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Morphofunctional Properties of the Thymus and Changes in the Effect of Biostimulants in Radiation Sickness

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Abstract

The thymus is a central organ of the immune system that plays a crucial role in the formation, differentiation, and functional maturation of immunocompetent cells. Age-related involution of the thymus and its sensitivity to external factors significantly affect immune homeostasis. The present study aimed to investigate the morphofunctional characteristics of the thymus and structural changes occurring under the influence of the biostimulator ASD-2 in experimental animals. The study was conducted on rats of different age groups using macroscopic, histological, and morphometric methods. Age-dependent changes in the vascular, capsular, trabecular, and parenchymal components of the thymus were identified. Administration of ASD-2 did not result in pronounced alterations at the macroscopic level; however, significant microscopic changes were observed, including disruption of the cortical-medullary differentiation and expansion of stromal elements. The findings indicate that ASD-2 exerts a targeted effect on the parenchymal-stromal relationships of the thymus, which may influence immune reactivity. The obtained results provide a morphological basis for evaluating the immunomodulatory effects of biostimulants and may be useful in developing preventive and therapeutic approaches for immunodeficiency conditions.
Keywords: thymus, morphofunctional characteristics, immune system, biostimulants, ASD-2, experimental study, rats, thymic involution, immunomodulation

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Морфофункциональные свойства тимуса и изменения в действии биостимуляторов при лучевой болезни

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Резюме

Вилочковая железа является центральным органом иммунной системы, играющим ключевую роль в формировании, дифференцировке и функциональном созревании иммунокомпетентных клеток. Возрастная инволюция тимуса и его высокая чувствительность к экзогенным факторам оказывают существенное влияние на иммунный гомеостаз. Целью настоящего исследования явилось изучение морфофункциональных особенностей вилочковой железы и характера ее структурных изменений под влиянием биостимулятора АСД-2 в эксперименте. Исследование выполнено на крысах различных возрастных групп с применением макроскопических, гистологических и морфометрических методов. Установлены возрастные изменения сосудистых, капсулярных, трабекулярных и паренхиматозных компонентов тимуса. Введение АСД-2 не сопровождалось выраженными анатомическими изменениями, однако на микроскопическом уровне выявлены нарушения кортико-медуллярной дифференциации и усиление стромального компонента. Полученные данные свидетельствуют о направленном воздействии АСД-2 на паренхиматозно-стромальные взаимоотношения тимуса, что может отражаться на иммунной реактивности организма. Результаты исследования имеют значение для обоснования иммунокорригирующих и профилактических мероприятий при иммунодефицитных состояниях.
Ключевые слова: тимус, морфофункциональные особенности, иммунная система, биостимуляторы, АСД-2, экспериментальное исследование, крысы, инволюция тимуса, иммуномодуляция

■ INTRODUCTION

The thymus serves as the primary organ of the immune system. During the early phases of development, it regulates and directs the structural and functional maturation of immunecompetent tissue; in later stages, it guarantees the integrity and efficiency of immune responses. The thymus is a lymphoepithelial organ, with its parenchyma comprising a threedimensional framework of epithelial cells, whose processes are interlinked by desmosomal contacts, creating a type of syncytium [3, 5, 6].

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The spaces between epithelial cells are occupied by lymphoid cells, predominantly Tlymphocytes, which are in various stages of differentiation. They constitute up to 90% of all thymic cells [2].

Additionally, there is a significant presence of cells of mesenchymal origin, mainly macrophages and interdigitating cells. The ratio of lymphocytes to nonlymphoid cells in the thymus cortex is roughly 100:1, while in the medulla it is 1 : 20 [3]. The medulla is also characterized by the presence of thymic bodies (Gassal's bodies), which are created by concentric layers of epithelial cells.

The histogenesis of the thymus is intricate. The thymus plays a pivotal role in the development (both functionally and morphologically) of the immunogenesis system and the initiation of the immune response in pathological conditions [1, 2]. In his context, there is no doubt that the thymus is one of the most crucial central immune organs that manage the body's protective resources in response to the introduction of various types of antigens, particularly genetically modified products [7].

However, despite substantial progress in understanding the structure and function of the human thymus and experimental animals, there is still a lack of data in the literature regarding the patterns of its morphogenesis following the application of ASD2. Such information is critically needed to comprehend the general principles of the integrated action of all components of the immune system after ASD use [4, 6].

The new findings will offer a morphological foundation for justifying the strategies of immunocorrecting and immunomodulating therapeutic interventions for various immunodeficiency states of the body. Furthermore, they will assist in establishing morphological criteria for the reserve capabilities of the immune system at both organ and systemic levels. Meanwhile, the subject of the patterns of morphological changes and cytodynamics in the thymus under the influence of a biostimulator is inadequately addressed in the literature. It has mainly been examined in various pathological scenarios of the thymus [1, 7]. Therefore, one can only indirectly infer the structural changes of the thymus from data concerning its morphogenesis in pathological states and the immune response to other antigens.

Therefore, there is a lack of data in the literature regarding the patterns of alterations in the structure and cytoarchitecture in different regions of the thymus due to the influence of genetically modified organisms. Available literature on the development of the thymus under the influence of ASD- 2 is either nonexistent or inadequate. Given that GMOs have been widely utilized in recent years, it is easy to comprehend the potential risks they may pose to humans.

■ MATERIALS AND METHODS

The research involved 50 mixedbreed rats; newborns, 3 months, and 6 months old. All subjects were maintained under identical vivarium conditions. Rats in both the control and experimental groups were given the same daily feed. All laboratory animals were categorized into 3 groups: the experimental group – animals whose diet included soy flour (at a dosage of 0,04 g per rat weighing 250 g for a duration of 30 days (n=20); the control group – animals that received only a standard diet, without soy flour (n=15). Group 3 – intact animals (n=15).

After the 30 day soy flour feeding period, the laboratory animal groups were euthanized humanely, and an autopsy was conducted. Throughout the euthanasia and autopsy of the laboratory animals, the guidelines for biological safety and ethical standards for handling laboratory animals were followed.

To investigate the morphological characteristics of the thymus, a macroscopic approach (anatomical dissection) was utilized. Following the excision of the tissue, fixation was performed in 10% buffered formalin, subsequently rinsed in water, dehydrated in alcohols, and embedded in benzene. Thereafter, sections 4–6 microns thick were embedded in paraffin and prepared, with staining performed using hematoxylin and eosin. The sections were assessed morphometrically using an eyepiece micrometer DN-107T/ Model CM001 CYAN cope (Belgium).

RESULTS AND DISCUSSION

In baby rats aged one month, the diameter of the right internal thoracic artery ranged from 121,4 to 165,4 microns, with an average of 146,7±0,42, while the left internal thoracic artery measured between 121,2 to 166,6 microns, averaging 150,9±0,70.

The width of the right internal thoracic vein varied from 186,4 to 223,6, averaging 200,9±0,71 microns, and the left vein ranged from 195,7 to 234,4, with an average of 210,2±0,25. The thickness of the thymus capsule in onemonth rats at the gate was on average 6,3±0,41 at the anterior end it averaged 8,7±0,32, and at the posterior end the average was 7,3±0,21 microns. The diameter of the trabecula in the proximal region averaged 13,4±0,6, while in the distal region it was on average 10,8±0,21 microns. The depth of the trabecula averaged 14,0±0,61.

The area of the thymus lobule in onemonth baby rats averaged 72,1%. The wall thickness of the trabecular artery in onemonth rats in the proximal region averaged 17,1±0,25 and for veins, it averaged 15,6±0,31 microns. The inner diameter of the artery averaged 23,4±0,13 while for veins, it was 22,8±0,19 microns. The wall thickness of the trabecular artery in onemonth rats in the distal region averaged 15,1±0,18, while veins averaged 15,2±0,23 microns. The inner diameter of the artery averaged 20,9±0,21 compared to 17,4±0,37 microns for veins.

The thickness of the wall of the arteriole of the cortical layer was on average – 14,1±0,24 and the venules on average – 13,4±0,18; the inner diameter of the arteriole of the cortical layer was on average – 16,3±0,41, and the venule is on average – 22,9±0,15 microns.

The thickness of the wall of the arteriole of the medulla was on average 13,4±0,26 and the venules on average 13,1±0,11; the inner diameter of the arteriole of the medulla was on average 15,6±0,3 and the venules is on average 17,2±0,12 microns.

In 3-month-old baby rats, the diameter of the right internal thoracic artery ranged from 167,4 to 190,1 microns, on average 176,3±0,92, the left internal thoracic artery was 153,5 to 187,7 microns, on average 172,8±0,28. The width of the right internal thoracic vein ranged from 226,1 to 263,2, on average 247,9±0,64 microns, and the left is equal to 202,8 to 251,4, on average 220,2±0,15.

The thickness of the thymus capsule of 3-month-old baby rats at the gate was on average 5,8±0,11, at the anterior end on average 8,1±0,19, and at the posterior end on average 6,9±0,31 microns. The diameter of the trabecula in the proximal part averaged 12,9±0,7, in the distal part it is on average 9,2±0,34 microns. The depth of the trabecula was on average – 13,2±0,51. The area of the thymus lobule of 3–month-old baby rats is on average – 68,4%.

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The wall thickness of the trabecular artery of 3-month-old rats in the proximal part averaged 18,4±0,22, and the veins averaged 16,3±0,21 microns, the inner diameter of the artery averaged 24,6±0,15 and the veins averaged 23,5±0,23 microns.

The wall thickness of the trabecular artery of 3-month-old rats in the distal part averaged 16,4±0,19, and the veins averaged 16,6±0,25 microns, the inner diameter of the artery averaged 21,6±0,31, and the veins averaged 18,7±0,22 microns.

The thickness of the wall of the arteriole of the cortical layer was on average – 15,7±0,14, and the venules on average – 14,1±0,13; the inner diameter of the arteriole of the cortical layer was on average – 17,4±0,43, and the venule is on average – 23,4±0,19 microns.

The thickness of the wall of the arteriole of the medulla was on average – 14,2±0,16, and the venules on average – 14,3±0,21; the inner diameter of the arteriole of the medulla was on average – 16,7±0,27, and the venule is on average – 17,9±0,13 microns.

The thickness of the wall of the arteriole of the medulla was on average – 15,6±0,36, and the venules on average – 15,1±0,11; the inner diameter of the arteriole of the medulla was on average – 17,8±0,17, and the venule is on average – 18,1±0,21 microns.

In 9-month-old rats, the diameter of the right internal thoracic artery varied from 221,5 to 252,8 microns, on average 242,3±0,34, the left internal thoracic artery was 218,2 to 259,4 microns, on average 230,8±0,25. The width of the right internal thoracic vein ranged from 206,7 to 323,2, on average 302,9±0,84 microns, and the left is equal to 272,5 to 311,3, on average 294,2±0,42.

The thickness of the thymus capsule of 9-month-old baby rats at the gate was on average 3,9±0,41, at the anterior end on average 7,3±0,29, and at the posterior end on average 5,6±0,11 microns. The diameter of the trabecula in the proximal part averaged 11,8±0,17, in the distal part it is on average 8,1±0,14 microns. The depth of the trabecula averaged 12,2±0,71. The area of the thymus lobule of 9-month-old baby rats is on average 53,7%.

The wall thickness of the trabecular artery of 9-month-old rats in the proximal part averaged -20,3±0,18, and the veins averaged 18,8±0,34 microns, the inner diameter of the artery averaged 26,3±0,25, and the veins averaged 25,6±0,13 microns.

The wall thickness of the trabecular artery of 9-month-old rats in the distal part averaged 17,7±0,17 and the veins averaged 18,3±0,21 microns, the inner diameter of the artery averaged 23,7±0,31 and the veins averaged 19,8±0,4 microns.

The thickness of the wall of the arteriole of the cortical layer was on average – 17,3±0,21 and the venules on average – 15,2±0,13; the inner diameter of the arteriole of the cortical layer was on average – 19,1±0,23 and the venules were on average – 25,2±0,35 microns.

The thickness of the wall of the arteriole of the medulla was on average – 16,1±0,19 and the venules on average – 16,6±0,23; the inner diameter of the arteriole of the medulla was on average – 18,3±0,33, and the venule is on average – 19,3±0,15 microns.

In 12-month-old rats, the diameter of the right internal thoracic artery varied from 224,5 to 262,8 microns, on average 248,5±0,74, the left internal thoracic artery was 210,2 to 249,7 microns, on average 236,8±0,24. The width of the right internal thoracic vein varied from 297,1 to 330,4 on average 315,5±0,24 microns and the left is 301,7 to 347,4, on average 311,2±0,76.

The thickness of the thymus capsule of 12-month-old baby rats at the gate was on average 3,6±0,17, at the anterior end on average 6,3±0,09, and at the posterior end on average 5,0±0,31 microns. The diameter of the trabecula in the proximal part averaged

10,7±0,8, in the distal part it is on average 7,4±0,34 microns. The depth of the trabecula averaged 11,5±0,71. The area of the thymus lobule of 12-month–old baby rats is on average 46.2%.

The wall thickness of the trabecular artery of 12-month-old rats in the proximal part averaged 21,6±0,12, and the veins averaged 19,3±0,14 microns, the inner diameter of the artery averaged 27,5±0,17, and the veins averaged 26,7±0,23 microns.

The wall thickness of the trabecular artery of 12-month-old rats in the distal part averaged 18,5±0,17, and the veins averaged 19,1±0,42 microns, the inner diameter of the artery averaged 24,5±0,23, and the veins averaged 20,6±0,43 microns.

The thickness of the wall of the arteriole of the cortical layer was on average – 18,2±0,19, and the venules on average – 16,3±0,18; the inner diameter of the arteriole of the cortical layer was on average – 20,5±0,14, and the venules were on average – 26,3±0,22 microns.

The thickness of the wall of the arteriole of the medulla was on average – 17.3±0.41, and the venules on average – 17.2±0.24; the inner diameter of the arteriole of the medulla was on average – 19.1±0.19, and the venule is on average – 20.2±0.23 microns.

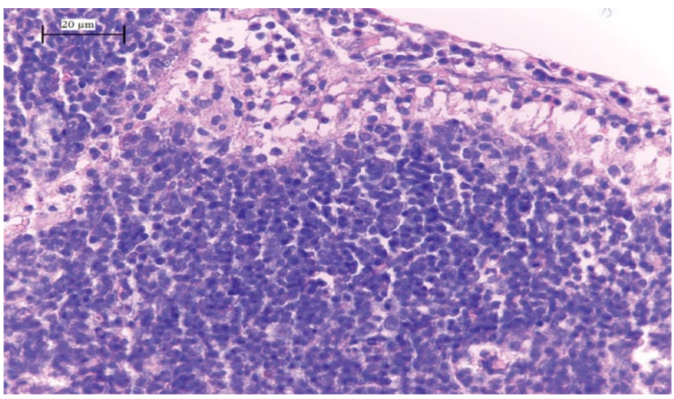

Fig. 1. A thymus lobule with a limited connective septum, numerous blood vessels in the septa are also visible. Staining with hemotoxylin and eosin, 10×10

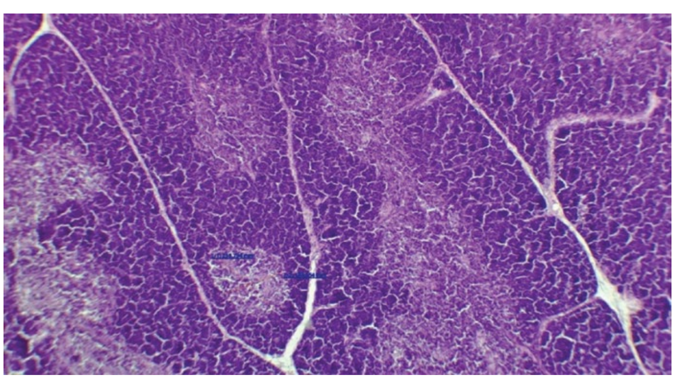

Fig. 2. The thymus lobule is represented by two clearly distinguishable areas-cortical and medullary matter, connective tissue strands and vessels are also visible. Staining with hemotoxylin and eosin, 10×10

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The main morphometric features (area on slices, in %) of the structural components of rats are normal

Structural components of the thymus	Area on slices (%)		
	X±Sx	min	max
1. Cortical substance	56.1±1.9	56.7	64.7
2. Medulla	23.9±1.9	18.2	28.3
3. Cortical-cerebral index	1.3±0.3	1.5	3.1
4. Stroma	12.8±0.6	8.6	11.6

Note: % the area of the structural component as a percentage of the area of the thymus.

The results of the study show that in 3 months of rats, the thymus gland in 67.6% of cases consisted of 2 lobes and in 32.4% of one, multi-lobed forms were not detected.

Under the action of ASD, we did not detect any noticeable changes in anatomical parameters. The most pronounced changes are observed in the microscopic structure of the thymus gland.

In the experiment, the parenchyma of the thymus gland does not have a distinct division into cortical and medulla, and there is also an increase in small and large diameters of the medulla compared to the control.

Microscopic examination of the thymus after feeding ASD in animals revealed that a sharp reaction develops after certain periods after administration, underdevelopment, which progresses with an increase in the duration of the experiment. Thus, the results of morphological studies have shown that pathological changes often occur in rats when using ASD-2. Changes in the thymus when using ASD-2 should be taken into account when choosing treatment tactics and developing preventive measures for both immunodeficiency conditions and its complications.

■ CONCLUSION

The impact of ASD on the thymus results in a targeted lesion between the parenchyma and stroma; these modifications may stem from a specific response of the thymus to the introduction of ASD, which culminates in reduced immunity, the emergence of allergic reactions, the development of antibiotic resistance, along with a decline in treatment efficacy and accumulation processes within the body.

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