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MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISTICS OF THE THYMUS AND BONE MARROW OF OUTBRED RATS DURING POSTNATAL ONTOGENESIS

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Abstract
This study investigated the morphological and morphometric characteristics of the thymus and bone marrow in outbred rats during postnatal ontogenesis. Histological and morphometric analyses were performed on tissues obtained from newborn, 2-month-old, and 4-month-old rats of the control group. The results revealed age-dependent structural changes characterized by gradual thickening of the thymic capsule and trabeculae, development of vascular components, redistribution of thymocytes between cortical and medullary zones, and signs of early involution processes. Morphometric indicators confirmed progressive structural maturation and functional reorganization of central immune organs during postnatal development. The obtained data may serve as baseline reference values for further experimental and immunomorphological studies.

Keywords: thymus, bone marrow, postnatal ontogenesis, outbred rats, morphology, morphometry, age-related changes, immune system development.

Introduction

Exogenous chemical immunosuppression in any form increases the risk of malignant tumor development, and the final outcome of this process is directly dependent on the state of the immune system [1]. It is well established that the development of oncological diseases is influenced not only by genetic factors but also by hormonal regulation, the body's immune responses, and a number of other factors. In recent years, however, scientific attention has increasingly focused on studying the status of immunity [2].

In mammals, hematopoietic stem cells (HSCs) play a key role throughout life in ensuring the continuity of hematopoiesis. These cells are localized in the bone marrow, where they maintain the homeostasis of blood cells and contribute critically to the effective functioning of the immune system. HSCs reside in perivascular niches within the bone marrow and spleen, interacting closely with sinusoidal blood vessels. These specialized venules are unique to hematopoietic tissues and support the maintenance of HSCs. The preservation and differentiation of HSCs into multiple restricted progenitor cells are regulated by specific molecules produced by endothelial and leptin-positive stromal cells. Additionally, biologically active substances synthesized by various niche cells modulate

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the development of certain progenitor populations or influence the metabolic activity of HSCs and their microenvironment [3].

Among carcinogenic factors, chemical carcinogens occupy a distinct place [4]. Lee H. (2021) demonstrated that immunosuppressive drugs enhance apoptosis in thymic lymphoid cells, with pronounced effects in 2-month-old rats and relatively mild effects in 4-month-old rats [5]. According to Montero-Herradón et al. (2025), the thymus, as a primary lymphoid organ, consists of a three-dimensional epithelial network that provides a specialized microenvironment for the phenotypic and functional maturation of lymphoid progenitor cells. Specialization of the pharyngeal endoderm into the thymus occurs independently of Foxn1 transcription factor expression during the initial stages of thymic organogenesis, whereas Foxn1 later regulates thymic development in coordination with lymphoid cells. Early thymic development resembles the ontogeny of other epithelium-derived organs [6].

Thymic T-lymphocyte differentiation is influenced by biogenic amines, including histamine, serotonin, and catecholamines. The thymus is unique in that its structural components—thymic lymphocytes and epithelial cells, along with their biogenic amine secretions—actively participate in neuroendocrine processes [7]. Glucocorticosteroid-resistant nephrotic syndrome, glomerulonephritis, and other kidney diseases, as well as rheumatic conditions such as rheumatoid arthritis, juvenile dermatomyositis, scleroderma, interstitial lung disease, vasculitis, and thrombocytopenic purpura, may also involve similar mechanisms of immunomodulation [8].

One of the most pressing issues in modern oncology is the treatment of malignant tumors, a challenge further complicated by the increasing incidence of late-stage diagnoses [9]. High proliferative potential of breast cancer underscores the need for intensified chemotherapy regimens, especially at early and locally advanced stages, where treatment effectiveness directly depends on the completeness of neoadjuvant chemotherapy. Optimization of dosages and treatment intervals is therefore crucial for successful therapeutic outcomes [10].

Aim of the Study

The aim of this study was to investigate the morphological and morphometric characteristics of the thymus and bone marrow in outbred rats during postnatal ontogenesis and to determine age-related structural changes reflecting the processes of functional maturation and early involution of central immune organs.

Materials and Methods

The study was conducted on 203 outbred white rats aged 2 and 4 months under standard vivarium conditions. At the beginning of the experiment, all animals underwent a one-week quarantine period during which somatic and infectious diseases were excluded. Following quarantine, the rats were maintained on the regular vivarium regimen with three daily feedings.

Experimental skin carcinoma was induced using 7,12-dimethylbenz[a]anthracene (DMBA), a polycyclic aromatic hydrocarbon that reliably models epithelial tumorigenesis by inducing DNA-damaging changes in epidermal cells.

The animals were divided into three groups:

- Group I (Control) – 2- and 4-month-old rats (n=73);

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• Group II (Experimental, Chemotherapy) – rats with DMBA-induced skin carcinoma receiving chemotherapy (n=68);
• Group III (Chemotherapy + Biocorrection) – rats treated with chemotherapy followed by plant-based biocorrection (n=62).

During the experiment, the growth, development, general condition, and behavior of the animals were monitored, and no significant deviations were observed. At designated time points, the rats were weighed and decapitated under ether anesthesia on an empty stomach. Animals were decapitated on days 1, 7, and 30 post-exposure for subsequent tissue collection.

Upon confirmation of papillomas and pre-cancerous changes, rats in Groups II and III underwent chemotherapy. Paclitaxel, a microtubule-stabilizing agent that inhibits cell division and enhances apoptosis in tumor cells, was used at a dose of 0.2 mg/kg, adjusted individually according to the body weight of each rat. In Group III, post-chemotherapy treatment included a standardized liquid extract of Silybum marianum (milk thistle), chosen for its rapid absorption, convenient dosing, and high bioavailability.

Immunohistochemical studies of thymus tissue were performed in collaboration with the "Ipsum Pathology" laboratory (Tashkent, Uzbekistan) using CD4 and CD138 markers.

Morphometric and histological data were processed using Microsoft Excel 7.0, and statistical analysis was conducted using STTGRAPH 5.1, calculating mean values, standard deviations, and representative errors.

Results

Histological and morphometric analysis of the thymus and bone marrow in control group outbred rats demonstrated distinct age-dependent structural changes during postnatal ontogenesis.

In newborn rats, the thymus exhibited signs of high functional and proliferative activity. The organ was covered by a thin connective tissue capsule forming a clearly defined lobular structure with cortical and medullary layers. Capsule thickness measured 6.0–7.0 μm in the hilar region (mean 7.1±0.26 μm), 5.5–6.5 μm in the anterior region (5.9±0.29 μm), and 6.5–7.5 μm posteriorly (7.1±0.31 μm). Subcapsular trabeculae were thin and short, with a thickness of 5.0–6.0 μm (5.6±0.19 μm) near the capsule and 7.0–8.0 μm (7.6 μm) centrally. Their average length ranged from 100 to 120 μm (118.1±0.23 μm), indicating incomplete stromal development at this stage.

The vascular component of trabeculae was relatively poorly developed. The wall thickness of proximal and distal arterioles ranged from 2.2 to 2.5 μm (2.3±0.06 μm), while cortical trabecular arterioles measured 1.8–2.2 μm (1.9±0.04 μm). Venular wall thickness reached 1.0–1.3 μm (1.1±0.04 μm) in the cortical layer and 1.5–1.7 μm (1.62±0.044 μm) in proximal and distal regions, reflecting incomplete maturation of the microcirculatory system.

The thymic parenchyma was dominated by the cortical layer, where thymocytes accounted for 75–85% of cellular elements, whereas the medulla contained 15–25%. Lymphocyte density reached 300–350 cells per field of view in the cortex and 90–120 cells in the medulla, confirming intense lymphopoiesis and active immune system formation.

In 2-month-old rats, structural maturation of the thymus was observed. Capsule thickness increased to 8.02–10.01 μm (8.67±0.22 μm) in the hilar region, 6.03–8.03 μm (6.77±0.21 μm) anteriorly, and 7.01–9.04 μm (8.22±0.22 μm) posteriorly. The capsule consisted predominantly of dense connective tissue containing collagen fibers, fibroblasts, and fibrosites. Trabecular thickness reached 5.01–7.21

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μm (6.22±0.21 μm) in the subcapsular zone and 8.12–10.11 μm (8.90±0.22 μm) centrally, with lengths of 120.22–140.01 μm (126.22±2.16 μm).

Microvascular structures also demonstrated development. Arteriolar wall thickness in proximal and distal trabeculae measured 2.51–3.04 μm (2.61±0.05 μm), while venular walls measured 1.52–2.02 μm (1.82±0.05 μm). Cortical arterioles had wall thicknesses of 2.01–2.52 μm (2.14±0.05 μm) and diameters of 7.01–9.05 μm (8.41±0.06 μm), whereas venules measured 10.02–12.11 μm (11.21±0.06 μm) in diameter. Thymocyte distribution changed, with cortical thymocytes comprising 65–70% and medullary thymocytes 30–35%.

In 4-month-old rats, further structural remodeling was identified. Capsule thickness increased significantly, reaching 12.01–14.02 μm (12.53±0.22 μm) in the hilar region, 9.02–11.03 μm (9.67±0.22 μm) anteriorly, and 10.02–12.01 μm (11.03±0.22 μm) posteriorly. Trabeculae became thicker and longer, measuring 9.12–11.02 μm (10.23±0.22 μm) subcapsularly and 12.04–14.32 μm (13.41±0.21 μm) centrally, with lengths of 200.02–220.11 μm (210.31±2.16 μm).

Vascular maturation was evident, as arteriolar wall thickness increased to 3.51–4.02 μm (3.64±0.05 μm), with internal diameters of 14.11–16.04 μm (14.91±0.06 μm). Venular walls measured 2.02–2.51 μm (1.63±0.06 μm) with diameters of 16.22–18.31 μm (17.81±0.06 μm). Cortical arterioles reached diameters of 11.02–13.11 μm (12.09 μm), while venules measured 14.02–16.04 μm (14.93±0.04 μm).

Cellular composition analysis revealed age-related redistribution of thymocytes: cortical thymocytes decreased to 55–60%, whereas medullary thymocytes increased to 40–45%. Histological examination demonstrated focal involutional changes characterized by reduction of T-lymphocytes and reticuloepithelial cells and partial replacement of lymphoid tissue by adipose tissue. Van Gieson staining revealed collagen fiber proliferation surrounding expanding adipose areas, indicating early thymic involution.

Overall, the obtained morphometric data demonstrate progressive structural maturation of stromal, vascular, and parenchymal components of the thymus during postnatal ontogenesis, accompanied by gradual initiation of involutional processes by the fourth month of development.

Conclusions

The conducted study demonstrated that postnatal ontogenesis in outbred rats is accompanied by consistent morphological and morphometric remodeling of the thymus and bone marrow. In the early postnatal period, the thymus is characterized by high functional activity, predominance of the cortical substance, high thymocyte density, and insufficient development of stromal and vascular components, reflecting intensive formation of the immune system. With increasing age, progressive thickening of the capsule and trabeculae, maturation of connective tissue structures, and development of the microcirculatory network were observed. Morphometric parameters confirmed gradual redistribution of thymocytes between cortical and medullary zones and structural stabilization of the organ.

By the fourth month of life, initial involutional changes become evident, including a decrease in lymphoid cellularity and focal replacement of thymic parenchyma with adipose tissue, accompanied by increased collagen fiber formation. These findings indicate that structural maturation of central immune organs during postnatal development is followed by the onset of age-related involution processes. The obtained data provide baseline morphometric reference values and may serve as a morphological foundation for further experimental and immunopathological studies.

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