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RESTRUCTURING OF VESSELS OF THE THYROID GLAND AFTER EXPERIMENTAL THERMAL INJURY ALONG WITH APPLICATION OF MINCED SUBSTRATE OF FREEZE-DRIED XENOGRAFT

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Alterations in the vessels of the albino rats' thyroid gland after experimental thermal injury in conditions of early necrectomy and the use of minced substrate of the freeze-dried xenograft have been studied. It has been established that the use of xenografts reduces the degree of destructive changes in the organ at the early stages and has a positive effect on the course of regenerative processes and normalization of structural components of the vascular wall of the organ at the late stages of the experiment.

Keywords: thyroid gland, vessels, micro- and submicroscopic alterations, thermal injury, substrate of freeze-dried xenografts.

The work is a fragment of the research project *"Identification of the features of the burn wound reparative processes and morphofunctional alterations in the internal organs and clinicopathogenetic rationale for application of freeze-dried xenografts in thermal injury", state registration No. 0115U001531.*

Thermal injuries are most commonly occurred during household and industrial activities [6]. Generally, in case of man-made disasters and during wartime, a thermal injury can be large-scaled and refers to one of the most severe types of military trauma [4, 5]. In local wars and military conflicts, thermal injuries occur in 1,5-3,6% of all losses. Apparently, persistent and fairly high lethality in burn shock and especially during the acute burn toxemia shows that the problem of thermal injuries is relevant to date [4, 8]. Thermal injury is a specific disease, usually poorly understand by surgeons, traumatologists or intensive care physicians and has significant differences from all other types of trauma: the prevalence of tissue damage, severity, duration of shock and intoxication, the incidence of generalized infection development and other complications of burn disease [2, 5 , 6, 7, 9]. Burn disease is the response of the body to severe thermal injury, resulting in irritation, damage and death of numerous skin receptors with subsequent changes in the vital functions of all internal organs and systems and disorder of all types of metabolism [2, 3, 7, 9, 13]. The burn disease has a complex multicomponent pathogenesis, the individual sections of which become predominant in different time periods after burn: hypovolaemia and circulatory disorders, especially microcirculation, on the first day after burn; dramatically expressed intoxication in the first two weeks; infection in the following weeks [5, 9].

Therefore, the use of materials that would reduce the level of toxins in the body is promising in treatment of severe burns [8, 15]. In recent years, in cases of deep burns early necrectomy of the affected tissues with subsequent closure of the wound with freeze-dried xenografts has been widely applied in combustiology. Such methods of treatment have a positive effect on the clinical course of burn disease and accelerate the healing of wounds [12, 14]. However, the study of the state of structures of the thyroid gland in severe burns along with the use of xenograft has not been fully elucidated in scientific publications to date [10, 11].

The purpose of the work was to detect micro- and submicroscopic alterations of vessels of the thyroid gland after experimental thermal injury and in conditions of use of substrate of freeze-dried xenograft.

Materials and methods. The total of 20 senior male albino rats were involved into the experiments. Animals were housed on a regular ration at the vivarium of the SHEE «I.Ya.Gorbachevskiy Ternopil State Medical University of MOH of Ukraine». Animal housing and experiments on them have been carried out in compliance with the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg , 1986), and "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics (Kyiv, 2001). Third-degree burn was induced by placing of copperplates, heated in boiled water to 97-100 °C under ketamine anesthesia. The affected area accounted for 18-20% of the epilated surface of the rats' body. Early necrectomy of damaged skin areas was carried out on the folowing day after burn induction. The originated wounds were covered with substrate of freeze-dried xenograft. During daily supervision, we monitored their overall state, the degree of manifestation of local changes in the area of thermal injury, body weight and mortality. The object of

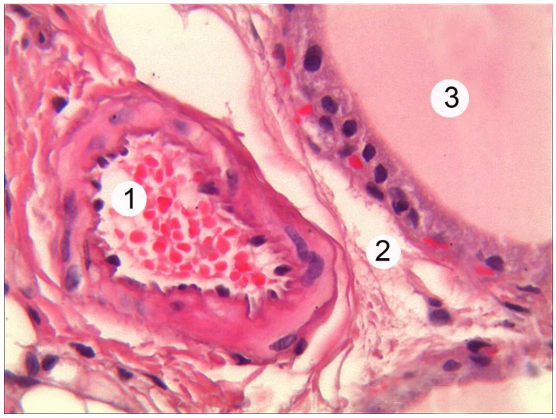
the study was thyroid gland. To study microscopic changes, animals were decapitated under ketamine anesthesia at 7, 14 and 21 days, which, according to contemporary concepts, corresponds to the stages of early and late toxemia, septicotoxicemia of burn disease [9].

For histological studies, pieces of thyroid gland were fixed in 10% neutral formalin solution. Subsequent processing of the material followed by embedment in paraffin blocks was carried out according to conventional technique. The sections obtained on the sliding microtome were stained with hematoxylin - eosin [1]. The histological specimens were studied using the optical microscope SEO SCAN and images were made with the Vision CCD Camera with a histological image display system.

For electron microscopic studies, the obtained pieces of the thyroid gland were fixed in 2.5% solution of glutaraldehyde, postfixed in 1% solution of osmium tetraoxide on phosphate buffer. Further processing was carried out in compliance with the conventional technique [1]. Ultra-thin sections made on the ultramicrotome UMPT-7 were contrasted with uraniacetate and lead citrate according to Reynolds and studied in the electron microscope PAM – 125K.

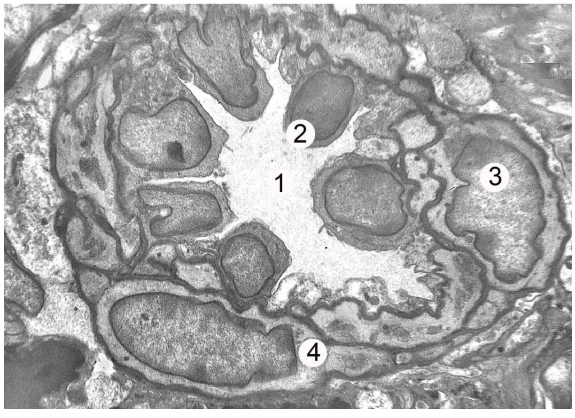
Results of the study and their discussion. Previous studies of the submicroscopy of the thyroid gland have established that thermal injury caused the development and progress of destructively degenerative changes in tyrocytes and hemocapillaries in the organ, the degree of which depends on the duration of the experiment. Histological studies of the thyroid gland of animals, experienced early necrectomy of damaged areas of the skin with the subsequent closure of the wound with minced substrate of freeze-dried xenografts, showed that the best preservation of the structural components of the vascular wall was revealed already on day 7 of the experiment and regenerative processes were activated. It has been established that the lumens of most vessels were insignificantly dilated and blood-filled; attenuation of perivascular edema, compared with animals that have not been corrected for thermal injury, was noted. Dilated lumens of some veins and venules, filled mostly with red blood cells, were noted. The degree of destructive changes and manifestations of vascular permeability of vascular walls, especially in microvasculature, was decreased; moderate leukocytic infiltration of the perivascular and stromal connective tissue was found (fig. 1, A).

At this stage of the experiment, arterioles were moderately altered. Dilated lumens contained red blood cells. The surface of endothelial cells was rough, their plasmolemma formed numerous evaginations. The nuclei of the endothelial cells protruded into the lumen of the vessel in the form of a palisade. The luminal and basal part of plasmolemma contained numerous small pits and pinocytic vesicles. Mitochondrial matrix was electron-dense; the outer and inner membranes retained their integrity. The nuclei of the endothelial cells of the arterioles had sporadic shallow invaginations of karyolemmas. Euchromatin prevailed in the karyoplasm. The heteromorphic changes of smooth myocytes were detected in their tunica media. However, cells without structural changes were also detected; they had elongated nuclei and cytoplasm filled with myofibrils. Some of the muscle cells had a swollen, cleared cytoplasm with thin loose myofibrils. Small mitochondria had densely packed cristae. The basal plate was dense. The outer adventitious connective tissue membrane was thinned, and in some areas it was poorly expressed (fig. 1, B).



A

Fig. 1. A. Microscopy of the rat thyroid gland following 7 days after experimental thermal injury and the use of the substrate of freeze-dried xenograft. Blood-filled artery lumen (1), moderate edema of adventitia (2). Follicle with colloid (3). H&E stain. 300 × magnification.



B

Fig. 1. B. Ultrastructure of arteriole of the thyroid gland following 7 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Lumen of the arteriole (1), evaginations of the nuclei of endotheliocytes (2), nucleus (3) and cytoplasm (4) of the smooth myocyte. 4000 × magnification.

Microscopically, on day 14 of the experiment blood vessels were moderately blood-filled. However, lumens of some veins and venules were dilated and filled with blood corpuscles. At this stage

of the experiment, less apparent changes in the vessels of the microvasculature were observed microscopically. Insignificant thickening, swelling of the wall and their moderate infiltration with leukocytes was characteristic. In the lumens, aggregation of erythrocytes and platelets was noted (fig. 2, A, B).

Fig. 2. A. Microscopy of the rat thyroid gland following the 14 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Lumen of the artery (1), smooth myocytes of the tunica media (2), moderate edema of adventitia (3). H&E stain. 300×magnification.

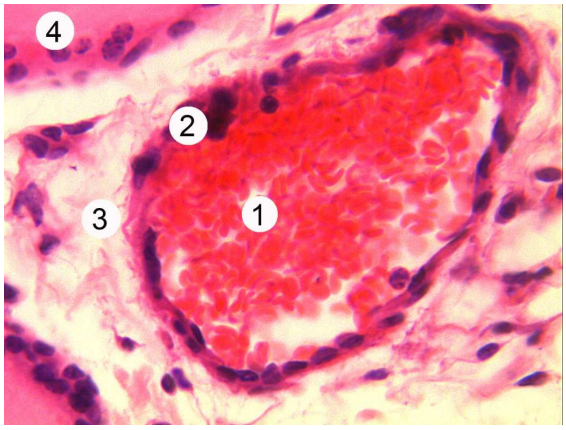


Fig. 2. B. Histology of the rat thyroid gland following the 14 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Lumen of the vein filled with blood (1), minor infiltration of the vascular wall (2), moderate edema of adventitia (3), fragment of follicle with colloid (4). H&E stain. 300×magnification.

At this stage of the experiment, ultrastructural studies established a high functional activity of microvessels. Lumens of most hemocapillaries were moderately enlarged, containing red blood cells. High content of pinocytic vesicles was found in moderately cleared cytoplasm of endothelial cells of the blood capillaries. However, local edema of the cellular cytoplasm was detected in some vessels. The luminal surface was twisting, forming cytoplasmic evaginations on separate sites. The nuclei of endothelial cells were hypertrophied, containing mainly euchromatin and large nucleoli in the karyoplasm. In the perinuclear part of the cytoplasm, well-defined, partially hypertrophied mitochondria, canaliculi of the endoplasmic reticulum, cisterns and vacuoles of the Golgi complex were detected. Peripheral zones of endothelial cells were saturated with small pits, vesicles and vacuoles; fenestrae were detected. Basement membrane thickness was uneven, and perivascular spaces were moderately enlarged (fig. 3, A).

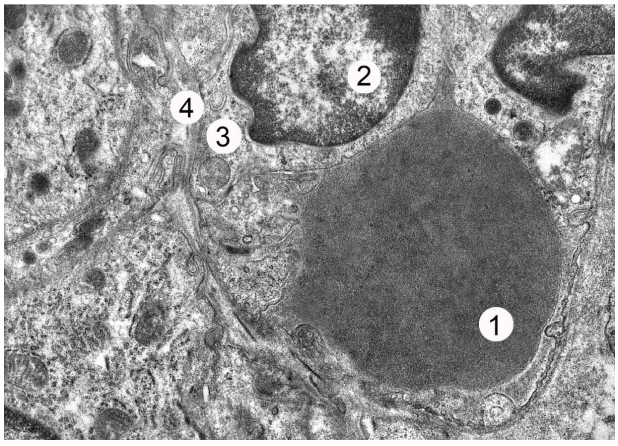


Fig. 3. A. Submicroscopy of the hemocapillary of the thyroid gland following the 14 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Capillary lumen with red blood cell (1), nucleus (2), and cytoplasm of the endotheliocyte (3), basement membrane (4). 12 000 × magnification.

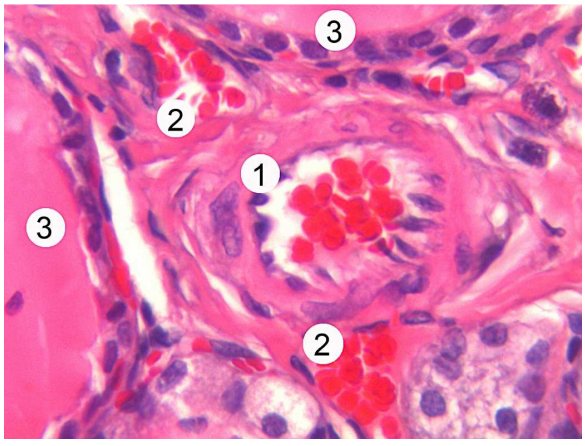


Fig. 3. B. Microscopy of the rat thyroid gland following the 21 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Arteriole (1), venules (2), follicles with colloid (3). H&E stain.400×magnification.

At this stage of the experiment the changes reflected the active course of intracellular reparative regeneration.

The use of substrate of freeze-dried xenografts showed significant restructuring of vessels in the thyroid gland on day 21 of the experiment. Insignificantly dilated lumens with its moderate blood filling and minor edema of the vascular wall were characteristic. Adventitious membrane of the vascular wall was insignificantly swollen with scanty infiltrated lymphocytes, macrophages and fibroblasts (fig. 3, B).

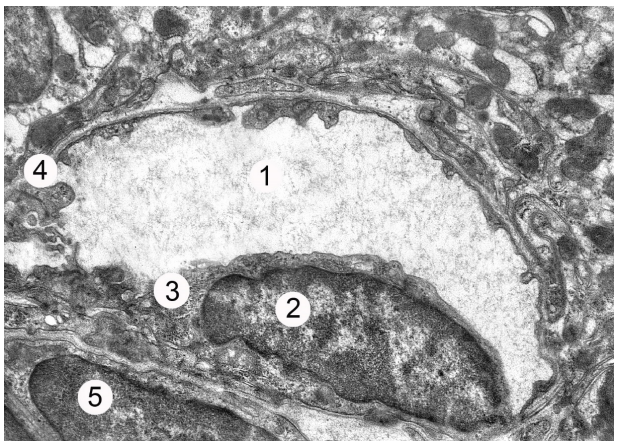


Fig. 4. Submicroscopy of the hemocapillary of the thyroid gland following the 21 days after experimental thermal injury and use of the substrate of freeze-dried xenograft. Capillary lumen (1), nucleus (2), and cytoplasm of the endotheliocyte (3), basement membrane (4), pericyte (5) 12 000 × magnification.

At this stage of the experiment lumens of the hemocpillaries were markedly dilated and filled with fine-dispersed substance. The luminal surface of the plasmolemma formed scarce finger-shaped evaginations and had moderate number of caveolas (fig. 4).

Nuclei of the endothelial cells were elongated with flexuous karyolemma, forming scarce invaginations. Euchromatin prevailed in karyoplasm where clusters of ribosomal granules, mainly located near the inner membrane of the nuclear membrane, were also found. Nucleoli were hypertrophied. Cytoplasm of the endothelial cells contained unmodified organelles and numerous pinocytic vesicles; fenestrae were pronounced. At this stage of the experiment, no edema of perivascular spaces was noted. The basement membrane was clearly defined and had a moderate thickness. The majority of hemocapillaries tightly adhered to the thyrocytes as part of the follicles, indicating the normalization of histohematogenous, transendothelial metabolic processes in the organ.

Conclusion

The findings of histological studies indicate that early excision of necrotic tissues after thermal injury and closure of wound by the minced substrate of freeze-dried xenograft eliminates the action of the pathogenic factor on the thyroid gland and creates conditions for active progress of reparatory regeneration. This contributes to the relative normalization of the structural components of the vascular wall at the final stage of the experiment.

Prospects of further research will encompass clarification of the morphology of the structural components of the thyroid gland in thermal injury and the use of different corrective factors during the experiment.

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Реферати

СТРУКТУРНА РЕОРГАНІЗАЦІЯ СУДИН ЩИТОПОДІБНОЇ ЗАЛОЗИ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ І ПРИ ЗАСТОСУВАННІ ПОДРІБНЕНОГО СУБСТРАТУ ЛІОФІЛІЗОВАНОЇ КСЕНОШКІРИ

Корицький В.Г., Небесна З.М., Крамар С.Б.

В експерименті на білих щурах досліджено зміни судин щитоподібної залози після тяжкої термічної травми в умовах проведення ранньої некректомії і застосування подрібненого субстрату ліофілізованої ксеношкіри. Встановлено, що використання ксенодермотрансплантати зменшує ступінь деструктивних змін в органі в ранні терміни і позитивно впливає на перебіг регенераторних процесів і нормалізацію структурних компонентів стінки судин органу в пізні терміни досліду.

Ключові слова: щитоподібна залоза, судини, мікро- і субмикроскопические зміни, термічна травма, субстрат ліофілізованих ксенодермотрансплантати.

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СТРУКТУРНАЯ РЕОРГАНИЗАЦИЯ СОСУДОВ ЩИТОВИДНОЙ ЖЕЛЕЗЫ ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОЙ ТЕРМИЧЕСКОЙ ТРАВМЫ И ПРИ ПРИМЕНЕНИИ ИЗМЕЛЬЧЕННОГО СУБСТРА ТА ЛИОФИЛИЗИРОВАННОЙ КСЕНОКОЖИ

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В эксперименте на белых крысах исследованы изменения сосудов щитовидной железы после тяжелой термической травмы в условиях проведения ранней некрэктомии и применении измельченного субстрата лиофилизированной ксенокожи. Установлено, что использование ксенодермотрансплантатов уменьшает степень деструктивных изменений в органе в ранние сроки и положительно влияет на протекание регенераторных процессов и нормализацию структурных компонентов стенки сосудов органа в поздние сроки опыта.

Ключевые слова: щитовидная железа, сосуды, микро- и субмикроскопические изменения, термическая травма, субстрат лиофилизированных ксенодермотрансплантатов.

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МОРФОФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА МІКРОЦИРКУЛЯТОРНОГО РУСЛА СУБХОНДРАЛЬНОЇ КІСТКИ ПРИ ВВЕДЕННІ КРІОКОНСЕРВОВАНОЇ ПЛАЦЕНТИ НА ТЛІ АСЕПТИЧНОГО ЗАПАЛЕННЯ СУГЛОБУ У ЩУРІВ

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Проведено дослідження морфологічно-функціональних особливостей судинного руслу субхондральних кісток щурів в нормі, в умовах створення стресової ситуації, експериментального неспецифічного артриту та при введенні кріоконсервованої плаценти. Аналіз варіантів судинної реакції кісткової тканини свідчить про ефективність застосування кріоконсервованої плаценти в профілактиці та лікуванні неспецифічних запальних процесів суглобу.

Ключові слова: мікроциркуляція кістки, кріоконсервована плацента, асептичне запалення.

Робота є фрагментом НДР «Експериментально-морфологічне вивчення дії трансплантатів кріоконсервованої плаценти та інших екзогенних чинників на морфофункціональний стан внутрішніх органів», № державної реєстрації 0113V006185.

Наявність ознак остеоартриту зустрічається майже у кожного п’ятого мешканця земної кулі, що робить дану проблему дуже актуальною. Незважаючи на значну кількість досліджень вказаної патології, розповсюдженість остеоартриту у світі збільшується. Звертає на себе увагу неоднозначність поглядів учених на питання етіопатогенезу, діагностики та лікування [6, 9]. Протягом значного терміну вважалось, що єдиним субстратом остеоартриту є суглобовий хрящ і лише в кінці ХХ сторіччя з’явились переконливі докази того, що ураження суглобового хрящу здійснюється за рахунок первинного ремоделювання субхондральної кістки [2]. В ініціації дегенеративних процесів суглобового хрящу значна роль відводиться стану васкулярної системи кістки, яка забезпечує функціонування кісткового мозку та процеси мінералізації кісткового матриксу [2]. У порівнянні із іншими відділами трубчастих кісток субхондральні відділи мають особливо розвинену судинну мережу, що зумовлює високу метаболічну активність вказаної ділянки. В той же час відсутність резервних колатеральних шляхів зумовлює високий ризик розвитку ішемії субхондральних відділів і відповідно