DOI 10.26724/2079-8334-2023-1-83-233-238
UDC 616.441:599.323.4:615.459

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WHITE RATS' THYROID GLAND MORPHOLOGICAL CHANGES THROUGHOUT THE EXPERIMENTAL THERMAL INJURY IN CONDITIONS OF LACTOPROTEIN WITH SORBITOL HYPEROSMOLAR SOLUTIONS ADMINISTRATION

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The purpose of the study was to establish the animal's thyroid gland histological changes after thermal skin injury in conditions of lactoprotein with sorbitol hyperosmolar colloid solution using. One day after the skin thermal injury and the abovenamed solution application one could reveal changes in the thyroid gland of animals manifested as the perivascular and interstitial edema, stasis in small vessels and the presence of a large number of resorption vacuoles inside the follicles' colloid. Three days after the skin thermal burn and the lactoprotein with sorbitol infusion the data did not show any progression of edematous phenomena neither around the blood vessels of the organ nor in the stromal connective tissue. On the 7th and the 14th days of a trial one could register the signs of thyroid gland capsule thickening and densification. Throughout the late stages of the trial there were no signs of the inflammatory process in the thyroid gland connective tissue. A moderate organs' vascular blood filling was observed on the sections stained with hematoxylin and eosin. Thus, the use of a hyperosmolar colloid lactoprotein with sorbitol solution as a corrective substance in experimental skin thermal injury reveals positive influence on the thyroid gland morphological state. The first signs of intraglandular environment recovery began to be registered from the 7th day of the trial, and the maximally expressed protective effect of lactoprotein with sorbitol hyperosmolar colloidal solution administration was observed from the 21st day till the end of the experiment.

Key words: thyroid gland, burning injury, morphological disturbances, hyperosmolar colloid solution, lactoprotein, sorbitol, pathogenetical correction.

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МОРФОЛОГІЧНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ БІЛИХ ЩУРІВ В ДИНАМІЦІ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ ЗА УМОВ ЗАСТОСУВАННЯ ГІПЕРОСМОЛЯРНИХ РОЗЧИНІВ ЛАКТОПРОТЕЇНУ З СОРБІТОЛОМ

Метою дослідження стало встановлення гістологічних змін щитоподібної залози тварин після термічної травми шкіри та за умов корекції гіперосмолярними колоїдними розчинами лактопротеїну з сорбітолом. Через 1 добу після термічної травми шкіри та застосування зазначеного розчину у щитоподібній залозі тварин виявлені зміни, що проявлялися периваскулярним та інтерстиційним набряком, стазами у судинах дрібного калібру, наявністю великої кількості вакуоль резорбції у колоїді фолікулів. Через три доби після термічного опіку шкіри та дозованого введення інфузійного препарату лактопротеїну з сорбітолом не спостерігали прогресування виникнення набрякових явищ ні довкола судин кровоносного русла органу, ні у стромальній сполучній тканині. На 7 та 14 доби досліду у щитоподібній залозі спостерігали ознаки потовщення та ущільнення капсули органу. У пізні терміни експерименту у сполучній тканині щитоподібної залози не відмічено ознак запального процесу. На зрізах, забарвлених гематоксиліном та еозином, спостерігалося помірне кровонаповнення судин органу. Таким чином, застосування гіперосмолярного розчину лактопротеїну з сорбітолом в якості коригуючого чинника при експериментальній термічній травмі шкіри позитивно впливає на морфологічний стан щитоподібної залози тварин. Перші ознаки відновлення внутрішньозалозистого оточення почали реєструватися, починаючи з 7-ї доби досліду, а максимально виражений проективний ефект від застосування гіперосмолярного колоїдного розчину лактопротеїну з сорбітолом спостерігався, починаючи з 21-ї доби і до кінця досліду.

Ключові слова: щитоподібна залоза, опікова травма, морфологічні порушення, гіперосмолярний колоїдний розчин, лактопротеїн, сорбітол, патогенетична корекція.

The work is fragments of scientific-research investigation "Peculiarities in micro-/ultramicroscopic structure and histochemical properties of body tissues during the development of compensatory-adaptive reactions", state registration No 0121U108204.

Thermal injuries are one of the most urgent medical and social problems of modern medicine in the world including Ukraine [8, 11]. More than 45000 people suffer from burns annually in Ukraine, such indices occupy the third place in the structure of mortality after all received injuries; these indexes higher only in case of traffic injuries [7]. The urgency of the burn injury problem determined by the following aspects: frequent lesions of adults and children, postburn treatment complexity and duration, 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 [1, 4, 13].

It is important that the thyroid gland in case of thermal factors of both of threshold and suprathreshold intensity influencing is one of the first organs which undergone to thermal shock taking into consideration the thyroid gland structural-functional organization and morpho-functional features, the massive duplicative feedback mechanisms of its regulatory activity, the wide spectrum of thyroid hormones physiological activity etc [5, 12].

We considered hypohydration as the leading clinical manifestations of thermal damage according to fundamental concepts that's why we tried to use 0.9 % physiological NaCl solution to eliminate the thyroid gland parenchyma and cellular composition damage – these efforts turned out to be ineffective both in case of morphological changes correction and certain functional disorders eradication [14, 15]. The analogous results were proved by [10]. Taking into account the known burn disease pathogenetic mechanisms with consecutive (and sometimes simultaneous) manifestations of hypoproteinemia, hemoconcentration, formation of toxic syndrome, inflammatory and autoimmune reaction, we decided to check the reasonability of colloid solutions administration with pharmacocorrective aim in conditions of thyroid gland thermally induced disturbances of structure and function.

The purpose of the study was to establish the experimental animal's thyroid gland histological changes dynamic after thermal skin injury in conditions of lactoprotein with sorbitol hyperosmolar colloid solution using.

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. Animal keeping, handling and manipulation were 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 were 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 rat's body for 10 sec, these rats were preheated for 6 min in water with a temperature of 100 °C [9]. The total area of skin lesions was 21-23 %.

Rats were infused with lactoprotein with sorbitol hyperosmolar solution (10 ml/kg) once per day throughout the first 7 days (the first administration was done 1 hr the skin burn) after the skin burn during 5-6 min into the lower femoral vein using a catheter. The catheter after placing was sutured under the skin, its lumen along its entire length after each hyperosmolar 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.

Colloidal hyperosmolar lactoprotein with sorbitol solution (Kyiv JSC "Biopharma", Certificate of state registration of the Ministry of Health of Ukraine N 464/09–300200000 dated 12.03.2009) is a protein-salt solution that contains donor albumin as a colloid base – 5 % , sorbitol – 6 %, sodium lactate – 2.1 %, sodium chloride – 0.8 %, calcium chloride – 0.01 %, potassium chloride – 0.0075 %, sodium bicarbonate – 0.01 %. The ionic composition of the drug has the following structure: Na^+ – 343.5 mmol/l, K^+ – 1.0 mmol/l, Ca^{2+} – 0.9 mmol/l, Cl^- – 139.7 mmol/l, HCO_3^- – 1.2 mmol/l, $CH_3CH(OH)COO^-$ – 187.4 mmol/l. Solution osmolarity equals to 1020 mosmol/l.

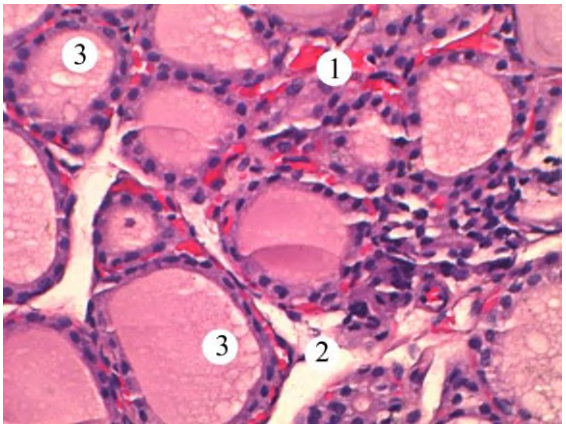
Tissues for microscopic studies were collected 1, 3, 7, 14, 21 and 30 days after the skin thermal injury using lactoprotein with sorbitol, and tissue histoprocessing was performed according to accepted methods [3]. The thyroid gland samples were fixed with 10 % neutral formalin solution, then dehydrated 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 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 [3]. Ultrathin sections were stained with methylene blue.

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.

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. One day after the skin thermal injury and the colloidal hyperosmolar lactoprotein with sorbitol solution application one could reveal changes in the thyroid gland of experimental animals manifested at the light-optical level by perivascular and interstitial edema, stasis in small vessels and the presence of a large number of resorption vacuoles inside the follicles' colloid (fig. 1 A).

Three days after the skin thermal burn and the lactoprotein with sorbitol infusion the histological specimens did not show any progression of edematous phenomena neither around the blood vessels of the organ nor in the stromal connective tissue. One could reveal the residual signs of the spaces between the follicles of the organ expansion. The majority of follicles of the organ during this term of the trial were lined with flat thyrocytes (fig. 1 B).



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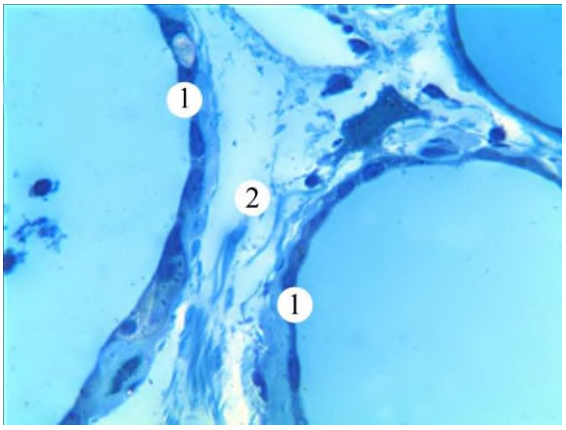

B

Fig. 1. Microscopic changes of the thyroid gland after skin experimental thermal injury under the lactoprotein with sorbitol solution administration. **A –** The 1st day of the trial. 1 – stasis in capillaries; 2 – interstitial edema; 3 – follicles with vacuoles resorption in colloid. Hematoxylin-Eosin staining. x 400. **B –** The 3rd day of the trial. 1 – follicles lined with flat thyrocytes; 2 – connective tissue. Ultrathin cut. Staining with methylene blue. x 1000.

On the 7th and the 14th days of a lactoprotein with sorbitol hyperosmolar solution administration to correct the thermal burn on the thyroid gland influence the signs of organs' capsule thickening and densification were observed. One could detect leukocyte's cells in the stromal connective tissue and mainly moderate both arterial and venous vessels blood filling. It should be mentioned that there were also somewhat overexpanded and stagnant vessels (fig. 2 A).

In this time of the trial we observed more follicles lined with cuboidal epithelium compared with the same data in early days after the burn using colloidal correction (fig. 2 B). Cellular detritus is present in the cavity of some follicles which may indicate apoptotic processes in the epithelium or its local destruction (fig. 2 C).

Throughout the late stages of the trial (21, 30 days) using lactoprotein with sorbitol solution there were no signs of the inflammatory process in the thyroid gland connective tissue. A moderate organs' vascular blood filling was observed on the sections stained with hematoxylin and eosin, their adventitia thickening was visible somewhere. Most follicles are lined by cuboidal follicular epithelium with basophilic nuclei and a small number of lumps of heterochromatin which is clearly visible on ultrathin sections. It should be noted the interfollicular islands presence inside the thyroid gland parenchyma in these terms of the experiment (fig. 3).

Thus, the use of a hyperosmolar colloid lactoprotein with sorbitol solution as a corrective substance in experimental skin thermal injury reveals positive influence on the thyroid gland morphological state. A structural condition of thyroid gland both the stromal and parenchymal components significant improvement was established in the dynamics of the experiment. One could register also their relative normalization in the late period of the trial.

It was proved that the first signs of intraglandular environment recovery began to be registered from the 7th day of the trial, and the maximally expressed protective effect of lactoprotein with sorbitol hyperosmolar colloidal solution administration was observed from the 21st day till the end of the experiment. Drawing parallels with the burn disease stages classification we note that the correction we applied showed effectiveness starting from the toxemia stage, and such a positive histological dynamics lasted for 30 days.

We tried to perform the thyroid gland morphological state and its microenvironment pharmacological correction taking into account burn disease pathogenetic mechanisms. Our choice of

lactoprotein with sorbitol hyperosmolar colloidal solution was due to both hypovolemia and oxygen deficiency correction failure [14, 15] which is somewhat consistent with the data [7, 8].

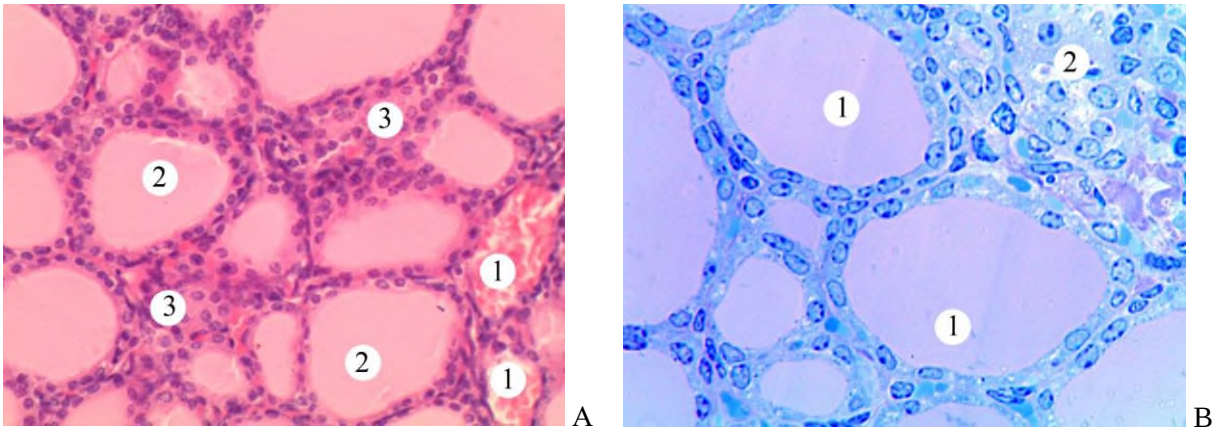

Fig. 3. Microscopic changes of the thyroid gland 30 days after skin experimental thermal injury under the lactoprotein with sorbitol solution administration. **A –** 1 – moderately blood-filled vessels; 2 – follicles; 3 – interfollicular islands. Hematoxylin-Eosin staining. x 400. **B –** 1 – follicles; 2 – interfollicular island. Ultrathin cut. Staining with methylene blue. x 400.

We suppose that we have chosen a sufficiently effective compound for the correction of thyroid gland parenchyma, stroma and vascular environment thermally induced morphological disorders - a hyperosmolar colloidal solution of lactoprotein with sorbitol. The positive component of this compound hyperosmolarity as well as lactate, sorbitol and at least albumin molecules presence in its composition induce the positive effect.

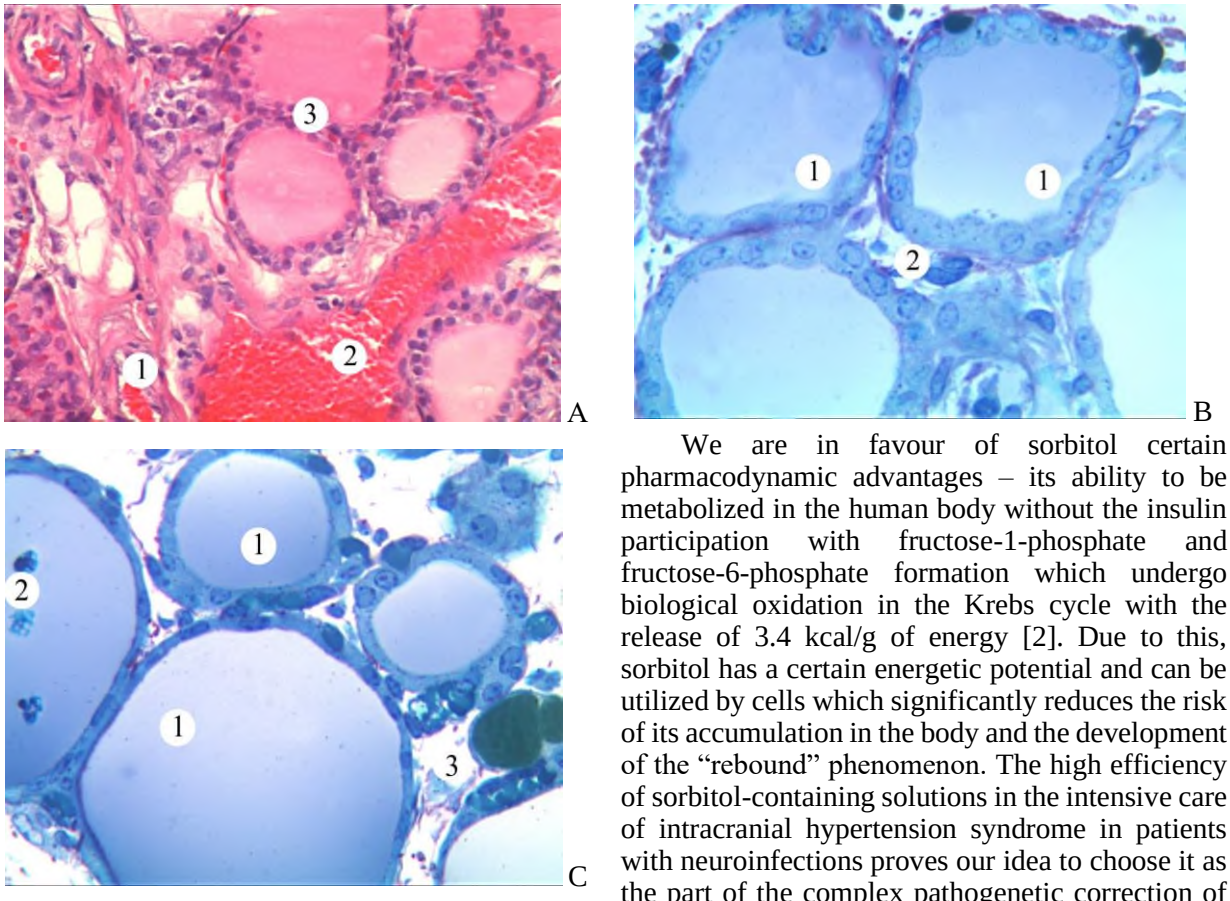

Fig. 2. Microscopic changes of the thyroid gland 7 and `14 days after skin experimental thermal injury under the under the lactoprotein with sorbitol solution administration. **A –** The 7th day of the trial. 1 – moderately blood-filled arteriole; 2 – venule; 3 – follicles. Hematoxylin-Eosin staining. x 400. **B –** The 14th day of the trial. 1 – follicles; 2 – connective tissue. Ultrathin cut. Staining with methylene blue. x 1000. **C –** The 7th day of the trial. 1 – follicles; 2 – cellular detritus; 3 – connective tissue. Ultrathin cut. Staining with methylene blue. x 1000.

We are in favour of sorbitol certain pharmacodynamic advantages – its ability to be metabolized in the human body without the insulin participation with fructose-1-phosphate and fructose-6-phosphate formation which undergo biological oxidation in the Krebs cycle with the release of 3.4 kcal/g of energy [2]. Due to this, sorbitol has a certain energetic potential and can be utilized by cells which significantly reduces the risk of its accumulation in the body and the development of the “rebound” phenomenon. The high efficiency of sorbitol-containing solutions in the intensive care of intracranial hypertension syndrome in patients with neuroinfections proves our idea to choose it as the part of the complex pathogenetic correction of thyroid gland ultrastructure in case of skin thermal burning [2].

The positive side of hypertonic solutions which contain sodium lactate is their ability to increase cardiac output, improve microcirculation and inhibit the vascular endothelium and neutrophilic granulocytes inflammatory response.

Albumin use, in our opinion, is reasonable by its high oncotic activity which makes it possible to eliminate hypovolemia. The following positive effects of its use we appreciate: antiplatelet effect and interaction with nitric oxide to form S-nitrosotoluenes which can improve local blood flow. Albumin is known to be involved in pyruvate dehydrogenase regulation in brain astrocytes and has a positive effect on the bioenergetics of neurons; can bind calcium ions and reduce the reperfusion effects of the calcium paradox; able to bind iron cations and inhibit lipid peroxidation, bind peroxide and peroxynitrite radicals.

One could suppose the pathogenetic reasonability of hyperosmolar colloid lactoprotein with sorbitol solution after such a listing of this solution effects mechanism of realization. We believe that hyperosmolar lactoprotein with sorbitol solution intravenous use under conditions of experimental skin burn injury induces adaptogenic (cyto- and angioprotective) properties, prevents both edema and hemorrhages development, avoids alteration and contributes also to thyroid gland intraglandular (stromal) composition repair.

In this aspect, it is important that colloid drugs, and most importantly - their early use, reduce mortality in burn disease early and postponed periods [7, 15]. It was proved that such compounds administration reduces the activity of the inflammatory cytokine, improves adaptation and compensatory disturbed functions of internal organs affected by burn disease [7].

Resuming, we state that earlier infusion (starting from the 1st day of the experiment) of hyperosmolar colloid lactoprotein with sorbitol solution showed pronounced efficacy starting from the 7th day till the end of the trial which indicates the experimentally proven principle of thyroid gland parenchyma and the periglandular environment adaptative, ischemic and necrotic changes.

We hope that in further experiments we will find out the likely positive effect of the tested compound in the thyroid gland functional activity restoration and establish the exact time0dependent aspects of its administration.

Conclusions

1. Hyperosmolar colloid lactoprotein with sorbitol solution use as a corrective substance in experimental skin thermal injury reveals positive influence on the thyroid gland morphological state.

2. A structural condition of thyroid gland both the stromal and parenchymal components significant improvement was established in the dynamics of the experiment.

3. Hyperosmolar colloid lactoprotein with sorbitol solution use normalizes thyroid gland stromal and parenchymal components in the late period of the trial.

4. The first signs of intraglandular environment recovery began to be registered from the 7th day of the trial, and the maximally expressed protective effect of lactoprotein with sorbitol hyperosmolar colloidal solution administration was observed from the 21st day till the end of the experiment.

Prospects for furthers researches include a comprehensive experimental investigation of principal possibility to improve the thyroid gland and periglandular morphological elements pathomorphological disorders in condition of burn disease. This will allow to develop the pathogenetically orientated therapy aimed to thyroid gland ultrastructure restoration and to renew the thyroid gland functional activity throughout the post-burn period.

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Стаття надійшла 14.03.2022 р.

DOI 10.26724/2079-8334-2023-1-83-238-242
UDC 616.36-008.6:[616.711/.714-001.3+617.55-001.31]-092.9

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FEATURES OF THE LIVER'S FUNCTIONAL STATE UNDER CONDITIONS OF CRANIO-SKELETAL INJURY COMBINED WITH BLUNT ABDOMINAL TRAUMA

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Blunt abdominal trauma and cranioskeletal trauma were simulated in mature male Wistar rats. In a separate group of rats, these injuries were combined. The control group consisted of intact animals. After 1, 3, 7, 14, 21 and 28 days, the rate of bile secretion was determined in the experimental animals. It was established that in the dynamics of the post-traumatic period, the additional infliction of blunt trauma to the abdomen significantly deepens the systemic impact of cranioskeletal trauma, which is manifested by the deepening of liver dysfunction, in particular, a significant decrease in the rate of bile secretion with a minimum of 14 days after inflicting the injury. By the 28th day of the experiment, the index increases, but does not reach the control level. Therefore, the complication of cranioskeletal trauma by abdominal trauma is accompanied by an increase in the systemic impact on the body with deepening liver dysfunction, which should be taken into account when developing measures to prevent and correct multiorgan dysfunction of traumatic origin.

Key words: abdominal and craniocerebral trauma, hip fracture, hepatic excretion.

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ОСОБЛИВОСТІ ФУНКЦІОНАЛЬНОГО СТАНУ ПЕЧІНКИ ЗА УМОВ КРАНІОСКЕЛЕТНОЇ ТРАВМИ, ПОЄДНАНОЇ З ТУПОЮ ТРАВМОЮ ЖИВОТА

У статевозрілих щурів-самців лінії Вістар моделювали тупу травму живота та краніоскелетну травму. В окремій групі щурів ці травми поєднували. Контрольну групу склали інтактні тварини. Через 1, 3, 7, 14, 21 та 28 діб у піддослідних тварин визначали швидкість жовчовиділення. Встановлено, що в динаміці посттравматичного періоду додаткове нанесення тупої травми живота суттєво поглиблює системний вплив краніоскелетної травми, що в'являють поглибленням дисфункції печінки, зокрема суттєвим зниженням швидкості жовчовиділення з мінімумом через 14 діб після нанесення травми. До 28 доби експерименту показник зростає, проте не досягає рівня контролю. Отже, ускладнення краніоскелетної травми абдомінальною травмою супроводжується посиленням системного впливу на організм з поглибленням дисфункції печінки, що доцільно враховувати при розробці заходів профілактики і корекції поліорганної дисфункції травматичного походження.

Ключові слова: абдомінальна і черепно-мозкова травма, перелом стегна, печінкова екскреція.

The study is a fragment of the research project "Pathogenetic features of systemic and organ disorders under the action of extraordinary factors on the body", state registration No. 0121U100071.

Traumatism is one of the urgent medical and social problems of today. Despite significant achievements in the diagnosis and treatment of the injured, even under the conditions of effective provision of emergency medical care, the level of mortality and lethality remains at a high level. In general, trauma is the fifth leading cause of death after coronary heart disease, lung cancer, stroke, and chronic obstructive pulmonary diseases [13].

The growth of traumatism in modern urbanized society is caused by a significant increase in a number of technogenic, natural, social and military emergencies. In the structure of modern peacetime trauma, the leading place is occupied by combined trauma, the prevalence of which, according to various authors, ranges from 23.5 to 85.0 %. Among such injuries, the combined trauma of the skull and bones of the skeleton (cranioskeleton trauma) stands out, which is characterized by a particularly severe course and high mortality [6].