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WHITE RATS THYROID GLAND MICRO- AND ULTRA-MICROSCOPIC CHANGES 7 DAYS AFTER THE EXPERIMENTAL THERMAL INJURY IN CONDITIONS OF PHYSIOLOGICAL SALINE ADMINISTRATION

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The purpose of the study was to determine the microscopic and ultrastructural changes in the experimental animals' thyroid gland 7 days after simulated thermal skin injury. Microscopic studies have established the intrathyroid changes of different degrees and of a predominantly destructive nature in the follicles, thyrocytes, vessels and stroma 7 days after the thyroid gland experimental thermal injury. Micro- and ultra-microscopic studies of thyroid tissue 7 days after thermal damage of the body revealed expressed changes of the mainly destructive nature. Follicles of various sizes with intracellular swelling, the presence of swollen thyrocytes with a changed shape, oxyphilic cytoplasm and destructively changed intracellular organelles, pathological changes in the surrounding connective tissue and impaired blood supply - the established pathomorphological correlates showed the irreversibility of the detected histological changes. Physiological solution injection and attempts to restore the circulating blood volume proved the failure of thyroid burn pharmacocorrection. Therefore, the results of micro- and ultra-microscopic studies of the thyroid gland of animals under the specified conditions against the background of NaCl administration established the processes thyrocytes structure remodeling, the capillaries walls and stromal connective tissue remodeling with signs of their destruction and alteration, which indicates the organ functional activity worsening.

Key words: thyroid gland, thermal injury, morphological disturbances, ultra-microscopic changes, destruction, irreversibility, decompensation

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МІКРО- ТА УЛЬТРАМІКРОСКОПІЧНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ БІЛИХ ЩУРІВ ЧЕРЕЗ 7 ДІБ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОГО ТЕРМІЧНОГО УРАЖЕННЯ В УМОВАХ ВВЕДЕННЯ ФІЗІОЛОГІЧНОГО РОЗЧИНУ

Метою дослідження стало встановлення мікроскопічних та ультраструктурних змін щитоподібної залози експериментальних тварин через 7 діб після змодельованої термічної травми шкіри. Через 7 діб після експериментальної термічної травми у щитоподібній залозі відзначаються різного ступеня зміни фолікулів, тиреоцитів, судин та строми переважно деструктивного характеру. Мікро- та ультрамікроскопічні дослідження тканини щитоподібної залози через 7 діб після термічного ураження організму свідчать про виражені зміни, які мають переважно деструктивний характер. Фолікули різного розміру з внутрішньоклітинним набряком, наявність набряклих тироцитів зі зміненою формою, оксифільною цитоплазмою та деструктивно зміненими внутрішньоклітинними органелами, патологічні зміни оточуючої сполучної тканини та порушення кровопостачання – встановлені патоморфологічні кореляти післяопікового процесу вказують на незворотність виявлених гістологічних змін. Введення фізіологічного розчину та намагання відновлення об'єму циркулюючої крові засвідчило неспроможність фармакокорекції опіку щитоподібної залози. Таким чином, результати проведених мікро- та ультрамікроскопічних досліджень щитоподібної залози тварин при експериментальній опіковій травмі на тлі введення NaCl встановили процеси ремоделювання структури тироцитів, стінки капілярів та стромальної сполучної тканини з проявами їх деструкції та альтерації, що свідчить про погіршення функціонування органу.

Ключові слова: щитоподібна залоза, опікова травма, морфологічні порушення, ультрамікроскопічні зміни, деструкція, незворотність, декомпенсація

The study is a fragment of the research project "Peculiarities in micro-/ultramicroscopic structure and histochemical properties of body tissues during the development of compensatory-adaptive reactions", state registration No. 0121U108204.

The problem of thermal burns is multifaceted from a medical point of view [2, 7, 15], and the urgency of its precise solution and clarification of the basic principles of adequate and effective medical care providing to this category of patients acquires extremely important medical, economic and social importance [8]. A significant number of pathological processes occur in the body after a thermal burn which can lead to the death of a person without immediate qualified medical assistance [9, 10]. But even in case of well-timed medical care and the formed disorders pharmacological correction, with the preservation of human life, a number of morphological and pathophysiological disorders and changes are "triggered" the result of which are vital organs and organ systems postponed dysfunctions [11, 14].

Studying the dynamics and severity of post-burn changes in the body, the thyroid gland intraparenchymal changes in the tissue, cells, and surrounding environment were found [1, 12]. This organ was preferred due to following factors: firstly, the thyroid gland is located superficially and is one of the most sensitive to temperature effects. Secondly, it's considered to be one of the leading organs in the body's

functions endocrine regulation, whereas the process of endocrine dysregulation is characteristic of a burn injury. Thirdly, from a fundamental point of view, the consequence of the pathological dysregulation of organs and systems stimulated by endocrine thyroid dysfunction is the formation of multiple organ failure, the pathogenetic mechanisms of which in case of thermal burning induction have not been sufficiently investigated. Fourthly, it should be understood that the researches conducted under the specified conditions aims both the excessive thermal factors pathogenic influence delicate mechanisms establishment and the potential thermoprotective pharmacological pharmacons selection, development and efficacy testing with the perspective of thyroid protective profile efficacy.

The predominant micro- and ultra-microscopic focus of our research is explained by the clear understanding that in the case of a clear explanation of a thermal factor induced "Virchow's triad" probable pathology with thyroid localization we will be able to test the efficacy of the studied pathology complex pharmacological therapy based on established mechanisms. It is clear that, in case of success, the mechanisms of the thyroid gland thermal burns pathogenetically justified complex pharmacocorrection implementation will be identical to this pathological condition sanogenetic mechanisms activation [13]. To implement the chosen direction of scientific research, it is important to follow the intrathyroid micro- and ultra-microscopic changes over a long postburn period.

The purpose of the study was to determine the microscopic and ultrastructural changes in the experimental animals' thyroid gland 7 days after simulated thermal skin injury.

Materials and methods. Experimental trials were performed on 90 white male rats weighing 160–180 g (obtained from the vivarium of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine) on the basis of the Research Center of N.I. Pirogov Vinnytsia National Medical University. Animals keeping, handling and manipulation was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013) and was guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985) and guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine on "Preclinical studies of drugs" (2001) as well as rules of humane treatment of experimental animals and conditions approved by the Committee on Bioethics of N.I. Pirogov Vinnytsia National Medical University (Prot. N1 from 14.01.2010).

Thermal skin burns of 2–3 degrees were modeled by four copper plates (each surface area equal to 13.86 cm^2) applying to pre-depilated side surfaces of the rats body for 10 sec, these rats were preheated for 6 min in water with a temperature of 100 °C [5]. The total area of skin lesions was 21–23 %. Rats were infused with 0.9 % NaCl solution into the inferior vena cava throughout the first 7 days of a trial. Animals were euthanized by decapitation after 7 days. Shaving, venous catheterization, skin burns and decapitation of rats were performed under propofol (i.v., 60 mg/kg) anesthesia.

Biological material collection for microscopic examination was done according to used method [3]. The thyroid gland samples were fixed with 10 % neutral formalin solution, then dehydrated by passing through increasing concentrations of alcohol and embedded into paraffin blocks. The obtained sections, 5–6 μm thick, were stained with Hematoxylin-Eosin [3].

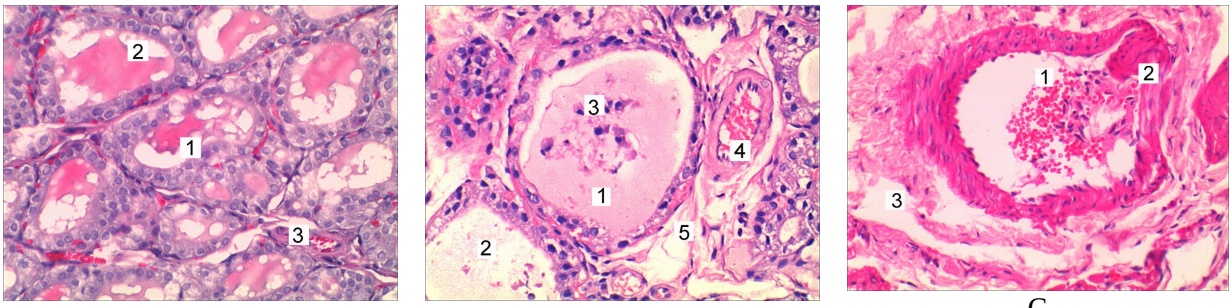
The histological sections were examined under the MIKROmed SEO SCAN light microscope ("Sumy Electron Optics", Sumy, Ukraine), the photomicrographs were taken with the Vision CCD Camera with an image output system for histological specimens. The thyroid gland samples collected for electron microscopic examination were fixed with 2.5 % glutaraldehyde solution, and then post-fixed with 1 % osmium tetroxide prepared with phosphate buffer. Further processing was done according to used method [3]. Ultrathin sections made with ultramicrotome UMPT–*7* were contrasted with uranyl acetate, lead citrate according to the Reynolds method and examined under the electron microscope PEM–125K.

All morphological researches were performed under the Agreements on Scientific Cooperation among the Histology, Cytology and Embryology Department of Odesa National Medical University and Research Center of N.I. Pirogov Vinnytsia National Medical University (from 01.01.2018) and Histology and Embryology Department of I. Gorbachevsky Ternopil National Medical University (from 01.01.2019).

Results of the study and their discussion. Microscopic studies have established the intrathyroid changes of different degrees and of a predominantly destructive nature in the follicles, thyroid epithelium, vessels and stroma 7 days after the thyroid gland experimental thermal injury. The intralobular follicles have different sizes, some of them are deformed. The centrally located small follicles contain little colloid, which is loose, finely dispersed and weakly oxyphilic with the large resorption vacuoles. These follicles thyrotropic epithelium is highly prismatic or cubic. The cells are characterized by intercellular swelling, hypochromic nuclei, unclear cellular borders (fig. 1 A).

Interfollicular islands are also mainly found in the central parts of the lobule. Stromal, interfollicular connective tissue is swollen, leukocyte infiltrates are present in some areas, mainly perivascularly. Large follicles of an altered shape with compacted or lightened colloid are determined in the peripheral areas of the lobules, no resorption vacuoles were detected. Thyrocytes of such follicles are flat-shaped, desquamated cells were observed in the lumen of the follicles (fig. 1 B).

The vessels of the organ - both arteries and veins - were filled with blood and had a swollen wall. In the arteries, the media was thickened, smooth myocytes had intercellular swelling and intensively basophilic nuclei. The endothelium of the intima is also destructively changed, with bulging nuclei in the lumen of the vessel, one could reveal the leukocytes marginal standing near the endothelium. The adventitia is swollen, with lymphocytes infiltration. Veins are characterized by marked full blood, wall swelling and local thinning (fig. 1 C).



A B C

Fig. 1. Microscopic changes of the thyroid gland (fragments A and B) and its vessels (frarment C) 7 days after the experimental thermal injury in conditions of physiological saline administration. Hematoxylin-Eosin staining. x 200.

A – 1 – malformed follicles; 2 – colloid with resorption vacuoles; 3 – arteriole. **B –** 1 – voluminous (enlarged) follicles; 2 – clarified colloid; 3 – desquamated thyrocytes in the follicular lumen; 4 – artery; 5 – swollen interstitial connective tissue. **C –** 1 – artery lumen; 2 – wall deformation; 3 – adventitial edema.

The vessels were revealed with narrowed and collapsed lumens together with deformed, thinned or thickened walls in some areas. The intraorganic hemodynamics disturbance is also manifested by microcirculatory vessels changes. Most of the venules were filled with blood, arterioles had a thickened, swollen wall with perivascular leukocyte infiltrates.

Ultramicroscopically one could register the hemocapillaries walls and the follicle wall thyrocytes ultrastructure impairment 7 days after the experimental thermal injury with physiological saline administration. The thyrocytes located in large follicles had a flattened shape, contained elongated nuclei characterized by an indistinct, sometimes homogeneous karyolemma with numerous intussusceptions.

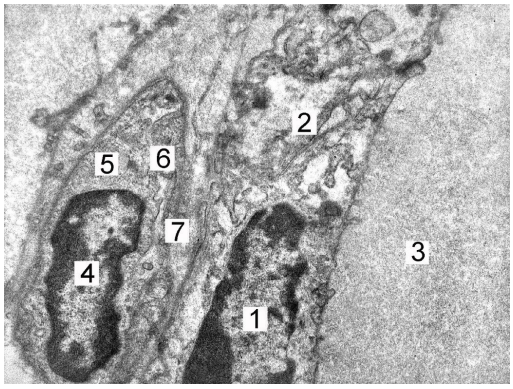
An expansion of the perinuclear space was found in some areas. Karyoplasm contained small loci of euchromatin and large, marginally located clumps of heterochromatin together with small, compacted nucleoli. There is swelling of the cytoplasm, as a result of which it becomes electron-bright with destructively changed organelles. The tubules of the granular endoplasmatic reticulum are significantly thickened or thinned. Mitochondria are few, they have a changed shape with vacuoles and with a light mitochondrial matrix, reduced cristae. Few free ribosomes and polysomes were detected in the cytoplasm. In the apical pole of thyrocytes, numerous vesicles and osmiophilic lysosomes are determined. The plasmolemma of cells forms individual low, microvilli (fig. 2 A).

The heterogeneous changes in the thyroid gland capillaries structural components were established ultramicroscopically 7 days after the experimental thermal injury with NaCl administration. The lumens of many of them were widened, there was significant blood filling with the erythrocytes stasis and sludge formation, wall swelling was noted. The endotheliocytes nuclei were deformed, their shape was elongated, elliptical, the karyoplasma contained mainly euchromatin and lumps of osmiophilic heterochromatin on the periphery. Local cariolema intussusceptions were detected, its outer and inner membranes were unclear and blurred. Locally expanded perinuclear space was observed.

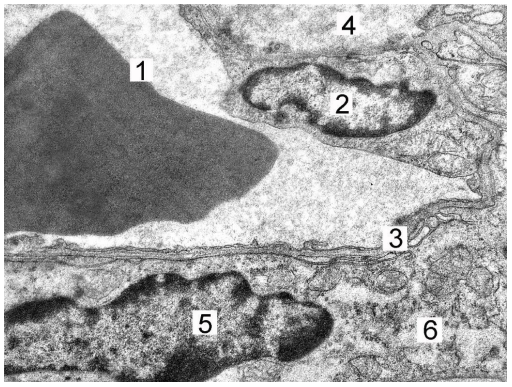
In the endotheliocytes cytoplasm there are poorly developed organelles characterized by signs of destruction. Tubules of the endoplasmic reticulum are significantly expanded and thickened with large vacuoles and cavities formation. Mitochondria had a changed shape, their outer and inner membranes were clearly contoured, and the enlightened mitochondrial matrix filled the spaces between the partially fragmented cristae.

Preservation of intercellular contacts was observed. The peripheral areas cytoplasm was moderately swollen, electron bright, with few micropinocytous vesicles and caveolae and a small number of fenestrae The lumenal surface, which faces the blood flow, is indistinct covered with single low microvilli. The basement membrane was slightly thickened or thinned, vaguely contoured (fig. 2 B).

Pericytes are characterized by a changed shape of the nuclei with enlightened areas of the cytoplasm and damaged membrane organelles.



A



B

Fig. 2. Ultramicroscopic changes of the thyroid gland 7 days after the experimental thermal injury in conditions of physiological saline administration. Electrogram. x 10000. **A –** 1 – nucleus and 2 –cytoplasm of thyrocyte; 3 – colloid; 4 – nucleus and 5 – cytoplasm of endotheliocyte; 6 – narrow capillary lumen; 7 – basal membrane. **B –** 1 – lumen of a capillary with an erythrocyte; 2 – nucleus of an endotheliocyte; 3 – basal membrane; 4 – interstitial connective tissue; 5 – nucleus and 6 – cytoplasm of a thyrocyte.

Thus, micro- and ultra-microscopic studies of thyroid tissue 7 days after thermal damage of the body revealed expressed changes of the mainly destructive nature. Follicles of various sizes with intracellular swelling, the presence of swollen thyrocytes with a changed shape, oxyphilic cytoplasm and destructively changed intracellular organelles, pathological changes in the surrounding connective tissue and impaired blood supply - even such a shortened list of the post-burn process established pathomorphological correlates is enough to understand the irreversibility of the detected histological changes.

Table 1

Thyroid gland and its components micro- and ultramicroscopic morphological changes at 1, 3 and 7 days after experimental thermal injury in conditions of physiological saline administration

Indices	Time of obseravtion		
	Day 1	Day 3	Day 7
The size of the follicles	Mostly medium in size	Most of the follicles in the composition of the lobules are overstretched by a dense homogeneous colloid and lined with flat thyrocytes.	Different. In the center of the lobe – small (highly prismatic epithelium), on the periphery - large (epithelium flat, with the phenomena of desquamation)
Thyrocytes	Low-prismatic form, resorption vacuoles are present. Mitochondria are swollen, with symptoms of cristae discomplexation. Lysosomes and phagosomes that cleave colloids on the apical surface. Available microvilli.	Cubic shape, oxyphilic cytoplasm and round nucleus with dense basophilic heterochromatin. Single locally swollen thyrocytes with lightened areas of the cytoplasm. On the apical surface - single microvilli	Cytoplasmic edema, destruction of organelles, mitochondria are few, their cristae are reduced. On the apical surface - a few vesicles and lysosomes. Single microvilli.
Connective tissue	Swelling of loose connective tissue, intercellular edema.		
Vessels	Hemocapillaries without signs of excessive blood supply, endothelial cytoplasm without signs of edema.	Arterial and venous full blood, stasis in the arterial link of the organ and components of the microcirculatory channel. Spasmed arteries with perivascular edema.	Blood supply to arteries and veins, endothelial destruction, marginal leukocytes. Blood filling of hemocapillaries, stasis and sludge of erythrocytes, deformation of endothelial cells.
Efficacy of NaCl injection	Ineffective		
The prevailing focus of pathomorphological processes	Destruction and alteration		
Expression of protective/compensatory mechanisms	Decompensatory		

It is interesting that all the micro- and ultra-microscopic changes in this time interval of the post-burn period were produced in case of the physiological solution injection. This is the second intermediate conclusion in the obtained results analysis which indicates the thyroid gland burn pharmacocorrection

failure by physiological solution introduction which have to restore the blood circulating volume, since such a therapeutic principle is based on the studied pathology pathogenetic mechanisms understanding.

One could follow a certain sequence of pathomorphological changes in the thyroid gland parenchyma during the first 7 days of the post-burn process, and our trials were methodologically the same with the 0.9% NaCl solution corrective introduction. 24 hrs after the burn of the body with physiological solution introduction we recorded reactive adjuvant and compensatory changes in the vascular, stromal and parenchymal components of the body, which characterized the initial manifestations of alteration and destruction of the thyroid gland parenchyma (Table 1).

We can trace the destructive irreversible pathomorphological changes progressive development during the first 7 days of the studied pathological process which indicates the complete organisms' maladaptation, its pathological dysintegration, the altering component prevalence in the long-term pathological reaction with low efficiency and/or lack of efficiency from the applied treatment. We searched the literary sources which revealed to be useless since there are no modern scientific clinical examinations and/or experimental studies in the direction we have chosen. According to one of the most powerful search services of the USA National Library of Medicine of the National Center for Biotechnology Information we found only lonely works devoted to thyroid gland functioning in conditions of thermal injury published on the 50s and 80s of the last century, i.e. Romani (1952), Reichlin & Lieberman (1958), Smeds et al. (1981), Becker et al. (1983), in which the gland hormonal activity changes were studied in response to its thermal damage. In a certain way, the results presented in these scientific works [4, 6] are consistent with ours where we showed the thyroid gland hormonal activity suppression throughout the 14 days of the post-burn period and hypothyroidism formation [13]. Unfortunately, insufficient attention was paid to intrathyroidal histological changes and disturbances in the surrounding structures study, which might provide a clear understanding of the existing intrathyroid micro- and ultra-microscopic changes during body burns as well as the pathophysiological mechanisms of gland damage [12].

Resuming, one should conclude that on the 7^{th} day of an experimental burn of the body, the pathomorphological signs of destruction prevail in the thyroid gland tissue, likelihood as a result of excessive thermal exposure and accelerated metabolism, which depletes the remaining energy balance in the body. The results of micro- and ultra-microscopic studies of the thyroid gland of animals under the specified conditions against the background of NaCl administration established the processes thyrocytes structure remodeling, the capillaries walls and stromal connective tissue remodeling with signs of their destruction and alteration, which indicates the organ functional activity worsening.

Conclusions

1. Micro- and ultra-microscopic studies of thyroid tissue 7 days after thermal damage of the body revealed expressed changes of the mainly destructive and irreversible nature.

2. Physiological solution injection and attempts to restore the circulating blood volume proved the failure of thyroid burn pharmacocorrection.

3. The results of micro- and ultra-microscopic studies of the thyroid gland of animals under the specified conditions against the background of NaCl administration established the processes thyrocytes structure remodeling, the capillaries walls and stromal connective tissue remodeling with signs of their destruction and alteration, which indicates the organ functional activity worsening.

Prospects for further research include a further pathomorphological study of the thyroid gland parenchyma and its surrounding tissue burn with efforts to find out the time interval of the intrathyroid adaptive processes restoration, as well as a obligatory effort to develop and test the efficacy of studied pathological condition pharmacological correction with a clear understanding of the gland thermal burn pathogenetic mechanisms with the prospect of sanogenetic systems of the body activation.

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TOPOGRAPHIC AND ANATOMICAL JUSTIFICATION OF THE APPROACH TO PERFORMING INTRAPERITONEAL INJECTION

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Performing intraperitoneal injections in rats is one of the most common and accessible methods of administering drugs and anesthetics in an experiment. In 74 % of cases (32/43), the cecum was located on the right and along the midline. A midline was performed in the laboratory animal from the external opening of the urethra to the xiphoid process. Through the point that divides it in half, a perpendicular was drawn along the left half of the abdomen's front wall. A bisector formed at an angle of 90° to the inguinal fold was lowered into the left inguinal region. The injection site was defined as a point lying on ½ of the line formed by the bisector of the angle. The proposed technical solution has significant advantages: it justifies the place and point of intraperitoneal injection and reduces the risk of iatrogenic damage to abdominal organs.

Key words: intraperitoneal injection, rats, cecum.

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ТОПОГРАФО-АНАТОМІЧНЕ ОБГРУНТУВАННЯ ПІДХОДУ ДО ВИКОНАННЯ ІНТРАПЕРИТОНЕАЛЬНОЇ ІН'ЄКЦІЇ

Виконання інтраперитонеальних ін'єкцій у щурів – один з найбільш поширених та доступних методів введення лікарських препаратів та анестетиків у експерименті. У 74 % випадків (32/43) сліпа кишка розташовувалась справа та по серединній лінії. Лабораторній тварині проводили середину лінію від зовнішнього отвору уретри до мечоподібного відростка. Через точку, що ділить її навпіл, проводили перпендикуляр по лівій половині передньої стінки живота. У ліву здухвинну ділянку опускали бісектрису, утвореного кута 90° до пахвинної складки. Місце для ін'єкції визначали як точку, що лежить на ½ лінії утвореної бісектрисою кута. Запропоноване технічне рішення має суттєві переваги: обгрунтовує місце та точку виконання інтраперитонеальної ін'єкції та знижує ризик ятрогенного ушкодження органів черевної порожнини.

Ключові слова: інтраперитонеальна ін'єкція, щурі, сліпа кишка.

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The choice of the method of administering the drug to a laboratory animal during the experiment involves the assessment of the main properties of the substance, in particular – sterility, pH, osmolarity, pharmacokinetic features and frequency of administration [7]. Performing intraperitoneal injections in rats is one of the most common and accessible methods of administering drugs and anesthetics in an experiment [2, 9].

Among its advantages over intravenous administration is primarily the ease of manipulation [3] and the absence of the need for catheterization of the lateral tail or iliac veins, the risk of phlebitis or