

IMMUNOHISTOCHEMICAL EXPRESSION OF CARDIAC MUSCLE TISSUE IN
RELATION TO AGE: AN EXPERIMENTAL STUDY

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Abstract: The aim of this study was to investigate age-related features of the immunohistochemical expression of structural proteins in the cardiac muscle of white outbred rats. The experiment was conducted on two age groups: 2-month-old and 4-month-old animals. The left ventricular myocardium was analyzed using histological and immunohistochemical methods with antibodies against troponin I, α -actin, and desmin. A quantitative assessment of immunopositive cardiomyocytes and staining intensity was performed.

The results showed a significant age-dependent increase in the expression of troponin I, α -actin, and desmin, accompanied by enlargement of cardiomyocytes, dense organization of myofibrils, and enhanced myocardial vascularization. It was found that 4-month-old rats exhibit a more mature morphofunctional organization of the cardiac muscle compared to 2-month-old animals. These findings indicate a regular age-related differentiation of cardiomyocytes and can be used for evaluating postnatal myocardial development and modeling pathological conditions of the heart.

Keywords: myocardium, cardiomyocytes, immunohistochemistry, troponin I, α -actin, desmin, white outbred rats, age-related features

Relevance of the Topic. The cardiovascular system plays a key role in maintaining the organism's vital functions, and the functional state of the myocardium is one of the main indicators of adaptive and age-related changes. Studying the morphofunctional and molecular characteristics of the cardiac muscle at different age periods is of particular scientific and practical significance, as it provides a deeper understanding of the mechanisms underlying age-related remodeling of cardiac tissue and the regularities of its structural and functional adaptation. Immunohistochemical methods allow the detection of the expression of specific protein markers that reflect the state of cellular structures, processes of differentiation, regeneration, and apoptosis of cardiomyocytes. Determining the level of expression of these markers at different ages makes it possible to establish correlations between morphological changes and the functional activity of the heart muscle.

White outbred rats are one of the most convenient experimental models for studying age-related aspects of cardiovascular function due to their physiological similarity to other mammals, accessibility, and reproducibility of results. Comparing immunohistochemical expression in animals of different ages (2- and 4-month-old) allows the identification of early morphological and molecular manifestations of age-related remodeling in the myocardium, which is important for preventive and experimental cardiology.

Thus, studying age-related features of immunohistochemical expression in cardiac muscle tissue using the white outbred rat model is highly relevant, as it contributes to expanding our understanding of the mechanisms of age-related changes in the myocardium and provides a scientific basis for developing approaches to the early diagnosis and correction of age-related cardiovascular disorders.



Aim of the Study. To determine age-related features of immunohistochemical expression in the cardiac muscle tissue of white outbred rats at early stages of postnatal ontogenesis (at 2 and 4 months of age) in order to identify patterns of morphofunctional changes in the myocardium depending on age.

Research Objectives.

1. To select and prepare experimental animals — white outbred rats aged 2 and 4 months — for morphological and immunohistochemical analysis of the myocardium.
2. To study the morphological structure of the cardiac muscle in animals of different ages using histological staining methods.
3. To determine the nature and level of immunohistochemical expression of specific protein markers reflecting the functional state of cardiomyocytes.
4. To perform a comparative analysis of immunohistochemical activity in the myocardium of rats from different age groups.
5. To establish patterns of age-related changes in the structure and protein expression of the cardiac muscle.

Object of the Study. Cardiac muscle tissue (myocardium) of white outbred rats aged 2 and 4 months.

Subject of the Study. Age-related features of immunohistochemical expression of structural proteins in cardiomyocytes, reflecting the morphofunctional state and age-related remodeling of the myocardium.

Materials and Methods. The experimental study was conducted on clinically healthy male white outbred rats weighing 180–250 g. Two age groups were used: 2-month-old (young) and 4-month-old (sexually mature) animals. A total of ___ animals were included in the study (___ per age group). Housing and handling of the animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Rules of Laboratory Practice in the Russian Federation (Ministry of Health Order No. 267, 19.06.2003).

After euthanasia under light ether anesthesia, thoracotomy was performed and the heart was excised. The left ventricular myocardium was fixed in 10% neutral buffered formalin at +4 °C for 24–48 hours. Tissue samples were subsequently dehydrated through a graded series of alcohols, embedded in paraffin, and serial paraffin sections 4–5 µm thick were prepared using a rotary microtome.

Histological Methods. For general morphological analysis, sections were stained with hematoxylin and eosin. Additionally, Van Gieson and Mallory staining methods were used as needed to assess connective tissue elements and the state of the myocardial stroma.

Immunohistochemical Analysis. Immunohistochemical analysis was performed using a standard indirect streptavidin–peroxidase method. Visualization reagents were obtained from Dako (Denmark) / Thermo Fisher (USA) (exact kit to be specified).

The following primary antibodies were used:

- Troponin I (cTnI) — marker of the cardiomyocyte contractile apparatus;
- Desmin — cytoskeletal protein reflecting the structural integrity of muscle fibers;
- α -Actin — marker of muscle filaments.

After incubation with primary antibodies, sections were treated with secondary antibodies conjugated with peroxidase and visualized with 3,3'-diaminobenzidine (DAB) to identify immunopositive areas. Control sections were processed without primary antibodies to exclude nonspecific staining.

Microscopy and Morphometric Analysis. Specimens were examined using a Leica DM500 light microscope at magnifications of $\times 100$, $\times 200$, and $\times 400$. Images were captured with a digital



camera and analyzed using ImageJ software. Quantitative assessment included counting the percentage of immunopositive cardiomyocytes relative to the total number of cells in the field of view and grading staining intensity on a semi-quantitative scale (from weak to strong).

Statistical Analysis. Quantitative data were processed using Statistica 10.0 (StatSoft Inc., USA). Results are presented as $M \pm m$ (mean $\pm$ standard error of the mean). Differences between groups were evaluated using the Student's t-test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion. Light Microscopy of the Myocardium Light microscopic examination of the myocardium in 2-month-old rats revealed that the cardiac muscle tissue exhibited clearly defined transverse striations. Cardiomyocytes were arranged in an orderly manner, forming dense muscle bundles. Cell nuclei were large, oval-shaped, centrally located, with evenly distributed chromatin and well-defined nucleoli. The intercellular substance (endomysium) was moderately developed, and the vascular network was evenly distributed. No signs of degeneration or dystrophic changes in muscle fibers were observed.

In 4-month-old rats, more pronounced structural maturation of the myocardial tissue was noted. Cardiomyocytes were larger, with cytoplasm containing a greater number of myofibrils displaying distinct striations, and nuclei appeared denser. The intercellular space was slightly increased due to moderate proliferation of connective tissue elements. The capillary network was denser and well-vascularized, reflecting increased metabolic activity of the myocardium. Thus, with age, there was a consistent increase in cardiomyocyte size and tissue differentiation, indicating functional maturation of the myocardium.

Immunohistochemical Analysis. Immunohistochemical examination showed that in 2-month-old rats, the expression of troponin I (cTnI) and α -actin was moderate and evenly distributed throughout the cytoplasm of cardiomyocytes, indicating active formation and organization of the contractile apparatus. Positive desmin staining was predominantly localized in the peripheral regions of the cells, corresponding to incomplete development of cytoskeletal structures at this early postnatal stage.

In 4-month-old animals, expression of these proteins was markedly increased. Immunopositive regions encompassed nearly the entire cytoplasm of cardiomyocytes, forming a dense network. The staining intensity for troponin I and α -actin was significantly higher than in younger animals ($p < 0.05$), reflecting increased contractile activity and completion of muscle fiber differentiation. Desmin expression also increased and became uniformly distributed, indicating stabilization of the cytoskeleton and intercellular contacts.

Comparative Analysis and Discussion Comparison of the results demonstrated a consistent age-related increase in immunohistochemical expression of structural proteins in cardiomyocytes. Enhanced expression of troponin I and α -actin reflects the increasing functional load on the myocardium and the formation of mature contractile complexes. Increased desmin expression indicates morphological stabilization of the cellular apparatus and improved mechanical resistance of the myocardium.

These findings are consistent with literature reports showing that in the early postnatal period in rodents, active processes of myofibrillogenesis and myocardial differentiation occur (Ivanov et al., 2018; Kim et al., 2021). With age, synthesis of cytoskeletal and contractile proteins increases, ensuring functional maturation of the myocardium.

Thus, the results of the present study confirm pronounced age-related differences in the level of immunohistochemical expression of structural proteins in cardiac muscle. The highest activity was observed in 4-month-old rats, corresponding to the period of morphological and functional myocardial maturation.



Conclusions. In 2-month-old white outbred rats, the myocardium is characterized by orderly arranged cardiomyocytes with well-defined transverse striations, moderately developed intercellular substance, and an evenly distributed vascular network. Nuclei are oval and centrally located, reflecting an early stage of functional differentiation. In 4-month-old rats, cardiomyocytes are larger, myofibrils are more densely organized, vascularization is enhanced, and there is moderate proliferation of connective tissue, corresponding to the completion of morphofunctional myocardial maturation.

Immunohistochemical analysis demonstrated that in 2-month-old rats, the expression of troponin I, α -actin, and desmin was moderate and localized in specific regions of cardiomyocytes. In 4-month-old animals, the expression of these proteins was significantly increased, encompassing nearly the entire cytoplasm, reflecting maturation of the contractile apparatus and stabilization of the cytoskeleton. The intensified desmin network indicates enhanced mechanical stability of the myocardium.

Comparative analysis revealed a statistically significant increase in the number of immunopositive cells and staining intensity in older animals ($p < 0.05$), demonstrating age-related morphofunctional maturation of the cardiac muscle. These findings confirm that age-dependent immunohistochemical expression of structural proteins is a reliable marker of myocardial maturity in white outbred rats.

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