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WHITE RATS' THYROID GLAND ULTRASTRUCTURAL CHANGES IN THE DYNAMICS OF EXPERIMENTAL THERMAL INJURY UNDER CONDITIONS OF LACTOPROTEIN WITH SORBITOL HYPEROSMOLAR SOLUTION USING

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The purpose of the study was to determine the experimental animals' thyroid gland ultrastructural changes throughout the thermal skin injury dynamics in condition of hyperosmolar lactoprotein with sorbitol solution injection. The provided ultramicroscopic studies have established the expressed hyperosmolar lactoprotein with sorbitol colloid solution corrective influence on thyroid gland ultrastructural changes induced by its burning. Hyperosmolar lactoprotein with sorbitol colloid solution administration results in normalization of thyroid gland morphological structure and ultramicroscopic intrathyroidal disorders during the post-burn period. A significant improvement of thyroid blood capillaries and follicular cells ultrastructure was established in the dynamics of the trial with the normalization of their morphology at the ultrastructural level in the late stages of the thyroid gland post-burn period. Initial lactoprotein with sorbitol solution protective effects were registered on the 3rd day after thyroid gland burning. The most expressed lactoprotein with sorbitol protective effects were registered from the 21st day of the trial which were continued till the end. Therefore, the obtained data concerning the lactoprotein with sorbitol solution protecting efficacy in thyroid burning have a pathogenetic background which we consider as the experimental confirmation of hyperosmolar colloidal solutions clinical efficacy testing reasonability in thyroid gland parenchyma burn lesions.

Key words: thyroid gland, thermal injury, ultrastructural changes, lactoprotein with sorbitol, pharmacocorrection, pathogenetic background

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СУБМІКРОСКОПІЧНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ БІЛИХ ЩУРІВ В ДИНАМІЦІ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ ЗА УМОВ ЗАСТОСУВАННЯ ГІПЕРОСМОЛЯРНОГО РОЗЧИНУ ЛАКТОПРОТЕЇНУ З СОРБІТОЛОМ

Метою дослідження є встановлення ультраструктурних змін щитоподібної залози експериментальних тварин в динаміці термічної травми шкіри за умов корекції даного патологічного стану гіперосмолярним розчином лактопротеїну з сорбітолом. Проведені ультрамікроскопічні дослідження виявили виражений корегуючий влив гіперосмолярного розчину лактопротеїну з сорбітолом на ультраструктурін зміни щитоподібної залози при її термічному ураженні. Введення гіперосмолярного колоїдного розчину лактопротеїну з сорбітолом спричиняє нормалізацію морфологічної структури щитоподібної залози та ультрамікроскопічних внутрішньотиреоїдних порушень протягом післяопікового періоду. Встановлено суттєве покращення ультраструктури кровоносних капілярів та фолікулярних клітин щитоподібної залози в динаміці досліду з нормалізацією їх морфології на субмікроскопічному рівні у пізні терміни досліду. Початкові захисні ефекти від застосування розчину лактопротеїну з сорбітолом були зареєстровані на 3-й добі досліду. Найбільш вираженими ці проективні ефекти були, починаючи з 21 доби досліду, та тривали до його кінця. Таким чином, отримані дані щодо захисної ефективності розчину лактопротеїну з сорбітолом при опіках щитоподібної залози мають патогенетичне підґрунтя, що ми вважаємо експериментальним підтвердженням доцільності клінічного тестування ефектів гіперосмолярних колоїдних розчинів при опікових ураженням паренхіми щитоподібної залози.

Ключові слова: щитоподібна залоза, опікова травма, субмікроскопічні зміни, лактопротеїн з сорбітолом, фармакокорекція, патогенетичне обгрунтування

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The problem of thermal burns is multifaceted from a medical point of view [8, 13], and the urgency of its quickest solution and the basic principles clarification of adequate and effective medical care providing to this category of patients obtains extremely important medical, economic and social importance [9, 11]. Big number of pathological processes occurs in the body after a thermal burn, which can result in patients' death without immediate qualified medical assistance [10, 12]. But even in case of timely and accurate medical care and pharmacological correction of the formed disorders, with the human life preservation, a number of morphological and pathophysiological disorders and changes are "triggered" resulting in vital organs and organ systems postponed dysfunctions [1-3, 13].

Thyroid gland is one of the first organs that falls under the alterative thermal influence. The cascade of pathomorphological and pathophysiological processes of mainly destructive and decompensatory nature with neurohumoral dysregulation followed thyroid burning [1, 5, 10].

Pathomorphological and dysfunctional manifestations in rats in the dynamics of the post-burn period were investigated, which revealed intraparenchymal thyroid tissue, cells, and surrounding environment changes [14]. We focused attention on number of pathological processes mediated by thermal exposure: hormonal dysfunction, lipoperoxidation acceleration and antioxidant protection inhibition within the thyroid gland parenchyma, liver and pancreas, dysfunction of the blood system, erythrocytes, kidneys, the structure of cellular membranes etc. [15].

We suppose the thyroid gland burning scheme of complex pathogenetically orientated pharmacological correction to be an important tool in pathogenetic mechanisms determination in case of organs and systems pathological disintegration in burning conditions. Our efforts to reveal the efficacy of hypovolemia recovery in conditions of thyroid gland thermal damage by physiological solution introduction failed. Therefore, we tried to investigate the thyroid gland thermal damage pharmacological correction principal possibility using a hyperosmolar lactoprotein with sorbitol (LPS) colloidal solution, since we already have encouraging results [14]. For that reason, we consider it reasonable to investigate the expression of intrathyroid ultramicroscopic changes under the conditions of LPS using.

The purpose of the study was to determine the experimental animals' thyroid gland ultrastructural changes throughout the thermal skin injury dynamics in condition of hyperosmolar lactoprotein with sorbitol solution injection.

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. No.1 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) preheated for 6 min in water with a temperature of 100 C applying to rats depilated side surfaces for 10 sec [14].

Rats were infused with hyperosmolar LPS solution (10 ml/kg) once per day throughout the first 7 days (the first administration was done 1 hr after the skin burn) after the skin burn during 5–6 min into the lower femoral vein via catheter. The catheter was sutured under the skin, its lumen along its entire length after each hyperosmolar LPS solution administration was filled by a titrated heparin solution (0.1 ml of heparin per 10 ml of 0.9 % NaCl solution). Animals were euthanized by decapitation (after 1, 3, 7, 14 and 21 days). Shaving, venous catheterization, skin burns and decapitation of rats were performed under propofol (i.v., 60 mg/kg) anesthesia.

Hyperosmolar LPS colloidal solution (Kyiv JSC "Biopharma", Certificate of state registration of the Ministry of Health of Ukraine N 464/09–300200000 dated 12.03.2009) has osmolarity equals to 1020 mosmol/l.

Tissues for ultrastructural studies were collected 1, 3, 7, 14, 21 and 30 days after the skin thermal injury with LPS administration. The thyroid gland samples collected for electron microscopic examination were fixed with 2.5 % glutaraldehyde solution, then post-fixed with 1 % osmium tetroxide prepared with phosphate buffer. Further processing was performed according to accepted methods [4].

Ultrathin sections made on an LKB-3 ultramicrotome were contrasted with uranyl acetate and lead citrate according to Reynolds method and then studied using PEM-125K electron microscope.

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. Horbachevsky Ternopil National Medical University (from 01.01.2019).

Results of the study and their discussion. In case of thyroid gland burning correction using hyperosmolar LPS solution one could establish that most of the follicles were lined with flat-shaped thyrocytes with partial destructuring of the organelles. These changes we received at the ultrastructural level throughout the 1–3 days of the trial. The nuclei of these cells are elongated, and their karyoplasm is filled with massive clumps of heterochromatin. The cytoplasm contains the granular endoplasmic reticulum

tubules, mitochondria as well as osmiophilic lysosomes. A moderate number of microvilli are observed on the plasmolemma apical surface of flat thyrocytes which protruded into the follicle lumen (fig. 1 A).

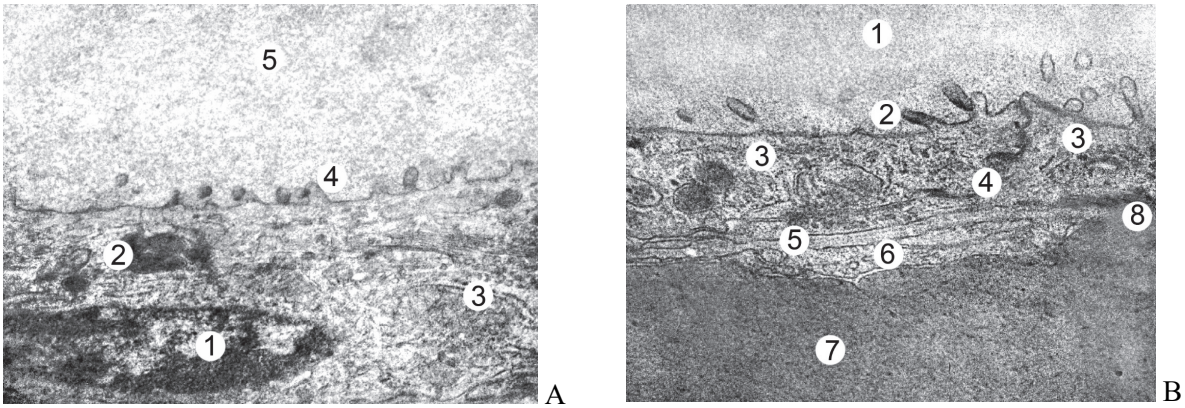

Fig. 1. Ultrastructural changes in the thyrocyte of the thyroid gland follicle wall (fragment A) and the wall of the follicle with a capillary (fragment B) 3 days after thyroid gland experimental thermal injury in case of LPS solution administration. Electrogram. x11000. **A –** 1 – nucleus, 2 – conglomerate of lysosomes, 3 – granular endoplasmic reticulum tubules, 4 – microvilli on the cellular apical surface, 5 – follicle lumen. **B –** 1 – follicle lumen, 2 – microvilli on the cellular apical surface, 3 – cytoplasm of neighboring thyrocytes, 4 – desmosomes, 5 – basal membrane, 6 – endotheliocyte cytoplasm, 7 – erythrocytes, 8 – osmiophilic material.

Desmosomal contacts between the neighboring cells plasmolemmas maintain their integrity. Fenestrated capillaries are observed within the interfollicular space, their lumen is densely filled with erythrocytes which indicate stasis development, and their basal membrane is equally thickened. Homogeneous osmiophilic masses, not surrounded by a membrane, are found within the pericapillary spaces. Their presence can be caused by blood components interference with burn endotoxins and a hyperosmolar LPS solution. Their exit outside the blood vessels is due to capillaries both endothelium and the base membrane increased permeability (fig. 1 B).

7–14 days after the thyroid gland experimental thermal injury in conditions of LPS application one could observe the follicles limited by cubic thyrocytes within the thyroid gland. Submicroscopically these cells nuclei characterized by polymorphism. Some of them have a rounded-oval shape with a euchromatin pattern of karyoplasm (fig. 2 A), and others contain numerous invaginations of karyolemma due to which they looked multilobed. The heterochromatin prevails in the latter (fig. 2 B).

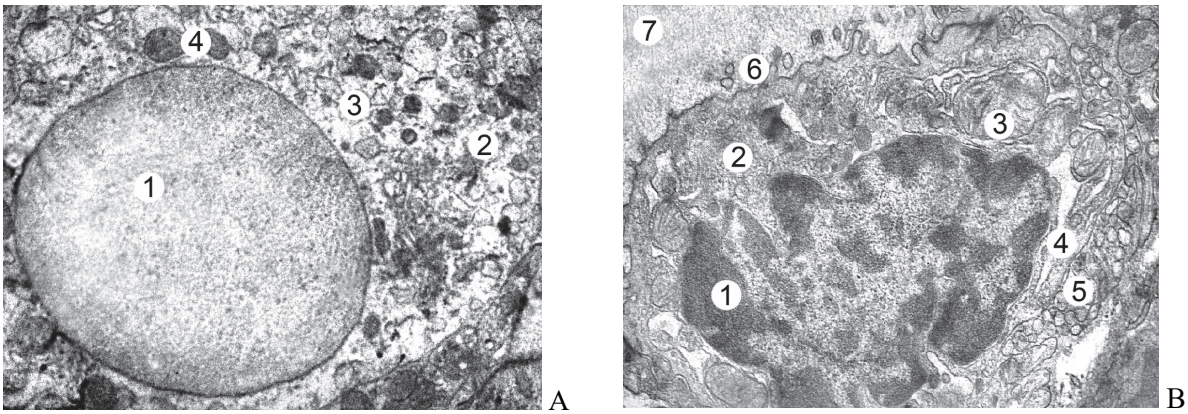

Fig. 2. Ultramicroscopic changes in the thyrocytes of the thyroid gland follicle wall of the rat 7 days (fragment A) and 14 days (fragment B) after thyroid gland experimental thermal injury in case of LPS solution administration. Electrogram. x17000 (fragment A) and x16000 (fragment B). **A –** 1 – nucleus filled with euchromatin, 2 – thyrocyte cytoplasm, 3 –granular endoplasmic reticulum tubules, 4 – lysosomes. **B –** 1 – heterochromatin within the thyrocyte nucleus, 2 – thyrocyte cytoplasm, 3 – mitochondria, 4 – granular endoplasmic reticulum tubules, 5 – microvesicles, 6 – microvilli, 7 – follicle lumen.

The follicular cells cytoplasm is saturated with organelles of the protein-synthesizing and energy apparatus. One could register a large number of mitochondria and expanded tubules of the granular endoplasmic reticulum. Many thyrocytes contain hypertrophied mitochondria with multi-armed cristae. Electron-dense lysosomes often form conglomerates in the paranuclear space. Single-membrane microvesicles quantitatively predominate in the basal pole of thyrocytes vs their apical pole, which indicates the activation of blood plasma substances absorption needed for thyroid hormones synthesis. A moderate number of microvilli are present at the apical pole.

Submicroscopically, moderate swelling of endothelial cells is observed in the wall of the thyroid gland fenestrated capillaries 7–14 days after the experimental burning in conditions of LPS using. Their

lumenal pole plasmolemma forms large microvilli, and the cytoplasm containes pinocytotic vesicles. Endotheliocyte nuclei contained marginally located heterochromatin, and their karyolem forms shallow intussusceptions. The basal membrane is structured and has a relatively identical thickness (fig. 3 A).

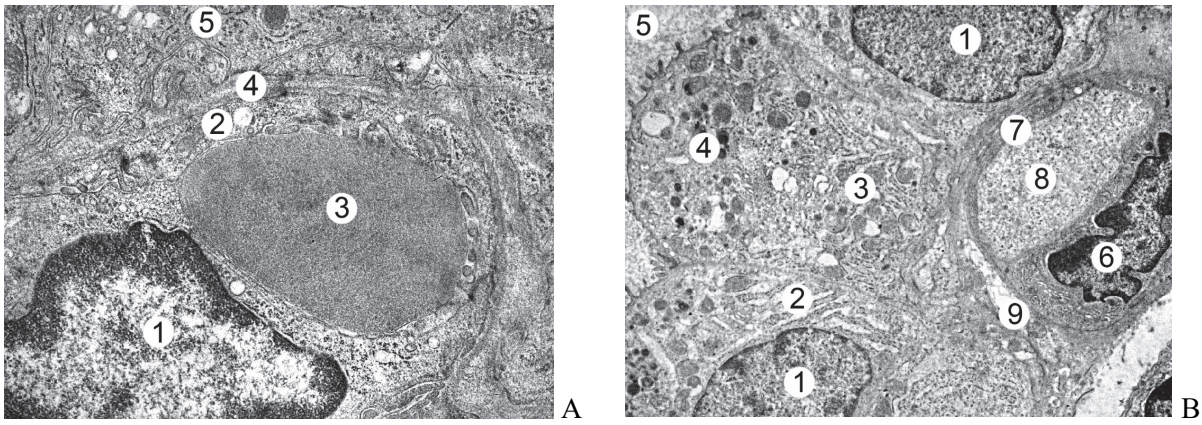

Fig. 3. Ultrastructure of the thyroid gland fenestrated capillary 14 days (fragment A) and 21 days (fragment B) after thyroid gland experimental thermal injury in case of LPS solution administration. Electrogram. x 11000 (fragment A) and x9000 (fragment B). **A –** 1 – nucleus, 2 – cytoplasm of endotheliocyte, 3 – erythrocyte within the capillary lumen, 4 – endotheliocyte and thyrocyte base membranes, 5 – cytoplasm of thyrocyte. **B –** 1 – nucleus filled with euchromatin, 2 – thyrocyte cytoplasm, 3 – granular endoplasmic reticulum tubules, 4 – lysosomes.

The ultrastructure of thyroid gland components 21–30 days after its thermal burning and pharmacological correction with a hyperosmolar LPS solution characterizes by morphological signs of thyroid hormones balanced synthesis and release, especially, the preservation of the structure of the protein-synthesizing apparatus organelles. The nuclei of mainly cubic and sometimes prismatic thyrocytes are clearly contoured, with the euchromatin prevalence in the karyoplasm. A widely branched network of granular endoplasmic reticulum tubules with a high density of ribosomes on their membrane and well-structured mitochondria were found around the nucleus located in the basal pole of the cell. The osmiophilic rounded vesicles, lysosomes, and the plasmolemma formed microvilli are observed in the apical pole.

The ultrastructural investigation throughout 21–30 days the thyroid gland burning and LPS using shows narrow lumens of thyroid gland blood capillaries that were lined with endotheliocytes of a typical structure. The endothelial layer cells nuclei often had karyolemma numerous invaginations with marginally located heterochromatin inside the karyoplasm. The peripheral zone contains many pinocytotic vesicles of various sizes which indicate active transendothelial exchange. One could see the phenomena of paravasal edema around some capillaries on the 21st day of the trial which was supported by the expanded spaces between the endothelial cells and follicular cells base membranes. We failed to record such phenomena on the 30th day of a trial (fig. 3 B).

Thus, the data obtained which outline the expressed hyperosmolar LPS solution corrective effect in conditions of thyroid gland thermal damage, complement the previously highlighted results regarding its protective effect with pronounced pathomorphological signs of thyroid tissue destruction in the early stages of its burning and indicate a proven normalization of thyroid gland morphological structure and ultramicroscopic intrathyroidal disorders during the post-burn period.

The obtained range of ultrastructural data additionally emphasizes the severity of the post-burn period manifestation within the thyroid gland parenchyma and demonstrates the fundamental possibility developed intrathyroid morphological and ultramicroscopic changes restore after hyperosmolar colloid LPS solution in case thyroid gland parenchyma thermal damage. From this point of view we indicate that together with hyperosmolar colloidal LPS solution we also tested the probable morphological and ultrastructural efficacy of HAES-LX 5 % under model conditions. Our data proved the identity of the thyroid-protective activity profile of both the hyperosmolar LPS colloid solution and HAES-LX 5 % which is also consistent with their proven comparative efficacy in endocrine function restoring and cellular activity of the small intestine reestablishing after skin thermal burns [6, 7].

We found initial LPS solution protective effects already on the 3rd day of the trial. The most expressed LPS protective effects we found from the 21st day of the trial which were continued till the end. We found no difference in both the LPS and HAES-LX 5 % antiburning efficacy and consider their restorative activity to be unidirectional and identical.

Resuming, we note that hyperosmolar LPS solution using for the experimental skin thermal injury correction has a positive effect on the experimental animals' thyroid gland functional condition. A significant improvement of thyroid blood capillaries and follicular cells ultrastructure was established in

the dynamics of the trial with the normalization of their morphology at the ultrastructural level in the late stages of the post-burn period.

The obtained data are consistent with results regarding the hormonal dysfunction restoration in thyroid burning under the influence of hyperosmolar LPS colloid solution and have a pathogenetic background which we consider as the experimental confirmation of hyperosmolar colloidal solutions clinical efficacy testing reasonability in thyroid gland parenchyma burn lesions.

Conclusions

1. Hyperosmolar lactoprotein with sorbitol colloid solution provides the expressed corrective influence on thyroid gland ultrastructural changes induced by its burning.

2. Hyperosmolar lactoprotein with sorbitol colloid solution administration results in normalization of thyroid gland morphological structure and ultramicroscopic intrathyroidal disorders during the post-burn period.

3. A significant improvement of thyroid blood capillaries and follicular cells ultrastructure was established in the dynamics of the trial with the normalization of their morphology at the ultrastructural level in the late stages of the thyroid gland post-burn period.

4. Initial lactoprotein with sorbitol solution protective effects were registered on the 3rd day after thyroid gland burning. The most expressed lactoprotein with sorbitol protective effects were registered from the 21st day of the trial which were continued till the end.

5. The obtained data concerning the lactoprotein with sorbitol solution protecting efficacy in thyroid burning have a pathogenetic background which we consider as the experimental confirmation of hyperosmolar colloidal solutions clinical efficacy testing reasonability in thyroid gland parenchyma burn lesions.

Prospects for further research include a further study of the thyroid gland thermal damage pathogenetically based pharmacological correction efficacy using the hyperosmolar lactoprotein with sorbitol colloidal administration in the aspect of organ and regulatory dysfunctions restoration as well as sanogenetic systems of the biological organism activation.

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