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SUBMICROSCOPIC CHANGES OF THE HEMOCAPILLARIES IN THE ADRENAL GLAND CORTEX IN DYNAMICS AFTER EXPERIMENTAL THERMAL INJURY

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The purpose of the study was to establish submicroscopic changes in the hemocapillaries of the adrenal gland of white rats in the dynamics after experimental thermal injury. The experiment was performed on 36 mature white male rats. Burns of 2B degree were simulated under thiopental-sodium anesthesia. Submicroscopic changes were studied on days 1st, 7th, 14th and 21st from the beginning of the experiment. Small pieces of adrenal cortex were taken for electron microscopic examination. Tissue processing was performed according to generally accepted methods. Ultrathin sections were studied in an electron microscope PEM-125K. In the early period after experimental thermal trauma there are initial destructive changes in the components of the blood capillaries wall of the organ, which were overfilled with blood, there were manifestations of excessive edema, destruction of the cytoplasm in the endothelial cells and pericytes, deformation of nuclei, uneven thickening of the basement membrane. In the late post-burn period, significant destructive-degenerative changes in the wall of hemocapillaries of the adrenal cortex were found. They were accompanied by uneven thickening and homogenization of the basement membrane, pyknosis of nuclei, destruction and fragmentation of organelles in the cytoplasm of endothelial cells, violation of fenestration and disappearance of micropinocytic vesicles, which lead to insufficiency of transendothelial metabolism in the body.

Key words: adrenal gland, hemocapillary ultrastructure, experimental burn injury.

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СУБМІКРОСКОПІЧНІ ЗМІНИ ГЕМОКАПІЛЯРІВ КОРИ НАДНИРКОВИХ ЗАЛОЗ У ДИНАМІЦІ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ

Метою даного дослідження було встановити субмікроскопічні зміни гемокапілярів надниркових залоз білих щурів в динаміці після експериментальної термічної травми. Експеримент проведено на 36 статевозрілих білих щурах-самцях. Опік ІІб ступеня моделювали під тіопентал-натрієвим наркозом. Субмікроскопічні зміни вивчали на 1, 7, 14 та 21 добу від початку експерименту. Для електронномікроскопічного дослідження забирали невеликі шматочки кори надниркових залоз. Обробку тканин проводили згідно загальноприйнятих методик. Ультратонкі зрізи вивчали в електронному мікроскопі ПЕМ-125К. В ранні терміни після експериментальної термічної травми відбуваються початкові деструктивні зміни складових елементів стінки кровоносних капілярів органу, що проявлялось їх надмірним кровонаповненням, набряком та деструкцією цитоплазми ендотеліоцитів, перицитів, деформацією ядер, нерівномірним потовщенням базальної мембрани. В пізні терміни після опіку, встановлено значні деструктивно-дегенеративні зміни в стінці гемокапілярів кори надниркових залоз, що супроводжувалось нерівномірним потовщенням та гомогенізацією базальної мембрани, пікнозом ядер, деструкцією і фрагментацією органел в цитоплазмі ендотеліоцитів, порушенням фенестрації та зникненням мікропіноцитозних пухирців, що призводить до недостатності трансендотеліального обміну в органі.

Ключові слова: надниркова залоза,ультраструктура гемокапіляра, експериментальна опікова травма.

The work is a fragment of the research project "Peculiarities of structural reorganization of the nervous, digestive, endocrine systems, hematopoietic and immune organs under conditions of thermal injury and the use of corrective factors", state registration No 0120U104152.

Burn injuries are common in everyday life and at work, as well as in the modern military conflicts. According to the WHO, the number of victims of thermal burns is constantly increasing, and the consequences of such injuries are a serious medical and economic problem for all countries [1, 11]. Deep burns cause destructive changes in the skin and significant morphological and functional changes in all organs and systems of the body [7]. The most common cause of death in burn patients are burn shock and intoxication, the source of which is a burn wound. From it toxic substances spread through blood vessels and cause disorders of the structural and functional state of organs [8, 10, 13]. It is known that the consequences of burn shock are a violation of the composition and volume of circulating blood due to significant plasma loss and hypovolemia [9], which leads to increased blood viscosity in general and circulatory disorders in organs, including the adrenal gland. This organ of the endocrine system are among the first to respond to this stressor, thus regulating the adaptive response and the processes of homeostasis in the body [2, 14]. However, currently in the scientific literature there is insufficient data on submicroscopic changes in the hemocapillaries of the adrenal gland cortex in thermal lesions.

The purpose of the study was to establish submicroscopic changes in the hemocapillaries of the adrenal gland cortex under conditions of experimental thermal trauma.

Materials and methods. The experiment was performed on 36 adult white male rats weighing 200–250 g. The animals were kept in the vivarium of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. All manipulations, animal care and experiments were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" (Strasbourg, 1986) and in accordance with the provisions of the "General Ethical Principles for Experiments on Animals", approved by the First National Congress Of Bioethics (Kyiv, 2001). The burn was applied under thiopental-sodium anesthesia with copper plates heated in boiled water to a temperature of 97–100^{0}C on the epilated surface of the animals' skin for 10–15 seconds. The size of the affected area was 18–20 % of the body surface of rats, which according to modern classification corresponds to burns of IIb degree. Animals were decapitated on 1st, 7th, 14th and 21st day of the experiment, which corresponds to the stages of shock, early and late toxemia and septicotoxemia of burn disease. For electron microscopic examination, small pieces of adrenal cortex were collected, fixed in 2.5–3 % glutaraldehyde solution, post-fixed in 1 % osmium tetroxide solution on phosphate buffer with pH 7.2–7.4. Dehydration was performed in alcohols of increasing concentration and propylene oxide, embedded into a mixture of epoxy resins with araldite. Ultrathin sections were made on an LMB-3 ultramicrotome, contrasted with uranyl acetate and lead citrate according to the Reynolds method and studied in an electron microscope PEM–125K [3].

Results of the study and their discussion. A submicroscopic examination a day after the experimental thermal injury found that reactive changes in hemocapillaries were observed in the organ. The lumens of microvessels were enlarged, which was accompanied by their significant blood filling, sludge effect of erythrocytes, single platelets and leukocytes were also present. The phenomenon of adhesion of thrombocytes to the lumenal surface of the endothelial cells plasmolemma was observed. Endothelial cells had elongated nuclei, a nuclear envelope with well-contoured membranes that had single invaginations. Euchromatin predominated in the karyoplasm of nuclei, and clumps of heterochromatin were located mainly under the karyolemma. The nucleoli of endothelial cells were fuzzy, disorganized (fig. 1).

Peripheral areas of the cytoplasm had an uneven thickness, there were thin areas with distinct fenestrates and thickened electron-dense areas. Dilated shortened tubules of the endoplasmic reticulum and an uneven number of pinocytic vesicles were found in the cytoplasm. The number of ribosomes on the surface of the rough endoplasmic reticulum was insignificant. Mitochondria were few, located mainly near the nucleus. Elements of the Golgi complex were represented by shortened fragmented cisternae and electron-light vacuoles. The basement membrane of hemocapillaries was thickened unevenly, perivascular edema was present. In the cleavage of the basement membrane, pericytes of irregular, elongated shape with thickened processes were visible, in the cytoplasm of which there were fuzzy tubules of the endoplasmic reticulum, single ribosomes, a few cisternae of the Golgi complex and rounded mitochondria. Nuclei of pericytes of round shape, electron-dense. In addition to hemocapillaries with fenestrated endothelium and dilated lumens, there were blood capillaries in which the lumen was significantly decreased due to edema of endothelium and perivascular lumen. The nuclei of endothelial cells of such capillaries were irregular in shape, the karyolemma contained iinvaginations and bulgings. The cytoplasm of the cells was enlightened, and the organelles of endothelial cells showed signs of destruction. The tubules of the rough endoplasmic reticulum looked like dilated shortened vacuoles with a small amount of ribosomes on their surface. Mitochondria increased in size, swollen, with an electron-light matrix. The cisternae of the Golgi complex are shortened and few. Basement membrane of these capillaries were with fuzzy contours due to its edema. Pericyte processes, which were observed in the cleavage of the basement membrane, were also swollen.

On the 7th day after the burn in all zones of the adrenal cortex there was a significant number of dilated and blood-filled hemocapillaries, which prove the progress of destructive changes in the walls of the microvessels in the organ. The lumens of the capillaries were filled with erythrocyte sludges that hade been in contact with the lumenal surface of endothelial cells. There was stasis of microvessels. Nuclei of cells were irregularly shaped with large clumps of marginally located heterochromatin (fig. 2).

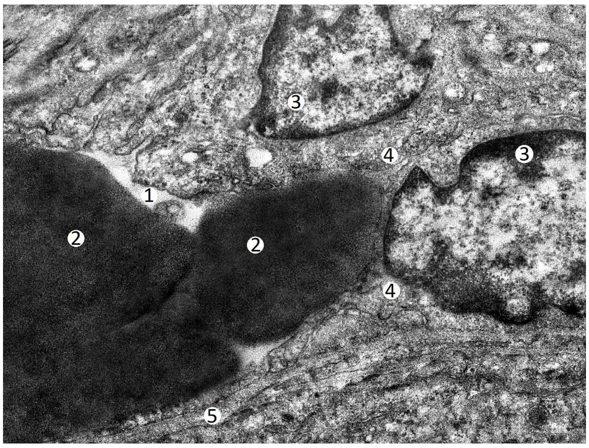

Fig. 1. Ultrastructural changes of hemocapillaries in the adrenal gland cortex a day after experimental thermal injury. Capillary lumen (1) with aggregation of erythrocytes in it (2), nuclei (3) and cytoplasm (4) of endothelial cells, basement membrane (5). x 9000.

Fig. 2. Submicroscopic condition of hemocapillaries of the adrenal cortex on the 7th day after experimental thermal injury. Capillary lumen (1) with erythrocytes in it (2), endothelial cell nuclei (3), single microvilli (4) on the lumenal surface of the endothelium, basement membrane (5). x 9000.

Small microvesicles were visible in some endotheliocytes. The presence of destructively changed round mitochondria with an enlightened electronic matrix and a small number of cristae was characteristic. Cisternae of the Golgi complex were expanded. The number of ribosomes in the cytoplasm was insignificant. Cell junctions between endothelial cells were disorganized. The basement membrane was unevenly thickened and partially stratified, its contours was indistinctly defined, there were some electron-dense areas.

The lumens of some capillaries were narrowed, and their walls were characterized by profound changes in the basement membrane and endothelium. In particular, the cytoplasmic areas of endothelial cells were thinned, fenestrae poorly contoured. The nuclei were enlarged, with numerous invaginations of the karyolema, the karyoplasm was dominated by marginally located heterochromatin. There was swelling of the perinuclear areas of the cytoplasm of endothelial cells, little amount of organelles and they were destructively changed. Mitochondria contain an electron-enlightened matrix and partially destroyed cristae. The tubules of the endoplasmic reticulum, as well as the cisternae of the Golgi complex, were dilated. The number of pinocytic vesicles was small, single microvilli were found on the lumenal surface of the endothelium. The basement membrane was unevenly thickened, the perivascular space was expanded.

On the 14th day after the experimental thermal trauma, along with destructively changed endocrinocytes, a large number of uneven blood-filled capillaries and significant changes in the structure of their wall were noted: the basement membrane was blurred, fenestration was disturbed, cell junctions between some endothelial cells were destructured. The nuclei of endothelial cells had uneven, fuzzy contours of membranes, they were pyknotically changed, osmiophilic. Chromatin clumps were located mainly under the karyolemma, deep invaginations were detected (fig. 3).

The cytoplasm of endothelial cells contained both enlightened and electron-dense areas, with destructively altered, fragmented organelles. The tubules of the rough endoplasmic reticulum were partially destroyed, with single ribosomes on their membranes. Cisternae of the Golgi complex were expanded, partially fragmented. Mitochondria were enlarged, containing an enlightened matrix and destroyed cristae. Single micropinocytic vesicles were found in cytoplasmic areas and a small number of fenestrae. The basement membrane was mostly thickened, with signs of edema and homogenization. Along with dilated blood-filled hemocapillaries, there were spasmic microvessels with narrowed lumens. The nuclei of endothelial cells were destructively altered, small in size, pyknotic, single nuclear pores were detected. The karyolema of the nuclei contained deep invaginations. There were areas where the perinuclear space was increased. The area in the cytoplasm near the nucleus of endothelial cells contained a few organelles that were destructively changed. The tubules of the endoplasmic reticulum and the Golgi complex cisternae were dilated, vacuolated, with fragmented membranes. Fenestra of cytoplasmic areas were weakly contoured, expanded. The basement membrane was destroyed in places, contained both thickened and thin areas, osmiophilic, they were indistinctly contoured, there were also gaps between some endotheliocytes.

Electron microscopic examinations of the adrenal gland cortex on the 21st day after the experimental thermal injury showed significant, deep damage to the wall of hemocapillaries of the microcirculatory tract of the cortical substance of the organ. The lumens of most capillaries were narrowed, there were destructured formed elements in them, and perivascular edema (fig. 4).

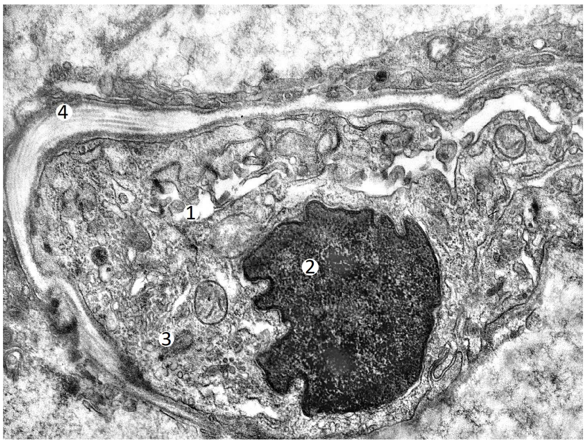

Fig. 3. Submicroscopic condition of hemocapillaries in the adrenal cortex on the 14th day after the experimental thermal injury. Narrowed capillary lumen (1), osmiophilic pyknotic nucleus of endothelial cell (2), destruction of organelles in the cytoplasm (3), swollen area of the basement membrane (4). x 9000.

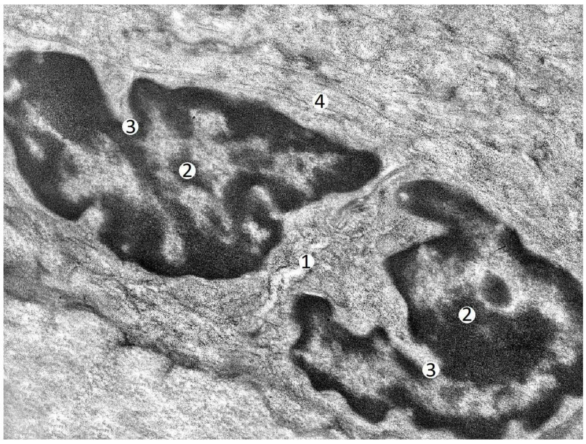

Fig. 4. Ultrastructural changes of hemocapillaries of the adrenal cortex on 21st day after experimental thermal injury. Narrow lumen of the hemocapillary (1), osmophilic pyknotic nuclei (2) with deep invaginations of the karyolemma (3), fuzzy homogeneous basement membrane (4). x 9000.

Endotheliocytes were destructively changed, swollen, with enlightened cytoplasm. The nuclei of the cells protruded into the lumen, they were electron-dense, elongated or rounded, small, pyknotically altered, with deep invaginations of the karyolemma. The karyoplasm contained condensed heterochromatin. Local areas of stratification of the outer and inner membranes of the karyolemma of the nuclei were observed, with the expansion of the perinuclear space. In the cytoplasm of endothelial cells there were a few organelles, tubules of the endoplasmic reticulum which were significantly dilated, partially fragmented. Round and oval mitochondria, hypertrophied due to edema, had an enlightened matrix and destroyed cristae. The cisternae of the Golgi complex were expanded in the form of elongated vacuoles. A small number of micropinocytic vesicles in the cytoplasm was characteristic. There were no microvilli on the lumenal surface of the endothelium. The basement membrane was unevenly thickened, indistinctly contoured, optically merged with the swollen perivascular space.

Numerous scientific researches are devoted to the study of the ultrastructure of the microcirculatory tract vessels of the adrenal glands [4, 5, 6, 12, 15]. In particular, the work of Knyazevych-Chorna [5], which aimed to establish structural changes in the hemocapillaries of the organ under the influence of cold injury, is consistent with the results of our research. The dynamics of the growth of destructive changes in the capillary wall under the action of this factor was similar to the results we found: dilation of the lumen, blood-filled capillaries, wall edema and destructive changes in endothelial cells. Accumulation of blood cells, diapedesis of leukocytes and macrophages are typical for both cold and thermal factors. Studies of remodeling of hemocapillaries under the influence of hypergravity [6], reorganization of the walls of microvessels of the organ under the influence of heavy metal salts [4], destructive changes in the walls and endothelial lining of capillaries in experimental diabetes [12, 15], showed similar changes in our study of the submicroscopic structure of hemocapillaries under the conditions of experimental thermal trauma.

Thus, typical for the influence of stressors of exogenous and endogenous origin is the growth of destructive-dystrophic changes in the hemocapillaries of the adrenal glands, which at the submicroscopic level is confirmed by such morphological changes as dilation, blood filling of hemocapillaries, violation of rheological properties of blood. formation of stasis, release of cellular elements and plasma into the extravascular space, violation of the ultrastructure of the endothelial organelles, decrease in the number of micropinocytic vesicles in the cytoplasm, thickening of the basement membrane, pyknosis of nuclei and edema of endothelial cytoplasm. The degree of destructive changes has a similar dynamics for various factors of stress genesis and reflects the violation of organ trophic and its hypofunctional state.

Conclusion

Electron microscopic studies of hemocapillaries of the adrenal cortex showed that in the early stages after experimental thermal trauma (1st-7th days of the experiment) there were initial destructive changes in the components of the blood capillaries of the organ, manifested by their excessive blood supply, edema and destruction of the cytoplasm of endothelial cells, pericytes, deformation of the nuclei, uneven thickening of the basement membrane.

In the late stage (14th and especially 21st day) after the burn injury, significant destructive-degenerative changes in the wall of hemocapillaries of the adrenal cortex were found, which was accompanied by uneven thickening and homogenization of the basement membrane, pyknosis of nuclei, destruction and fragmentation of organelles in the cytoplasm of endothelial cells, violation of fenestration and disappearance of micropinocytic vesicles, which leads to insufficiency of transendothelial metabolism in the body.

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