, характеризующейся снижением интенсивности адаптации.

Ключевые слова: *Гипобарическая гипоксия, брыжеечный лимфоузел, лимфоцит, фолликул, структура*