AGE-RELATED MORPHOFUNCTIONAL ALTERATIONS OF THE THYMUS IN WHITE RATS UNDER CONDITIONS OF EXPERIMENTAL ALIMENTARY ZINC DEFICIENCY

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Abstract. This article presents data from a comparative analysis of morphofunctional changes occurring in the thymus of white rats at different age periods under conditions of experimental alimentary zinc (Zn) deficiency. During the experiment, dynamic changes in the lymphoid structures of the thymus, the degree of thymocyte differentiation, the cortico-medullary ratio, as well as the response of stromal elements were assessed morphometrically. Disturbances of the parenchymal–stromal balance of the thymus, changes in morphometric parameters of lymphoid tissue, a decrease in the number of lymphoid cells, as well as thickening of the vessel walls of the organ and a reduction of their internal diameter were established. The obtained results reflect the important role of zinc in maintaining immune homeostasis and create a scientific basis for a deeper understanding of the immunopathological mechanisms of microelement deficiencies.

Keywords: zinc deficiency, thymus, white rat, T lymphocytes, immune system.

ВОЗРАСТНЫЕ МОРФОФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ ТИМУСА У БЕЛЫХ КРЫС В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО АЛИМЕНТАРНОГО ДЕФИЦИТА ЦИНКА

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Аннотация. В данной статье представлены данные сравнительного анализа морфофункциональных изменений, происходящих в тимусе белых крыс в различные возрастные периоды в условиях экспериментального алиментарного дефицита цинка (Zn). В ходе эксперимента морфометрически оценивались динамические изменения лимфоидных структур тимуса, степень дифференцировки тимоцитов, кортико-медуллярное соотношение, а также реакция стромальных элементов. Установлены нарушения паренхиматозно-стромального баланса тимуса, изменения морфометрических показателей лимфоидной ткани, уменьшение количества лимфоидных клеток, а также

утолщение стенок сосудов органа и уменьшение их внутреннего диаметра. Полученные результаты отражают важную роль цинка в поддержании иммунного гомеостаза и создают научную основу для более глубокого понимания иммунопатологических механизмов, обусловленных дефицитом микроэлементов.

Ключевые слова: дефицит цинка, тимус, белые крысы, Т-лимфоциты, иммунная система.

Relevance. The immune system ensures the adaptation of the organism to the effects of various damaging factors and provides its protection. Immunocompetent organs play an important role in maintaining the immunological homeostasis of the organism under experimental and clinical conditions [1]. The thymus is one of the most important organs responsible for maintaining the immune status of the organism and protecting it from aging processes and the development of diseases. The size, architecture, and functions of this gland change with age [7, 10].

Various chemical elements, particularly trace elements, are of great importance for maintaining physiological activity and overall health. Trace elements are necessary to ensure the processes of growth, development, reproduction, lactation, hematopoiesis, and other vital processes. The activity of the immune system depends on their content in the organism. Trace elements ensure mineral metabolism and participate in the regulation of all types of metabolic processes [8].

According to many specialists, human health is determined by the nature of the food products consumed. Among alimentary factors that are of great importance for the preservation of health, life expectancy, and quality of life, trace elements occupy one of the leading positions [4, 5, 9].

Zinc is a trace element essential for all living organisms and is involved in numerous biochemical processes occurring in cells [2, 6, 11, 13, 14]. Zinc participates in the formation of the antioxidant potential of cells during immune reactions and is an important element for the functioning of T and B lymphocytes [3]. Normal Zn levels ensure the implementation of the Th1 response and the maintenance of the integrity of mucous membranes. Additional zinc intake activates cellular components of innate immunity and antibody production. This trace element exhibits antioxidant activity and exerts a protective effect against oxidative stress [12].

Aim of the study. To assess the morphofunctional features of the thymus of white rats at different age periods under conditions of experimental alimentary zinc deficiency.

Materials and Methods. The study was conducted on 80 outbred white male rats. During the experiment, ethical standards for the use of animals and the requirements of the Helsinki Declaration were observed. The rats were kept under standard vivarium conditions. The age, sex, body weight, type of diet, and housing conditions of the laboratory animals met the requirements of the experiment. To determine the morphofunctional parameters of the thymus structure, two groups of animals were formed: Group I — control (n = 40); Group II — rats receiving a diet with magnesium deficiency (n = 40). To model microelement deficiency, a specialized feed manufactured by ALTROMIN Spezialfutter GmbH & Co. KG (Germany) was used. The feeds were provided with an official certificate No. 36/2024. Animals of the control group were fed a standard diet twice daily. In the experimental group, the specialized feed was administered at a dose of 20 g, taking body weight into account, twice daily.

Animals of the control and experimental groups were withdrawn from the experiment and subjected to decapitation under ether anesthesia. After opening the thoracic cavity, the thymus

was isolated. Fragments of thymic tissue were fixed in 10% neutral buffered formalin, after which they were washed in running water for 2–4 hours and dehydrated in alcohols of increasing concentration and chloroform; subsequently, paraffin blocks were prepared according to standard methods. Paraffin sections 4–6 μm thick were stained with hematoxylin and eosin, as well as by the Van Gieson method. Structural elements of the thymus were subjected to morphometric examination using an ocular micrometer. The relative area of thymic lobules, the cortical and medullary layers (in relation to the total section area), the thickness of the cortical layer, as well as the thickness of vessel walls and their internal diameter were determined. Measurements were performed in five randomly selected fields of view of each histological section.

To study the cellular composition of the lymphoid structures of the thymus, cell counting was performed in its structural compartments (the subcapsular zone, cortical layer, and medulla) under oil immersion using a **NOVEL Model NLCD-307** microscope (China). Cell counts were carried out using a morphometric grid installed in the microscope eyepiece.

Morphological and morphometric data obtained during the study were subjected to mathematical processing on a Pentium IV personal computer using the Microsoft Office Excel 7.0 software package. Standard deviation and standard errors were determined.

Variation series of numerical data were formed, arithmetic mean values, mean error, coefficient of variation, as well as the percentage deviation of parameters from control values were calculated. The statistical significance of differences relative to the control was assessed using a parametric method—the Student's *t*-test for two independent samples (with normal data distribution). Differences were considered statistically significant at $p \leq 0.05$. During the organization and conduct of the study, the principles of evidence-based medicine were observed.

Results and Discussion. The thymus of laboratory animals in the control group consisted of two interconnected lobes and was located in the lower third of the manubrium of the sternum. The thickness of the capsule in the region of the thymic hilum in healthy outbred white rats aged 6 and 9 months was 5.82 ± 0.32 μm and 5.97 ± 0.38 μm, respectively. The diameter of the trabeculae in the proximal part was 13.36 ± 0.27 μm and 13.52 ± 0.22 μm, while in the distal part it was 10.28 ± 0.14 μm and 10.43 ± 0.22 μm. The area of thymic lobules in the corresponding age periods amounted to 64.27 ± 0.12% and 52.27 ± 0.48%.

In outbred white rats of the zinc-deficient group, the thickness of the thymic capsule in the hilum region, compared with the control group values, increased by 1.07-fold at 6 months of age and by 1.08-fold at 9 months of age. The diameter of the proximal and distal parts of the trabeculae in both age groups increased by 6.5% and 7.3%, respectively. The area of thymic lobules decreased by 1.07-fold in 6-month-old animals and by 1.08-fold in 9-month-old animals.

In histological specimens of the thymus of healthy outbred white rats, the cortical and medullary layers are distinguished. The boundary between the cortical and medullary layers is indistinct. Areas partially replaced by adipose tissue are identified in the parenchyma. In control group animals aged 6 and 9 months, the area of the cortical layer of the thymus was 63.86 ± 0.37% and 58.83 ± 0.26%, respectively, while the area of the medullary layer was 28.52 ± 0.38% and 32.92 ± 0.42%. The cortico-medullary index in the corresponding age periods was 2.24 ± 0.16 and 1.78 ± 0.36. The thickness of the cortical layer was 248.27 ± 11.54 μm in 6-month-old animals and 165.27 ± 9.76 μm in 9-month-old animals.

In outbred white rats of the zinc-deficient group, the area of the cortical layer of the thymus, compared with intact animals, decreased by 7.6% at 6 months of age and by 8.3% at 9 months of age. The area of the medullary layer in both age groups decreased by 1.07-fold. The

cortico-medullary index also decreased in both age periods. The thickness of the cortical layer, compared with healthy white rats, decreased by 1.07-fold in 6-month-old animals and by 1.08-fold in 9-month-old animals.

Analysis of the population composition of T lymphocytes in the thymus of healthy laboratory animals aged 6 months showed that in the subcapsular zone of the cortical layer, the proportion of small lymphocytes was 38.42 ± 0.58%, in the cortical zone — 64.78 ± 0.44%, and in the medullary layer — 34.28 ± 0.17%. The proportion of medium-sized lymphocytes in the subcapsular zone of the cortical layer was 17.58 ± 0.26%, in the cortical zone — 16.37 ± 0.22%, and in the medullary layer — 31.26 ± 0.18%. The proportion of large lymphocytes in the subcapsular zone of the cortical layer was 17.64 ± 0.28%, in the cortical zone — 6.32 ± 0.12%, and in the medullary layer — 4.26 ± 0.10%.

In 9-month-old outbred white rats of the control group, the proportion of small lymphocytes in the subcapsular zone of the cortical layer was 33.46 ± 0.41%, in the cortical zone — 52.92 ± 0.36%, and in the medullary layer — 26.12 ± 0.14%. The proportion of medium-sized lymphocytes in the subcapsular zone of the cortical layer was 12.73 ± 0.18%, in the cortical zone — 11.94 ± 0.16%, and in the medullary layer — 32.27 ± 0.22%. The proportion of large lymphocytes in the subcapsular zone of the cortical layer was 12.68 ± 0.14%, in the cortical zone — 4.26 ± 0.10%, and in the medullary layer — 3.19 ± 0.16%.

In the group with induced zinc deficiency, in 6-month-old outbred white rats, the number of small lymphocytes in the structural compartments of the thymus decreased by 7.0%, 8.2%, and 7.2%, respectively; the number of medium-sized lymphocytes decreased by 4.1%, 4.0%, and 7.0%; and the number of large lymphocytes decreased by 6.1%, 2.1%, and 2.0%. In the experimental group of 9-month-old outbred white rats, the decrease in the proportion of small lymphocytes in the corresponding structural components of the thymus was 8.1%, 9.1%, and 8.0%; of medium-sized lymphocytes — 5.1%, 5.2%, and 6.8%; and of large lymphocytes — 7.1%, 2.0%, and 2.1%.

In the control group of 6-month-old outbred white rats, the thickness of the wall of the trabecular arteriole of the thymus was 17.38 ± 0.43 μm, with an internal diameter of 19.42 ± 0.18 μm; the wall thickness of capillaries was 5.27 ± 0.16 μm, with an internal diameter of 5.79 ± 0.42 μm. In the cortical layer, the wall thickness of arterioles was 16.36 ± 0.22 μm, with an internal diameter of 17.94 ± 0.27 μm; the wall thickness of capillaries was 4.83 ± 0.17 μm, with an internal diameter of 5.67 ± 0.24 μm. In the medullary layer, the wall thickness of arterioles was 14.78 ± 0.16 μm, with an internal diameter of 17.66 ± 0.23 μm; the wall thickness of capillaries was 4.97 ± 0.18 μm, with an internal diameter of 5.67 ± 0.28 μm.

In laboratory animals aged 9 months, the thickness of the wall of the trabecular arteriole of the thymus was 18.14 ± 0.12 μm, and its internal diameter was 19.78 ± 0.32 μm; the wall thickness of capillaries was 5.52 ± 0.17 μm, with an internal diameter of 5.87 ± 0.14 μm. In the cortical layer, the wall thickness of arterioles reached 16.82 ± 0.18 μm, with an internal diameter of 18.38 ± 0.28 μm; the wall thickness of capillaries was 4.96 ± 0.14 μm, with an internal diameter of 5.82 ± 0.13 μm. In the medullary layer, the wall thickness of arterioles was 15.48 ± 0.26 μm, with an internal diameter of 17.92 ± 0.38 μm; the wall thickness of capillaries was 5.24 ± 0.12 μm, with an internal diameter of 5.89 ± 0.24 μm.

In the group of outbred white rats with induced zinc deficiency, the wall thickness of the trabecular arteriole increased by 1.07-fold and 1.05-fold at 6 and 9 months of age, respectively, whereas its internal diameter decreased by 1.02-fold. The wall thickness of trabecular capillaries increased by 1.04-fold at 6 months of age and by 1.02-fold at 9 months of age, while the internal diameter of these vessels decreased slightly. The wall thickness of arterioles and capillaries of

the cortical layer at 6 and 9 months of age increased by 1.06-fold and 1.04-fold, respectively, whereas their internal diameter decreased by 1.02-fold and 1.03-fold. In the medullary layer, the wall thickness of arterioles increased by 1.07-fold, while the internal diameter decreased by 1.05-fold. The wall thickness of capillaries at 6 and 9 months of age increased by 1.04-fold, with a concomitant decrease in their internal diameter by 1.03-fold.

Conclusion. In healthy animals, the capsule, trabeculae, and the cortical and medullary layers of the thymus were formed relatively normally, while physiological involutional processes characteristic of the corresponding age periods were observed. Under conditions of zinc deficiency, an increase in the thickness of the capsule and trabeculae of the organ was noted, as well as a pronounced reduction in the area of thymic lobules, indicating an increase in the relative area of stromal components and a decrease in the proportion of parenchyma. The thickness and area of the cortical layer decreased, and the cortico-medullary index declined, which indicates a weakening of the processes of proliferation and differentiation of T lymphocytes. The relative area of the medullary layer also decreased, reflecting a reduction in the immunogenic activity of the thymus. A decrease in the number of all types of T lymphocytes (small, medium, and large) was observed, confirming suppression of the processes of T-cell division and maturation, as well as of the overall immune response under zinc deficiency. In blood vessels (arterioles and capillaries), thickening of the walls and a reduction in the internal diameter were detected, indicating the development of angiopathic changes. This may lead to impaired trophic supply of thymic tissue and metabolic processes. The obtained results indicate the important role of the trace element zinc in maintaining thymic function and preserving cellular immunity of the organism.

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