

Structural changes of lymph nodes under high calorie diet and melatonin correction

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The article presents and analyzes data from an experimental study conducted on white rats in females and males of reproductive age. The purpose of the study to study the morphometric and histological changes in the parenchyma of the lymph nodes of rats under the high-calorie diet (HCD) and with the correction of melatonin. The study was performed on 80 white rats of reproductive age. Microanatomy of the structural components of lymph nodes of white rats under physiological norms was examined in 10 intact animals. Experimental animals are divided into 5 groups. Statistical processing of digital data was performed using "Excel" software and "STATISTICA 6.0" using the parametric method. Eight weeks after HCD, there was a significant decrease in the relative area of the cortical substance in the parenchyma of lymph nodes of white rats of males and females by 10.3 % and 8.3 %, respectively, and an increase in the relative area of the medullary substance by 16.1 % and 13.2 %, respectively, greater than the intact animal group parameter. Corticomedullary index (CMI) decreased by 22.9 % and 19.0 %. After six weeks of HCD and the next six weeks of standard vivarium diet and melatonin administration, the relative area of cortical substance in the parenchyma of lymph nodes of white rats in males and females was 2.0 % and 2.9 %, respectively, greater than the parameters of the intact group of animals. Accordingly, the relative area of the medullary substance is 3.1 % and 4.6 % less than the parameters of the intact group of animals. CMI in both male and female rats was 5.1 % and 7.6 %, respectively, greater than the intact animal group parameter. Under the conditions of melatonin correction, it was found that on the histological preparations of lymph nodes the vein and artery were full-blooded. Empty hemocapillaries with thickened wall are observed. In the paracortical region, the number of high endothelial capillary venules decreases. Thus, long-term administration of melatonin improves the morphometric parameters of the parenchyma of the lymph nodes of rats, restores the morphological structure of the organ.

Keywords: experiment, lymph node, sodium glutamate, melatonin, correction.

Introduction

Obesity is a risk factor for human health. This is a complex medico-social problem of today, as most overweight people do not see this condition as a real threat to health [9, 11, 22]. The current issue is the study of the effect of obesity on organs and tissues, as well as the possibility of correction of these changes [1, 14, 15]. Of particular interest are the organs of the immune system, in particular lymph nodes, which provide protection of the body against genetically foreign cells and substances coming exo- or endo-pathways [6, 7, 13, 18, 20]. They are also called biological "filters".

In most cases, a high-calorie diet is used to develop experimental obesity. We choose sodium glutamate as a

dietary supplement to the standard vivarium diet. The monosodium salt of glutamic acid (glutamate sodium), known as the "flavor enhancer", is used in most groceries.

Melatonin, a pineal hormone, was selected for correction. It is an important regulator of sleep and circadian rhythms [4, 16, 19, 24]. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized by pinealocytes of the epiphysis under the control of the suprachiasmatic nucleus of the hypothalamus (the principal driver of circadian rhythms) [5, 12, 17]. It can also be formed in the heart, kidneys, digestive canal, genitals and other cells [2]. Interest in the healing properties of melatonin increased when it was noted that it

was not only found in humans but also identified in many foods - olive oil, tomatoes, fish, wine and others [8].

Aim of the study: to study the morphometric and histological changes of the parenchyma of the lymph nodes of rats under high-calorie diet and with melatonin correction.

Materials and methods

The study was conducted on 80 white rats females and males of reproductive age (2.5-6.5 months) weighing 120-280 g.

The structure of lymph nodes of white rats under normal conditions was examined in ten intact animals. All experimental animals were divided into 5 groups: the first group (10 animals), which were fed HCD for 8 weeks; the second group (10 animals), which were fed a HCD for 8 weeks and then transferred to a standard vivarium diet (for 8 weeks); a third group (10 animals) fed a HCD for 6 weeks, then transferred to a standard vivarium diet and administered melatonin for 2 weeks; the fourth group (10 animals) and the fifth group (10 animals) are similar to the previous one, but melatonin was administered for 4 and 6 weeks, respectively. Each group consisted of 5 male rats and 5 female rats. HCD was achieved by adding 0.07 g/kg of rat body weight to food glutamate. The dose of melatonin was 10 mg/kg of body weight of the rat. The drug was administered orally daily in the afternoon at the same time. The control was served by 20 white rats who received a standard vivarium diet instead of the high-calorie diet.

All the test animals were in the vivarium of Danylo Halytsky Lviv National Medical University. The studies were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On the protection of animals from ill-treatment", general ethical principles of animal experiments approved by the First National Congress of Ukraine on Bioethics (2001).

Morphometric studies were performed on histological specimens stained with hematoxylin and eosin, as well as with Heidenhain's azan using Videotest-5.0, KAAPA Image Base, Stepanizer and Microsoft Exel on a personal computer.

Statistical processing of digital data was performed using "Excel" and "STATISTICA 6.0" software using parametric methods. The numerical values of the parameters are represented by sample averages (M), standard deviation (σ), standard error of the mean (m), Student's t test (t). The results of the calculations were presented in graphical form in histograms using Microsoft Office, indicating confidence intervals at 95 % confidence level ($p=0.95$).

Results

The structure of mesenteric lymph nodes in rats of intact and control groups corresponded to the species norm, as

evidenced by the results of histological examinations. Lymph nodes are surrounded externally by the connective tissue capsule, from which go deep into the parenchyma numerous cortex and medulla trabeculae. On the cramped part of the node there is hilum. The parenchyma of the lymph node consists of located on the periphery cortex, and closer to the hilum - medulla. Under the capsule contains the marginal sinus. The primary and secondary lymph nodes are located in the cortex. Secondary nodes contain an illumination center - a germinal center or breeding center. A darker boundary zone is located around such a center. The medulla consists of medullar cords and medullar sinuses (Fig. 1).

After 8 weeks of HCD, a significant decrease in the relative area of the cortex in the parenchyma of lymph nodes of white rats of males and females was found, by 10.3 % and 8.3 %, respectively, compared to the intact group of animals (Tables 1, 2). The relative area of the medulla increases and exceeds the parameters of the intact group of animals by 16.1 % and 13.2 %, respectively (see Tables 1, 2). CMI decreases in both male and female rats by 22.9 % and 19.0%, respectively.

In both male rats and female rats, cortical, medullar, and marginal lymphatic sinuses were dilated and deformed after 8 weeks of experiment. In their lumen, the proportion of reticular connective tissue increased, which was accompanied by a decrease in the proportion of lymphocytes (Fig. 2). Dense B-lymphocytes, plasmocytes, and macrophages were observed in the medullar cord. Empty hemocapillaries with thickened walls were often noticeable. The number of capillary venules increased, especially in the paracortical region of the lymph nodes. The process of migration of lymphocytes from the bloodstream to the parenchyma of the lymph node was pronounced, as evidenced by the large number of them in the lumen of the vessels and the wall. This confirms the

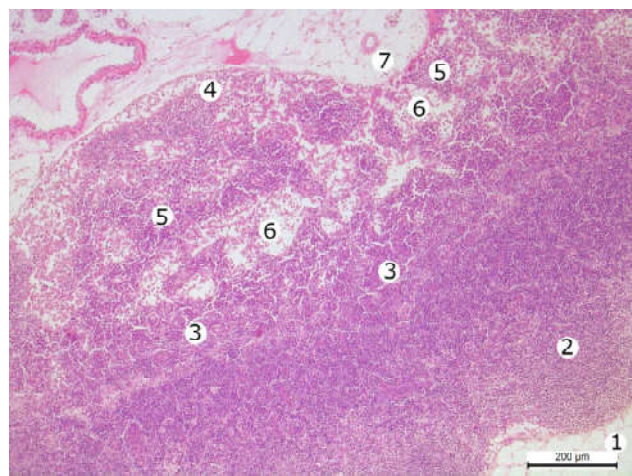


Fig. 1. Mesenteric lymph node of an intact white male rat. 1 - capsule of the lymph node; 2 - cortex; 3 - paracortical region; 4 - medulla; 5 - medullary cord; 6 - medullary sinus; 7 - hilum of the lymph node. Hematoxylin-eosin. Objective x10, eyepiece x10.

Table 1. Dynamics of changes in the relative area of cortex and medulla and corticomedullary index (CMI) of lymph nodes of white male rats in control and experimental groups (M±m).

Group of animals	S _{cortex} , %	S _{medulla} , %	CMI
Intact animals	61.08±1.56	38.92±0.78	1.569±0.112
Group 1 (8 weeks of HCD)	54.80±1.61*	45.20±0.87*	1.212±0.211*
Group 2 (8 weeks of HCD, 8 weeks of cancellation)	53.67±1.45*	46.33±0.81*	1.158±0.179*
Group 3 (6 weeks of HCD, 2 weeks of melatonin)	55.05±1.21*	44.95±0.76*	1.225±0.119*
Group 4 (6 weeks of HCD, 4 weeks of melatonin)	58.94±1.19*	41.06±0.83*	1.435±0.113*
Group 5 (6 weeks of HCD, 6 weeks of melatonin)	62.29±1.34	37.71±0.71	1.652±0.171

Notes: here and in the future, * - values that are statistically significantly different from those of the intact group of animals (p<0.05).

Table 2. Dynamics of changes in the relative area of cortex and medulla and corticomedullary index (CMI) of lymph nodes of white female rats in control and experimental groups (M±m).

Group of animals	S _{cortex} , %	S _{medulla} , %	CMI
Intact animals	61.23±1.70	38.77±0.76	1.579±0.109
Group 1 (8 weeks of HCD)	56.12±1.65*	43.88±0.78*	1.279±0.123*
Group 2 (8 weeks of HCD, 8 weeks of cancellation)	55.46±1.55*	44.54±0.67*	1.245±0.157*
Group 3 (6 weeks of HCD, 2 weeks of melatonin)	57.75±1.45*	42.25±0.71*	1.367±0.147*
Group 4 (6 weeks of HCD, 4 weeks of melatonin)	59.05±1.54	40.95±0.75*	1.442±0.211*
Group 5 (6 weeks of HCD, 6 weeks of melatonin)	63.01±1.45	36.99±0.81	1.703±0.185*

common belief among morphologists that obesity is a chronic inflammatory process that leads to the continued activity of the immune protection units. The number of secondary lymph nodes in the cortex of lymph nodes of white rats increases, they contain extended centers of reproduction. The relative area of the paracortex decreases. The enlarged, full-blooded and deformed arteries and veins contain platelets and erythrocytes with signs of adhesion and aggregation in their lumen. Often, vessels of the hemomicrocirculatory bed with a damaged wall are found, which leads to bleeding in the parenchyma of the organ.

After 8 weeks of abrogation of HCD, the relative area of the cortex in the parenchyma of lymph nodes of white rats of males and females decreased by only 2.1 % and 1.2 %, which is 12.1 % and 9.4 % less than the parameters of the intact group of animals (see Tables 1, 2). Accordingly, the relative area of the medulla increases by 2.5 % and 1.5 %,

which is 19.0 % and 14.9 % higher than the parameters of the intact group of animals (see Tables 1, 2). CMI decreases in both male and female rats by 26.1 % and 20.1 %, respectively. Histologically, both in male rats and female rats, the structure of the parenchyma of the lymph nodes differed little from the previous experimental group (Fig. 3). The fat content was significantly increased around the organ compared to the control group of animals. Trabeculae extending from the capsule are clearly expressed, thickened. Arteries with thickened wall, full-blooded, veins deformed, enlarged and full-blooded. The marginal sinus is unevenly expanded and deformed. The medullary sinus is enlarged, tortuous. The relative area of the paracortex decreases.

Morphometric indices in group 3 of animals (six weeks of HCD followed by 2 weeks of melatonin administration) indicate that the relative area of the cortex in the lymph nodes parenchyma of white rats of males and females decreased by 9.9 % compared to intact group of animals, by 9.9 % and 5.7 % (see Tables 1, 2). Accordingly, the relative area of the medulla increased by 15.5 % and 9.0 %, respectively, exceeding the similar parameters of the intact group of animals (see Tables 1, 2). CMI in both male and female rats was 22.3 % and 13.3 %, respectively, smaller than the intact animal parameter.

Histologically in the parenchyma of the lymph nodes of rats revealed that the number of secondary lymph nodes is slightly reduced compared with the previous group of animals, decreases the proportion of adipose tissue around the organ. However, the vessels are full-blooded, dilated, deformed. B-lymphocytes, plasmocytes and macrophages are densely located in the medullary cord. The number of monocytes, plasmocytes and macrophages increases (Fig. 4). An increase in the proportion of B-dependent zones and a decrease in T-dependent zones were detected. Such changes can lead to a redistribution

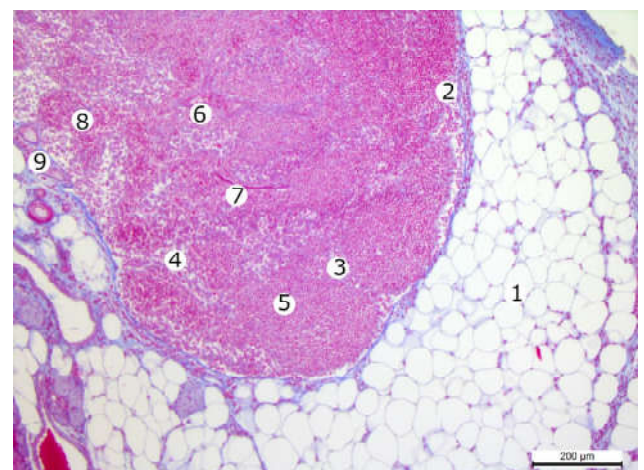


Fig. 2. Fragment of a lymph node of a white rat male after 8 weeks of HCD. 1 - adipose tissue in the thickness of the capsule and around the lymph node; 2 - marginal sinus; 3 - cortex sinus; 4 - medullary sinus; 5 - secondary lymph node; 6 - capillary venula; 7 - hemocapillary; 8 - medullary cord; 9 - hilum of the lymph node. Heidenhain's azan staining. Objective x10, eyepiece x10.

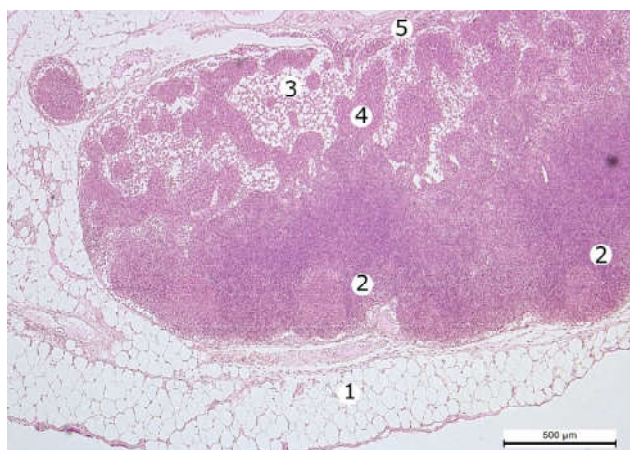


Fig. 3. Fragment of the lymph node of the white rat male after 8 weeks of HCD, followed by its cancellation after 8 weeks. 1 - adipose tissue in the thickness of the capsule and around the lymph node; 2 - secondary lymph node in the cortex; 3 - enlarged medullary sinus; 4 - medullary cord; 5 - hilum of the lymph node. Hematoxylin-eosin. Objective x5, eyepiece x10.

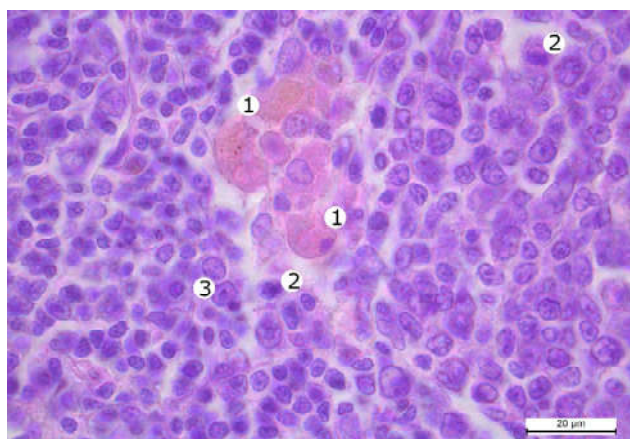


Fig. 4. Fragment of the cortex of the white rat female lymph node after 6 weeks of HCD and subsequent administration of melatonin for 2 weeks. 1 - macrophages; 2 - cells with signs of apoptosis; 3 - lymphocytes. Hematoxylin-eosin. Objective x100, eyepiece x10.

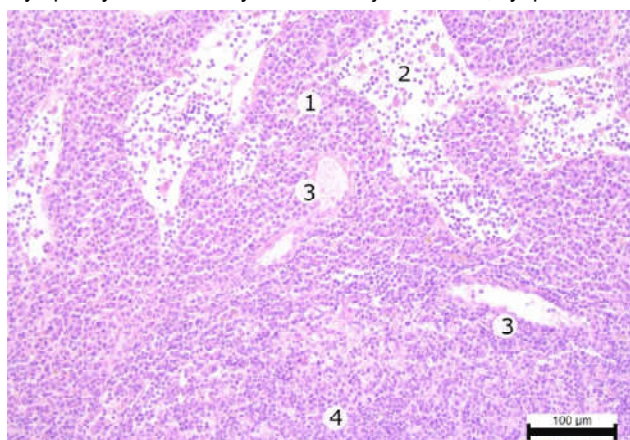


Fig. 5. A fragment of the medulla of the lymph node of the white rat female after 6 weeks HCD, followed by the introduction of melatonin for 6 weeks. 1 - medullary cord; 2 - medullary sinus; 3 - arterioles with thickened wall. Hematoxylin-eosin. Objective x10, eyepiece x10.

of activity toward a humoral immune response.

Morphometric indices in the fourth group of animals (6 weeks of HCD followed by 4 weeks of melatonin administration) indicate that the relative area of the cortex in the lymph nodes parenchyma of white rats in male and female rats increased by 7.1 % and 2.3 %, respectively compare with the previous group of animals. These figures are 3.5 % and 3.6 % lower than similar parameters of intact animals (see Tables 1, 2). Accordingly, the relative area of the medulla in the parenchyma of lymph nodes of white rats-males and females decreased by 8.7 % and 3.1 % compared with the previous group of animals, which is 5.5 % and 5.6 % higher than the same parameter of intact animals (see Tables 1, 2). CMI in both male and female rats was 8.3 % and 8.7 %, respectively, smaller than the intact animal group parameter.

Morphometric indices in the fifth group of animals (6 weeks of HCD followed by 6 weeks of melatonin administration) indicated that the relative area of the cortex in the lymph node parenchyma of white rats in males and females increased by 5.7 % and 6.7 %, respectively compare with the previous group of animals and is on 2.0 % and 2.9 % higher similar parameters of the intact group of animals (see Tables 1, 2). Accordingly, the relative area of the medulla decreases by 8.2 % and 9.7 % compared to the previous group of animals and is 3.1 % and 4.6 % less than the parameters of the intact group of animals (see Tables 1, 2). CMI in both male and female rats was 5.1 % and 7.6 %, respectively, greater than the intact animal parameter. Histologically, both full-blood veins and arteries occur in male and female rats of the fifth experimental group of animals, the artery and arterioles wall are thickened (Fig. 5). In the paracortex, the number of high endothelial capillary venules decreases. Empty hemocapillaries with thickened wall are found.

Discussion

Obesity reduces the size of inguinal lymph nodes, impairs lymphatic transport and migration of dendritic cells to peripheral lymph nodes, and reduces the number of T lymphocytes in lymph nodes. In general, obesity disrupts the integrity of the immune system and leads to changes in the development of leukocytes and lymphocytes, their migration and diversity [3].

Diet-induced obesity in mice leads to significant disruption of the lymphatic system, which is reflected by a decrease in lymph flow, changes in lymph node architecture and impaired dendritic cell migration. Using different research methods, it was discovered [23] that obesity leads to impaired lymphatic transport in the subcutaneous tissue and drainage it to the lymph nodes.

It has been found that body weight gain has a linear negative correlation with lymphatic function and that obesity leads to a decrease in lymphatic vessel density and a decrease in vascular filling. In addition, it has been found that increasing body weight and the degree of obesity lead to the progression of the surrounding lymphatic accumulation

of inflammatory cells [14].

Some authors [21] have argued that melatonin can correct structural and functional changes in the small intestinal wall that are caused by obesity. They also note that evening administration of the drug to experimental animals with obesity is more effective than morning administration.

In experiments on type 2 diabetes mellitus with resistant hypertension and in rats with metabolic syndrome [17], it was shown that the administration of melatonin significantly facilitates the course of hypertension.

Experimentally, [10] it was found that administration of melatonin leads to a decrease in body weight of rats, triglyceride levels, LDL, and an increase in plasma cholesterol and HDL. Ghada M. et al. [10] concluded that melatonin should be used as a treatment to prevent the harmful effects of obesity and related metabolic disorders such as dyslipidemia and insulin resistance.

The prospects for further development are related to the further study of the morphometric and submicroscopic changes in the structural organization of the lymph nodes of rats at different times of experimental obesity and its correction.

Conclusions

1. After 8 weeks of HCD a significant decrease in the relative area of the cortex in the parenchyma of lymph nodes of white rats of males and females by 10.3 % and 8.3 %, respectively, and an increase in the relative area of the medulla by 16.1 % and 13.2 % compared with an intact group of animals.

2. After 6 weeks of HCD, followed by melatonin for 6 weeks, the relative area of the cortex in the parenchyma of lymph nodes of white rats in males and females increased by 2.0 % and 2.9 %, respectively, compared with the intact group of animals. Accordingly, the relative area of the medulla decreased by 3.1 % and 4.6 %.

3. Under conditions of melatonin correction, it was found that the histological samples of lymph nodes in both male rats and female rats revealed full-blooded veins and arteries. Empty hemocapillars with thickened wall are available. In the paracortex, the number of high endothelial capillary venules decreases. Thus, long-term administration of melatonin "improves" the morphometric parameters of the parenchyma of the lymph nodes of rats and restores the morphological structure of the organ.

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СТРУКТУРНІ ЗМІНИ ЛІМФАТИЧНИХ ВУЗЛІВ ЗА УМОВ ВИСОКОКАЛОРИЙНОЇ ДІЄТИ ТА КОРЕКЦІЇ МЕЛАТОНИНОМ

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В статті наведені та проаналізовані дані експериментального дослідження, яке проводили на 80 білих щурах-самцях і самцях репродуктивного віку. Мета дослідження - вивчити морфометричні та гістологічні зміни паренхіми лімфатичних вузлів щурів за умов висококалорійної дієти (ВКД) та при корекції мелатоніном. Мікроанатомію структурних компонентів лімфатичних вузлів білих щурів за умов фізіологічної норми дослідили на 10 інтактних тваринах. Експериментальні тварини поділені на 5 груп. Статистичну обробку цифрових даних проводили за допомогою програмного забезпечення "Excel" та "STATISTICA 6.0" з використанням параметричного методу. Через 8 тижнів ВКД спостерігали достовірне зменшення відносної площі кіркової речовини у паренхімі лімфатичних вузлів білих щурів-самців та самиць на 10,3 % та 8,3 % та відповідно, збільшення відносної площі мозкової речовини на 16,1 % та 13,2 % у порівнянні з інтактною групою тварин. Кірково-мозковий індекс (КМІ) зменшився на 22,9 % та 19,0 %. Через 6 тижнів ВКД і наступних 6 тижнів стандартного харчового раціону віварію та введення мелатоніну відносна площа коркової речовини в паренхімі лімфатичних вузлів білих щурів-самців та самиць становила відповідно на 2,0 % та 2,9 % більше параметрів інтактної групи тварин. Відповідно відносна площа мозкової речовини зменшилася на 3,1 % та 4,6 % порівняно з інтактною групою тварин. Кірково-мозковий індекс (КМІ) як у щурів-самців, так і у щурів-самиць збільшився, відповідно, на 5,1 % та 7,6 % порівняно з інтактною групою тварин. Після корекції мелатоніном виявлено, що на гістологічних препаратах лімфатичних вузлів вени та артерії повнокровні. Наявні "порожні" гемокапіляри з потовщеною стінкою. У прикорковій ділянці кількість закапілярних венул з високим ендотелієм зменшується. Таким чином, тривале введення мелатоніну "покрощує" морфометричні показники паренхіми лімфатичних вузлів щурів, відновлює морфологічну будову органу.

Ключові слова: експеримент, лімфатичний вузол, глутамат натрію, мелатонін, корекція.

СТРУКТУРНЫЕ ИЗМЕНЕНИЯ ЛИМФАТИЧЕСКИХ УЗЛОВ В УСЛОВИЯХ ВЫСОКОКАЛОРИЙНОЙ ДИЕТЫ И КОРРЕКЦИИ МЕЛАТОНИНОМ

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В статье приведены и проанализированы данные экспериментального исследования, которое проводилось на 80 белых крысах самцах и самцах репродуктивного возраста. Цель исследования - изучить морфометрические и гистологические изменения паренхимы лимфатических узлов крыс в условиях высококалорийной диеты (ВКД) и при коррекции мелатонином. Микроанатомию структурных компонентов лимфатических узлов белых крыс в условиях физиологической нормы исследовали на 10 интактных животных. Экспериментальные животные разделены на 5 групп. Статистическую обработку цифровых данных проводили с помощью программного обеспечения "Excel" и "STATISTICA 6.0" с использованием параметрического метода. Через 8 недель ВКД наблюдали достоверное уменьшение относительной площади коркового вещества в паренхиме лимфатических узлов белых крыс-самцов и самок на 10,3 % и 8,3 % и, соответственно, увеличение относительной площади мозгового вещества на 16,1 % и 13,2 % по сравнению с интактной группой животных. Кірково-мозговой индекс (КМІ) уменьшился на 22,9 % и 19,0 %. Через 6 недель ВКД и последующих 6 недель стандартного пищевого рациона вивария с применением мелатонина относительная площадь коркового вещества в паренхиме лимфатических узлов белых крыс самцов и самок составляла на 2,0 % и 2,9 % больше параметров интактной группы животных. Соответственно относительная площадь мозгового вещества на 3,1 % и 4,6 % меньше параметров интактной группы животных. КМІ как у крыс-самцов, так и у крыс-самок на 5,1 % и 7,6 % соответственно больше параметра интактной группы животных. В условиях коррекции мелатонином выявлено, что на гистологических препаратах лимфатических узлов вены и артерии полнокровные. Наблюдаются "пустые" гемокапилляры с утолщенной стенкой. В околокорковом веществе количество закапиллярных венул с высоким эндотелием уменьшается. Таким образом, длительное введение мелатонина "улучшает" морфометрические показатели паренхимы лимфатических узлов крыс, восстанавливает морфологическое строение органа.

Ключевые слова: эксперимент, лимфатический узел, глутамат натрия, мелатонин, коррекция.