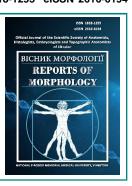
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Rats' thyroid gland histological and ultrastructural changes 30 days after the experimental thermal injury on the background of NaCl injection

Tiron O. I.Odesa National Medical University, Odesa, Ukraine

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CORRESPONDING AUTHOR

e-mail: chekina.o@ukr.net Tiron O. I.

CONFLICT OF INTEREST

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Very frequent natural and man-made disasters as well as current military conflicts are accompanied by injuries complicated by acute blood loss, burns and shock of different degrees of severity. Thermal injuries are one of the world modern medicine most urgent medical and social problems including the same in Ukraine. In response to a burn injury a significant number of pathological processes develop in the body which manifestation involves almost all organs and systems, leading to an expressed homeostasis disturbance and adaptative processes disorder. Thyroid gland is the one of the first which receives the alterative influence in conditions of both threshold and suprathreshold thermal exposure. The purpose of the work is to establish histological and ultrastructural changes in the thyroid gland of experimental animals 30 days after thermal skin injury. Experimental studies were conducted on 90 white male rats. Skin thermal burns were simulated using four copper plates application to previously depilated lateral surfaces of the rats' body for 10 s. Rats were injected with a 0.9 % saline into the vena cava inferior during the first 7 days of the post-burn period. Thyroid gland pieces were fixed in a 10 % neutral formalin solution, dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. The prepared sections of 5-6 µm thickness were stained with hematoxylin-eosin. In the rats' thyroid gland the presence of complex changes of a destructive, adaptive-compensatory and regenerative nature was established on the 30th day after a skin burn against the background of 0.9 % NaCl administration. These changes indicate a relative organs hemodynamics normalization together with the structure of various diameters vessels walls which creates the basis for the follicular cells typical structure restoration and new follicles synthesis. The expressed osmiophily and thyrocyte nuclei pyknotization, cytoplasm vacuolization and follicle's lumen cells desquamation are revealed in the gland parenchyma. These are signs of the organ fibrosis which are supported by connective tissue amount in the stromal component increase. The thyroid gland parenchyma and the vessels surrounding it micro- and ultrastructural changes caused by the thermal altering influence have a timedependent expression which transforms from predominantly destructive to predominantly restorative throughout the 30-day post-burn period. The authors are sure that the detected morphological changes of the gland have a time-dependent reversibility which is important to consider from both the time-course and the quantitative aspects taking into account the pathogenetically based scheme of pharmacocorrection. The 30th day of the post-burn pathological process manifestation characterizes by a certain imbalance in the compensatory, restorative and synthetic processes manifestation as well as in the destructive and decompensatory processes manifestation which gives reason to hope for the thyroid gland functioning restoration together with organs and systems in case of pathogenetically determined pharmacological treatment administration. Taking into account the thyroid gland morpho-functional changes wave-like dynamics the authors believe that sanogenetic mechanisms activation might occurs which will allow to prescribe the complex pathogenetically determined correction assuming the above-mentioned patho- and sanogenetic mechanisms dynamics.

Keywords: thyroid gland, thermal burn, post-burn period, morphological changes, restorative processes, pathogenetic mechanisms.

Introduction

Very frequent natural and man-made disasters, as well as current military conflicts, are accompanied by injuries complicated by acute blood loss, burns and shock of varying degrees of severity.

Thermal injuries are one of the most urgent medical and social problems of modern medicine in the world, including in Ukraine [4, 10]. In our country, more than 45,000 people suffer from burns every year, they occupy the third place in the structure of mortality, as a result of all received injuries, inferior in frequency only to traffic injuries [13, 18]. The urgency of the burn injury problem is determined by the frequent damage to adults and children, the complexity and duration of treatment, long-term disability and relatively high mortality. Despite the significant progress achieved in the treatment of this pathology, the mortality rate among severely burned patients remains high, especially with critical (40-50% of the body surface) and supercritical (over 50%) deep burns [11, 20].

In response to a burn injury, a significant number of pathological processes develop in the body, the manifestation of which involves almost all organs and systems, leading to a marked violation of homeostasis, disruption of adaptation processes, etc. [9]. One of the most important aspects of a burn injury, which directly affects the severity of its pathogenetic mechanisms, is endocrine dysregulation at the initial stages of the pathological process, which manifests itself as significant metabolic disorders, the direction and severity of which are directly related to the level of endogenous hormones [1].

In this aspect, we were interested in the changes that occur during thermal burns in the thyroid gland, since it plays one of the leading roles in the endocrine regulation of most body functions [2, 3, 14]. Secondly, this gland of internal secretion occupies an important place in the structure of hierarchical hypothalamic-pituitary regulation, and, thirdly, taking into account the anatomical and morphological features of the localization and structure of the thyroid gland, it primarily "takes on" altering traumatic influences, thereby modifying the most functional state of organs and tissues of the human body [7, 9].

We traced the histological changes in the structure of the thyroid gland and the periglandular environment, starting from 24-72 hours post-thermal period [16, 17]. In the first hours and days after a skin burn, the structural changes in the thyroid gland affected mainly the structure of the vascular component of the gland, its stroma and parenchyma and had mainly the character of adaptation and/or compensation [16]. In the urgent aspect, our research lasted 30 days, which prompted us to carefully examine the volume of morphological changes in the structure of the thyroid gland during this time interval. On the other hand, we were guided by a wide range of regulatory hormonal effects of the thyroid gland on organs, organ systems, and regulatory systems of the biological organism, which, in our opinion, determines the primary reactions in response to thermal injuries [1-3, 5,

12, 14].

It is important to imagine that the thyroid gland under the influence of temperature factors of threshold and suprathreshold intensity, taking into account the large-scale redundant feedback mechanisms, the wide spectrum of physiological activity of thyroid hormones, the structuralfunctional organization and morpho-functional features, is one of the first to fall under thermal shock [12]. With structural and functional changes in the organs of the affected and/or burned organism, including the thyroid gland, dysfunctions of many organs and systems are involved in the mediation of the pathological process, in particular, the blood, cardiovascular, respiratory, and other systems [1, 10, 20]. Pathogenetic mechanisms of the primary and related pathological processes induced by burn damage to the thyroid gland are insufficiently studied, which is a consequence of, firstly, unexplained morpho-functional dysfunctions of the parenchyma of the gland in the dynamics of the burn effect and, secondly, unexplored chains of the "vicious circle" pathological processes that are determined by thyroid dysfunction and occur with the participation of other organs and body systems.

However, studies of the structural reconstruction of the vascular bed of the thyroid gland during burns, especially in the time-delayed stages after the application of thermal exposure, are insufficient and require further research, especially with an emphasis on efforts to correct the morphofunctional disorders formed during a burn injury.

The aim of the work is to determine histological and ultrastructural changes in the thyroid gland of experimental animals 30 days after simulated thermal skin injury.

Materials and methods

Experimental studies were conducted 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 Sciences of Ukraine) on the basis of the research center of National Pirogov Memorial Medical University, Vinnytsya. Keeping, processing and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), while being guided by the recommendations of the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasburg. 1985), methodological recommendations of the Ministry of Health of Ukraine "Preclinical studies of drugs" (2001) and the rules of humane treatment of experimental animals and conditions approved by the Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya (protocol No. 1 dated January 14, 2010).

Thermal skin burns of the 2nd-3rd degree were simulated by pressing four copper plates (the surface area of each was 13.86 cm²) to the prematurely depilated lateral surfaces of the body of rats for 10 s. Experimental animals

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were heated in water with a temperature of 100°C for 6 minutes before the start of the experiment [6]. The total area of skin damage was equal to 21-23 %. During the first 7 days of the post-burn period, rats were injected with 0.9 % physiological NaCl solution into the inferior vena cava. Rats were shaved, vein catheterized, skin burned, and decapitated under propofol (i/v, 60 mg/kg) anesthesia.

Material collection for microscopic studies was carried out according to generally accepted methods [8]. Pieces of the thyroid gland were fixed in a 10 % neutral formalin solution, dehydrated in alcohols of increasing concentration, and embedded in paraffin blocks. The prepared sections, 5-6 µm thick, were stained with hematoxylin-eosin [8].

Histological preparations were studied with the help of a light microscope MICROmed SEO SCAN ("Sumi Electron Optics", Sumy, Ukraine) and photo-documented with the help of a Vision CCD Camera with a system of image output from histological preparations. For electron microscopic studies, pieces of the thyroid gland were taken, fixed in a 2.5 % glutaraldehyde solution, and postfixed with a 1 % osmium tetroxide solution in a phosphate buffer. Further processing was carried out according to the generally accepted methodology [8]. Semi-thin sections were stained with methylene blue. Ultrathin sections, made on an LKB-3 ultramicrotome, were contrasted with uranyl acetate, lead citrate according to the Reynolds method and studied in a PEM-125K electron microscope.

All morphological studies were carried out within the scope of the Agreements on Scientific Cooperation between the Department of Histology, Cytology and Embryology of Odesa National Medical University and the Research Center of National Pirogov Memorial Medical University, Vinnytsya (from 01.01.2018), as well as the Department of Histology and Embryology of I. Horbachevsky Ternopil National Medical University (from 01.01.2019).

Results

Histologically, 30 days after a skin burn against the background of the introduction of a 0.9 % NaCl solution in the thyroid gland of experimental animals, we found destructive, adaptive-compensatory and regenerative processes in the follicle wall, vascular bed and stromal component.

In this period of the experiment, at the morphological level between the lobes of the organ and in the interfollicular space, wide layers of connective tissue were observed in places, which is a consequence of the activation of fibroblasts and the process of collagen formation in the previous periods. Single neutrophils, basophils, and macrophages were present among the collagen and elastic fibers of the connective tissue framework (Fig. 1).

30 days after the skin burn, against the background of administration of 0.9 % NaCl solution, moderate blood filling and isolated stasis were noted in the vascular network of the organ. The vessel walls were characterized by moderate edema, which was mainly found in the adventitia. Arterioles, hemocapillaries, and venules had a relatively preserved wall

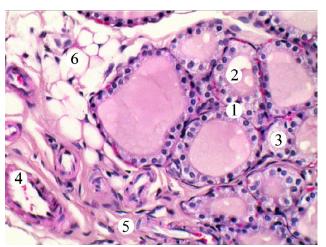


Fig. 1. Histological changes in the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9% NaCl solution. 1 - thyrocytes with areas of light cytoplasm; 2 - colloid with resorption vacuoles, 3 - interfollicular island, 4 - blood vessel, 5 - layers of connective tissue, 6 - adipocytes. Staining with hematoxylin and eosin. Magnification x400.

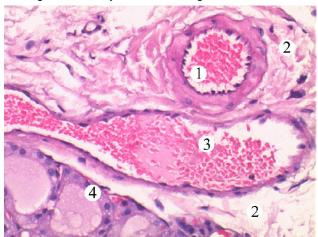


Fig. 2. Microscopic changes in the vessels of the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9 % NaCl solution. 1 - arteriole with 2 - perivascular edema, 3 - venule, 4 - follicle. Staining with hematoxylin and eosin. Magnification x400.

structure, with well-contoured endothelium and moderate lumen width (Fig. 2).

At the microscopic level, 30 days after the skin burn, follicles, mostly of medium size, filled with oxyphilic colloid of a homogeneous structure and lined with low-prismatic thyrocytes, were observed against the background of saline injection. Their rounded-oval nuclei were intensely basophilic, which indicates the predominance of heterochromatin in the karyoplasm. The cytoplasm of many follicular cells had areas of lightening (see Fig. 1). There were also follicles in the composition of the lobes of the organ, the wall of which was formed by flat epitheliocytes with their desquamation into the lumen, and resorption vacuoles were contained in the colloid. It should be noted that interfollicular islands of various sizes were histologically

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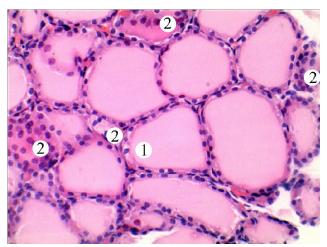


Fig. 3. Histological changes in the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9 % NaCl solution. 1 - follicle lined with flat thyrocytes and 2 - interfollicular islands. Staining with hematoxylin and eosin. Magnification x400.

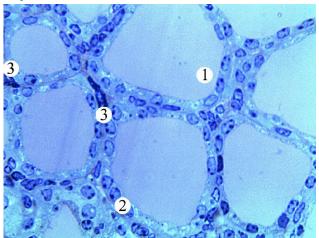


Fig. 4. Microscopic changes in the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9 % NaCl solution. 1 - homogeneous colloid, 2 - thyrocytes, 3 - basophils. Semi-thin cut. Staining with methylene blue. Magnification x400.

detected, the cells of which had moderately basophilic nuclei and moderately oxyphilic cytoplasm. This may indicate reparative processes in the organ (Fig. 3).

On semi-thin sections stained with methylene blue, basophils, the cytoplasm of which contained numerous intensively basophilic granules, were clearly visible in the interfollicular lumens at this time of the experiment. There were thyrocytes with a completely heterochromatin pattern of the nucleus (Fig. 4).

The condition of the thyroid gland of the experimental animals at the ultrastructural level 30 days after the skin burn against the background of the introduction of 0.9 % NaCl solution testified to the changes established by us during microscopic analysis. The low-prismatic thyrocytes of the middle follicles contained an osmiophilic nucleus with

numerous lumps of heterochromatin and intussusceptions of karyolemma. A significant number of organelles of the protein-synthesizing apparatus were observed in their cytoplasm.

In particular, most mitochondria were hypertrophied, with partially reduced cristae. The tubules of the granular endoplasmic reticulum are locally significantly expanded, and in some places they are vacuole-like, which could create areas of illumination at the light-optical level. The plasmalemma of the apical pole of thyrocytes formed a moderate number of microvilli. The basement membrane was characterized by tortuosity and contouring (Fig. 5).

Flat thyrocytes contained a pyknotically altered nucleus in the center of the cell. Damaged tubules of the granular endoplasmic reticulum, mitochondria with indistinct cristae and vacuole-like structures with homogeneous content were observed in their cytoplasm, and clusters of osmiophilic lysosomes were observed near the apex of the cell. The plasmalemma at the apical pole contained wide microvilli (Fig. 6).

The ultrastructure of the blood capillaries of the thyroid gland of experimental animals 30 days after a skin burn against the background of administration of a 0.9 % NaCl solution was characterized by the preservation of the wall and the moderate width of the lumen. Endotheliocyte nuclei had shallow invaginations of the karyolemma and a significant amount of heterochromatin in the karyoplasm. In the cytoplasm, the main mass of pinocytotic vesicles is concentrated near the lumenal surface, but the number of microvilli on it is insignificant.

The basement membrane is slightly thickened. Single cells of the leukocyte row and platelets were often observed in the lumen (Fig. 7).

30 days after a skin burn against the background of the

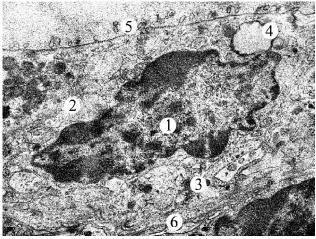


Fig. 5. Ultrastructure of the wall of the follicle of the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9% NaCl solution. 1 - thyrocyte nucleus, 2 - mitochondrion, 3 - tubules of the granular endoplasmic reticulum, 4 - vacuole-like structure, 5 - microvilli on the apical surface, 6 - basement membrane. Electrogram. Magnification x14 000.

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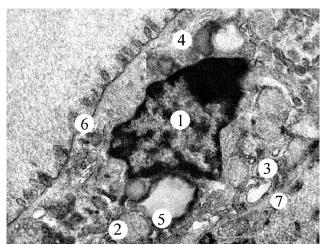


Fig. 6. Ultrastructure of the wall of the follicle of the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9% NaCl solution. 1 - thyrocyte nucleus, 2 - mitochondrion, 3 - tubules of granular endoplasmic reticulum, 4 - Golgi complex, 5 - vacuole-like structure with homogeneous content, 6 - microvilli on the apical surface, 7 - basement membrane. Electrogram. Magnification x14 000.

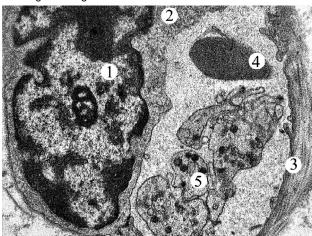


Fig. 7. Ultrastructural organization of the hemocapillary of the thyroid gland of an animal 30 days after a skin burn against the background of administration of a 0.9 % NaCl solution. 1 - nucleus and 2 - cytoplasm of endotheliocyte, 3 - basement membrane, 4 - erythrocyte and aggregation of platelets in the capillary lumen. Electrogram. Magnification x12 000.

introduction of a 0.9 % NaCl solution, a large number of thin collagen fibrils and already well-formed collagen fibers were noted in the stroma of the thyroid gland of experimental animals. The ultrastructure of fibroblasts was characteristic of the state of functional activity: euchromatin pattern of the nucleus, expanded tubules of the granular endoplasmic reticulum in the cytoplasm, with numerous mitochondria between them.

Discussion

Thus, 30 days after skin burn against the background of administration of 0.9 % physiological NaCl solution in the

thyroid gland of rats, the presence of complex changes of a destructive, adaptive-compensatory and regenerative nature was established. At the ultrastructural level, the relative normalization of the hemodynamics of the organ and the structure of the vessel walls of various calibers has been proven, which, in our opinion, creates the basis for the restoration of the typical structure of follicular cells and the synthesis of new follicles, which is evidenced by a significant number of interfollicular islands. Therefore, the obtained data can be verified as intensifying synthetic processes in the structural units of the endocrine gland.

However, along with optimistic intraglandular changes, we note that during this period of the experiment, signs of destructive changes are preserved directly in the parenchyma of the gland, which are manifested by significant osmiophily and pyknotization of thyrocyte nuclei, vacuolization of the cytoplasm, and desquamation of cells into the lumen of the follicle. The result of the active activity of fibroblasts in the earlier periods of the experiment are signs of fibrosis of the organ, as evidenced by an increase in the amount of connective tissue in the stromal component.

Summarizing, we note that the 30th day of the course of the post-burn pathological process is characterized by a certain imbalance in the manifestation of both compensatory, restorative and synthetic processes, on the one hand, and destructive, decompensatory processes, on the one hand, which in general gives us reason to hope that in the case of assigning rats model conditions of pathogenetically determined pharmacological treatment of the functioning of the thyroid gland, as well as organs and systems in the animal body will be not only in a compensated, but also in a pronounced restorative state and in the presence of stimulating hormonal and biochemical processes.

Tracking the course of the 30-day period after the skin burn and analyzing the morpho-functional changes of the thyroid gland, we note that they underwent a significant reversal from the supposedly irreversible and degenerative during the first 7 days of the experiment to those noted according to the results of the reported studies and which have a more pronounced restorative effect orientation We will recall that micro- and sub-microscopic studies 1 day after thermal damage revealed only primary and/or reactive auxiliary-compensatory changes and initial manifestations of destruction of the thyroid parenchyma [16]. At the same time, only after 3 days, the initial signs of a heat-induced "stress reaction" in the form of intrathyroidal parenchymal, stromal, and vascular lesions were revealed [17]. Note that the dependence of the morpho-functional changes of the thyroid gland on time that we discovered is, firstly, inherent in such changes during post-traumatic and post-stress periods [19] and, secondly, in a certain way consistent with the dynamics of the secretory activity of the gland and the anterior lobe of the pituitary gland [1, 3, 5, 12, 15].

Logically connecting the obtained results with efforts to develop a scheme for pathogenetically justified pharmacocorrection of burn damage to the thyroid gland,

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the following should be stated: micro- and ultrastructural changes in the parenchyma of the thyroid gland and the vessels surrounding it due to the thermal altering effect have a time-dependent severity, which within 30-day post-fire period transforms from predominantly destructive to predominantly restorative. Secondly, the morphological changes of the gland traced by us have a time-dependent reversibility, which is important to take into account from the urgent aspect and from the quantitative aspect of the composition of the scheme of pathogenetically justified pharmacocorrection. Thirdly, we consider it probable, taking into account the wave-like dynamics of changes in the morpho-functional changes of the thyroid gland, to assume the activation of sanogenetic mechanisms by the mechanism of systemic-anti-systemic regulation, which will make it possible, under the conditions of a thorough study of the specified systemic-anti-systemic regulatory mechanisms, to prescribe a complex pathogenetically determined correction taking into account the abovementioned dynamics patho- and sanogenetic mechanisms.

Conclusions

- 1. 30 days after a skin burn against the background of administration of a 0.9 % physiological solution of NaCl in the thyroid gland of rats, the presence of complex changes of a destructive, adaptive-compensatory and regenerative nature was established.
- The indicated changes indicate relative normalization of the hemodynamics of the organ and the structure of the vessel walls of various sizes, which creates the basis for the restoration of the typical structure of follicular cells and

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- 3. The micro- and ultrastructural changes of the parenchyma of the thyroid gland and the vessels surrounding it caused by the thermal altering effect have a time-dependent severity, which during the 30-day post-burn period transforms from predominantly destructive to predominantly restorative.
- 4. The morphological changes of the gland traced by us have a time-dependent reversibility, which is important to take into account from the urgent aspect and from the quantitative aspect of the composition of the pathogenetically justified pharmacocorrection scheme.
- 5. 30 days of the course of the post-burn pathological process is characterized by a certain imbalance in the manifestation of compensatory, restorative and synthetic processes, as well as destructive and decompensatory processes, which gives reason to hope for the restoration of the functioning of the thyroid gland, as well as organs and systems in the event of the appointment of pathogenetically determined pharmacological treatment.
- 6. Taking into account the wave-like dynamics of changes in the morpho-functional changes of the thyroid gland, we consider the activation of sanogenetic mechanisms to be probable, which will make it possible to prescribe a complex pathogenetically determined correction, taking into account the above-mentioned dynamics of patho- and sanogenetic mechanisms.
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ГІСТОЛОГІЧНІ ТА УЛЬТРАСТРУКТУРНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ ЩУРІВ ЧЕРЕЗ 30 ДІБ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ НА ФОНІ ВВЕДЕННЯ NaCI $Tipoh\ O.\ I.$

Дуже часті в даний час природні і техногенні катастрофи, а також поточні військові конфлікти супроводжуються травмами, ускладненими гострою крововтратою, опіком і шоком різного ступеня тяжкості. Термічні ураження є однією з найактуальніших медико-соціальних проблем сучасної медицини у світі, в тому числі, в Україні. У відповідь на опікову травму в організмі розвивається значна кількість патологічних процесів, до маніфестації яких залучені практично всі органи і системи, що призводить до вираженого порушення гомеостазу, зриву адаптаційних процесів. Щитоподібна залоза за умов впливу температурних чинників порогової та надпорогової інтенсивності однією з перших підпадає під термічний удар. Мета роботи - встановлення гістологічних та ультраструктурних змін щитоподібної залози експериментальних тварин через 30 діб після змодельованої термічної травми шкіри. Експериментальні дослідження проводили на 90 білих щурах-самцях. Термічні опіки шкіри моделювали шляхом притискання чотирьох мідних пластин до завчасно депільованих бокових поверхонь тіла щурів протягом 10 с. Протягом перших 7 діб післяопікового періоду щурам у нижню порожнисту вену вводили 0,9 % фізіологічний розчин NaCl. Шматочки щитоподібної залози фіксували в 10 % нейтральному розчині формаліну, проводили дегідратацію в спиртах зростаючої концентрації, заливали у парафінові блоки. Виготовлені зрізи, товщиною 5-6 мкм, забарвлювали гематоксиліном-еозином. На 30 добу після опіку шкіри на фоні введення 0,9 % фізіологічного розчину NaCl у щитоподібній залозі щурів встановлено наявність комплексних змін, які мають деструктивний, пристосувально-компенсаторний та регенераторний характер. Вказані зміни свідчать про відносну нормалізацію гемодинаміки органу та структури стінок судин різного діаметра, що створює підґрунтя для відновлення типової структури фолікулярних клітин та синтезу нових фолікулів. В паренхімі залози виявляється значна осміофілія та пікнотизація ядер тироцитів, вакуолізація цитоплазми та десквамація клітин у просвіт фолікула. Наявні ознаки фіброзу органу, про що свідчить збільшення кількості сполучної тканини у стромальному компоненті. Обумовлені термічним альтеруючим впливом мікро- та ультраструктурні зміни паренхіми щитоподібної залози та судин, які її оточують, мають залежну від часу вираженість і упродовж 30-денного післяопікового періоду трансформуються із переважно деструктивних до переважно відновлювальних. Автори впевнені, що виявлені морфологічні зміни залози мають залежну від часу зворотність, що важливо враховувати з термінового аспекту та з кількісного аспекту складу схеми патогенетично обґрунтованої фармакокорекції. 30 доба перебігу післяопікового патологічного процесу характеризується певним дисбалансом у прояві компенсаторних, відновлювальних і синтетичних процесів, а також деструктивних і декомпенсаторних процесів, що дає підстави сподіватися на відновлення функціонування щитоподібної залози, а також органів і систем в разі призначення патогенетично обумовленого фармакологічного лікування. З урахуванням хвилеподібної динаміки морфо-функціональних змін щитоподібної залози, автори вважають ймовірним активацію саногенетичних механізмів, що дасть можливість призначати комплексну патогенетично обумовлену корекцію з урахуванням вищевказаної динаміки пато- та саногенетичних механізмів.

Ключові слова: щитоподібна залоза, термічний опік, післяопіковий період, морфологічні зміни, відновлювальні процеси, патогенетичні механізми.

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