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ULTRASTRUCTURAL ORGANIZATION OF TESTICULAR BLOOD VESSELS UNDER CONDITIONS OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

Abstract. *Microangiopathy is the most frequent and prognostic unfavourable complication of diabetes mellitus. Changes in the links of the testicular blood microcirculation bed in case of diabetes mellitus are the basis for the development of pathological changes in the testicular structures under conditions of diabetes mellitus. In spite of topicality and importance of the problem, professional medical literature contains fragmental information concerning reorganization of the testicles in case of different pathological conditions, and the data concerning ultra-structural organization in the links of the blood microcirculation bed under conditions of diabetes mellitus are practically absent. Therefore, the objective of our study was to determine peculiarities of the testicular link ultrastructure of rats within the norm and in the dynamics of streptozotocin-induced diabetes mellitus. The study was performed on 30 albino mature male rats aged 4,5-7,5 months and the body weight 130-150 grams. The material of the study was presented by ultramicroscopic sections of the testes. Diabetes mellitus was simulated by a single intraperitoneal injection of streptozotocin («Sigma» USA), prepared on 0,1 M citrate buffer, pH =4,5 at the rate of 7 mg per 100 g of the body weight of an animal. Development of diabetes mellitus was controlled by increasing level of glucose in the blood measured by means of glucose-oxidase method. The study was performed on animals with glucose level over13,4 μmol/L in 2,4,6,8 weeks after the beginning of the experiment. Electron microscopy method was applied. The material of the study was examined and pictures were made by means of the microscope УЕМВ-100 К with acceleration voltage of 75 kV and magnification on the microscope screen1000-124000 x. The results of the study showed that the first changes in the ultra-structural organization of the testicular blood microcirculation bed of a rat occurred even 2 weeks after simulation of streptozotocin-induced diabetes mellitus and increased during the following terms of the experiment. Angiopathy is a trigger mechanism promoting the development of structural changes in the testicles in case of diabetes mellitus. The results obtained are the basis for further investigations of morphologists and clinicians with the purpose to elaborate new methods of diagnostics, prevention and treatment of testicular pathology in case of diabetes mellitus.*

Key words: *testicles, blood microcirculation bed, diabetes mellitus.*

Introduction. Microangiopathy is the most frequent and prognostic unfavourable complication of diabetes mellitus [1, 4, 6]. Changes in the links of the testicular blood microcirculation bed in case of diabetes mellitus are the basis for the development of pathological changes in the testicular structures under conditions of diabetes mellitus [3]. In spite of topicality and importance of the problem, professional medical literature contains fragmental information concerning reorganization of the testicles in case of different pathological conditions [2, 5, 7, 8], and the data concerning ultra-structural organization in the links of the blood microcirculation bed under conditions of diabetes mellitus are practically absent.

Objective: to determine peculiarities of the testicular link ultrastructure of rats within the norm and in the dynamics of streptozotocin-induced diabetes mellitus.

Materials and methods. The study was performed on 30 albino mature male rats aged 4,5-7,5 months and the body weight 130-150

grams. The material of the study was presented by ultramicroscopic sections of the testes. Diabetes mellitus was simulated by a single intraperitoneal injection of streptozotocin («Sigma» USA), prepared on 0,1 M citrate buffer, pH =4,5 at the rate of 7 mg per 100 g of the body weight of an animal. Development of diabetes mellitus was controlled by increasing level of glucose in the blood measured by means of glucose-oxidase method. The study was performed on animals with glucose level over13,4 μmol/L in 2, 4, 6, 8 weeks after the beginning of the experiment.

The animals were kept and the experiments were conducted according to the regularities of the European Convention on Protection of Vertebrate Animals Used in Experimental and Other Scientific Purposes (Strasbourg, 1985), General Ethical Principles of the Experiments on Animals approved by the First National Congress of Ukraine on Bioethics (Kyiv, 2001).

Electron microscopic method was applied in the study. Animals were taken from the experiment by means of overdosage of intraperitoneal narcosis with sodium thiopental (25 mg/kg). Immediately after death of animals the material was taken and prepared for the standard electron microscopy. Ultrathin sections were prepared on the ultramicrotome УЖТП–3 by means of glass knives. The strips of a silver or tender lemon yellow colour were taken for the study. First the sections were contrasted with 2% uranyl acetate solution followed by lead citrate. The material of the study was examined and pictures were made by means of the microscope УЕМВ-100 К with acceleration voltage of 75 kV and magnification on the microscope screen1000-124000 x.

Results and discussion. The links of the testicular blood microcirculation bed are located in the connective tissue round the winding seminiferous tubules and in their wall. The arterioles pass between the seminiferous tubules. The arteriole wall consists of three membranes. Endotheliocytes are elongated in shape on transverse sections, sometimes they protrude into the lumen of the arterioles. Plasmolemma forms numerous micro-projections, the cytoplasm has a mean electric optic density and contains a number of organelles and micropinocytic vesicles. Adjacent endotheliocytes contact forming desmosomes and interdigitations. The nuclei are of an elongated shape, non-condensed chromatin prevails in them, although a strip of the peripheral condensed chromatin is clearly seen close to the nucleome. The basal membrane is with clear outline and unbroken. Internal elastic membrane is determined from outside. It is thin and of irregular wave-like outline. Smooth myocytes of the middle membrane in the arteriole wall form one layer. They have electric-light cytoplasm, contain nuclei of an elongated shape, the points of myofibril attachment are clearly seen along the whole plasmolemma. Adjacent smooth myocytes have desmosomal contacts. Arteriole lumens are filled with blood cells (erythrocytes, platelets). The capillaries are transverse and longitudinal. The capillaries passing longitudinally interlace with those passing transversally forming capillary networks in the winding tubules. A part of the capillaries has a narrow lumen and does not contain blood cells, and their second part has a wide lumen filled with erythrocytes. On the ultra-structural level the capillary wall is typical and consists of 2-4 endotheliocytes located on the basal membrane possessing clear regular outline. Every endotheliocyte contain nucleus-containing and peripheral zones. Endotheliocyte nuclei are elongated, their outlines are clear. The nuclei contain mostly non-condensed chromatin as well as a thin strip of the peripheral condensed chromatin. Condensed chromatin is evenly distributed in karyoplasm in small amounts. Granular endoplasmic network is located in the perinuclear zone. It is presented by the canaliculi and cisterns with a number of ribosomes, Golgi apparatus and mitochondria on their membranes. In some places plasmolemma forms small microvilli. A continuous basal membrane with pericytes located between its layers adjoins closely to the basal surfaces of endotheliocytes. The pericytic nucleus is oval in shape with chromatin evenly distributed in the nucleoplasm. Close to the nucleus there is Golgi apparatus, granular endoplasmic network, mitochondria and single free ribosomes and vesicles. Contrary to arterioles venules have a wide lumen of an irregular shape. The venular wall consists of a thin layer of endothelial cells on the basal membrane. Endotheliocyte cytoplasm is electronic light containing inconsiderable number of organelles.

Venular lumens are filled with blood elements.

2 weeks after initiation of streptozotocin-induced diabetes mellitus the first signs of angiopathy appear in the links of the testicular blood microcirculation bed. Endotheliocyte swelling is seen in the capillaries, capillary lumens are of an irregular shape. Adhesion and aggregation of erythrocytes occur in the capillary lumen. Electron thick nuclei of endotheliocytes protrude into the lumen of vessels. Endotheliocyte nuclei become excessive elongated shape, the nucleoma forms many protrusions and invaginations, and chromatin is located on the margins. Nucleus-free areas of the endotheliocytes are thinned. Electron optic density of the endotheliocyte cytoplasm increases, and the number of organelles decreases. The fissures between adjacent endotheliocytes are widened. Single mitochondria have matrix bleaching and destruction of single cristae. The plasmolemma forms single protrusions into the capillary lumen, and as a result, thickening of the basal membrane. The basal membrane does not have regular external outline, but it is continuous with expressed three-layer structure. Most pericytes maintain connection with the basal membrane, but they exfoliate from it in some places. Pericytes penetrating through the basal membrane contact directly with endotheliocytes, the capillary lumen narrows. Bundles of collagen fibers are flaked and exfoliated from one another by the spaces of amorphous substance. Mast cells and interstitial swelling are found close to blood capillaries. Testicular arteriole lumens at this term of the experiment are narrowed a little. Some endotheliocytes of arterioles are damaged, the basal membrane is thickened and loses clear outline. Internal elastic membrane is thickened as well. The nuclei of the smooth myocytes become rod-shaped, their outline is regular. The structure of the venule walls is preserved although venule lumens are dilated partially.

4 weeks after the beginning of the experimental diabetes mellitus electron optic density of the capillary endotheliocytes decreases, endothelium in the testicular capillaries partially exfoliate, and due to this fact the basal membrane is bare in some places. Destroyed nuclei of endotheliocytes project into the capillary lumen. Swelling of the vascular walls intensifies, and the basal membrane thickens. The basal membrane is of an uneven passage, irregular outlines, and contains some pores. Marginal exfoliation of the nuclear chromatin is found in the pericytes, crests are dilated and fragmented in the mitochondria. Nuclear pores are found in the endotheliocytes of the testicular arterioles along the periphery of nuclei. The borders of condensed and non-condensed chromatin are difficult to determine. The cytoplasm of smooth myocytes is of an average electronic-optic density, the bundles of myofibrils are partially destructured. The adventitial membrane of the arterioles is also swollen, thickened, and a considerable amount of amorphous substance is seen between the bundles of collagen fibers. The venule lumen is often of irregular, sometimes stellate shape. Parietal clots are found in small venules.

6 weeks after the beginning of the experiment a number of ruined testicular capillaries are found, the wall is thickened, the lumens of the remained capillaries are narrowed. Platelet adhesion and erythrocyte sludge are determined in the capillary lumen. Endotheliocyte nuclei in the remained capillaries are excessively elongated and swollen, protrude deeply into the capillary lumen (Fig. 1).

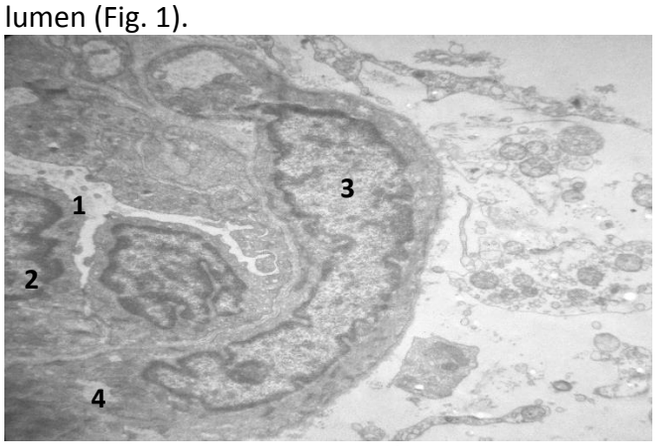

Figure 1 – Testicular capillary of a rat 6 weeks since diabetes mellitus simulation. Electronic photo. Magnification: x 4000. 1– capillary lumen; 2– endothelial cell; 3– pericyte; 4 – capillary basal membrane.

The nuclei are not found. The structural elements of Golgi complex are ruined in the cytoplasm of endothelial cells, and the internal membrane of mitochondria is destructed with vacuole formation. Microclasmatosis is determined. Plasmolemma forms protrusions in

the lumen of microvessels. The basal membrane is thickened without clear borders. Nuclei of pericytes become elongated in shape with fine invagination, marginalization of the nuclear chromatin, dilation and fragmentation of the mitochondrial crests. Arteriole lumens are filled with blood elements. Endothelial cells of arterioles are thickened, their cytoplasm contains a number of mitochondria and free chromosomes. The crests are destroyed in mitochondria. The cytoplasm of endothelial cells is vacuolated, with reduced electronic-optic density, and their plasmolemma forms micro-projections. The nucleolemma forms numerous fine protrusions. Condensed chromatin is fine-grained. The cytoplasm of smooth myocytes in the middle membrane of arterioles is of an average electronic-optic density and partially destructed. In some places structurally preserved areas of myofibril attachment are determined.

8 weeks after beginning of streptozotocin-induced diabetes mellitus deep destructive changes occur in all the links of testicular blood circulation of a rat. The lumen of capillaries is narrowed. In preserved capillaries endothelial cells become of columnar elevation which is a characteristic sign of tissue hypoxia. Nucleus-containing areas of endothelial cells project deeply into the capillary lumen, therefore, they are of fissure-like shape. Plasmolemma forms long processes, curled sometimes, penetrating into the lumen of capillaries (Fig. 2). Intercellular fissures between adjacent preserved endothelial cells are narrow, disorganized, desmosomes are found in the apical and basal areas of endothelial cells contact. The cytoplasm is of an increased electronic-optic density, mitochondria are swollen and vacuolated; a part of them is with destructive crests and damaged membranes. Round electronic-optic dense bodies are found in the cytoplasm. Micropinocytic vesicles in endothelial cells are practically absent which is indicative of decreased transendothelial transport. The nuclei of endothelial cells are elongated, contain condensed chromatin, and nuclear pores are dilated. Nucleoli are not found. In the basal membrane the areas of increased and decreased electronic-optic density are determined in turn, pericytes are swollen. The nuclei of sericytes are electronically dense with destructed chromatin, the nucleoli are not found, mitochondria contain destroyed crests. At this period of the experiment arterioles are dilated, although their lumens are narrowed at the expense of high columnar endothelial cells penetrating deeply into the arteriole lumen. The cytoplasm of endothelial cells is dark, without structure, single mitochondria are found. The nucleoma of the endothelial cells nuclei forms numerous long processes, chromatin is condensed. The basal membrane is thickened and contains electronic-optic dense bodies, and the internal elastic membrane is not detected. The cytoplasm of smooth myocytes is destructed. Every myocyte is surrounded by electronic dense plasmolemma with clearing areas.

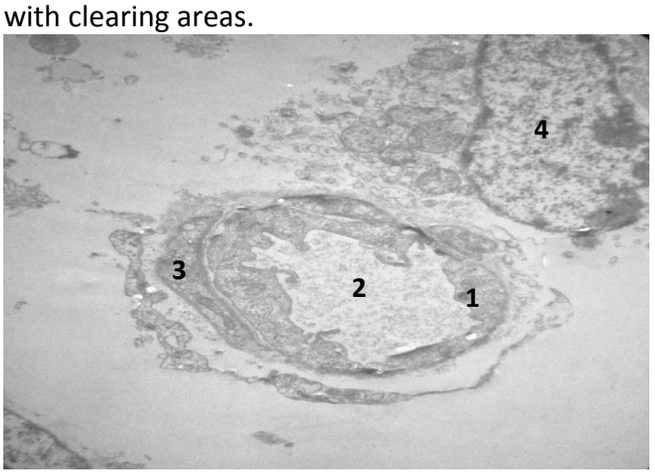

Figure 2 – Testicular capillary of a rat 8 weeks since diabetes mellitus simulation. Electronic microphoto. Magnification: x 4000. 1 – capillary lumen; 2 – endothelial cell; 3 – pericyte; 4 –endocrinocyte.

10 weeks after the beginning of experimental diabetes considerable changes occur in blood circulation. Due to protrusions of large nuclei of endothelial cells into the lumen of capillaries the latter become of an irregular fissure-like shape. Nucleoplasm in the nuclei of endothelial cells and pericytes is of low electronic density, chromatin is condensed close to the nucleoma. Perinuclear lumen is narrow. Endoplasmatic network is characterized by dilations, vacuolated cisterns with uneven outline, and they lose ribosomes attached to their surface. Mitochondria are swollen and have clear matrix and destroyed crests. The cytoplasm of endothelial cells and pericytes are of low electronic-optic density, they contain fine and small vacuoles. In some places junctions between endothelial cells diverge. The basal membrane is thickened and becomes of

irregular borders. The pericytes are of irregular shape, enlarged in size, their outlines are uneven. Cytoplasmic processes of sericytes contain a great number of vesicles and vacuoles. Erythrocyte sludge and platelet aggregates are found in capillary lumens. The nuclei of endothelial cells contain marginally located chromatin and deep invaginations of karyolemma. Close to the nucleus dilated and destroyed cisterns of Golgi complex and granular endoplasmic network are located, as well as destructively changes mitochondria. Luminal surface of plasmolemma forms numerous invaginations into the capillary lumen. The basal membrane is thickened unevenly. The signs of capillary sclerosis increase, pericapillary spaces are dilated.

Conclusions.

1. The first changes in the ultrastructural organization of the testicular blood circulation bed links of a rat are found even 2 weeks after the beginning of streptozotocin-induced diabetes mellitus and intensify during the following terms of the experiment.

2. Angiopathy is a target mechanism promoting the development of structural changes in the testicles in case of diabetes mellitus.

Prospects of further studies. The results obtained are the basis for further investigations of morphologists and clinicians with the purpose to elaborate new methods of diagnostics and treatment of testicular pathology in case of diabetes mellitus.

References:

1. Borovkova SO, Iftodly AG. Pitannya patogenezu dIabetichnih angIopatIy. Bukovinskiy medichniy vIsnik. 2006;(2):132-5.

2. Ivasyuk IY. ZmIni v krovonosnih sudinah I parenhImI yaEchka pIslya yogo travmuvannya. Naukoviy vIsnik Uzhgorodskogo unIversitetu, SerIya «Meditsina». 2004;(23):18-9.

3. Luchitskiy EV, Kobyakov SK, Slavnoye VM. Strukturno-funktsIonalniy stan ta krovopostachannya yaEchok u hvorih na tsukroviy dIabet. Bukovinskiy medichniy vIsnik. 2002;(1):79-81.

4. Mogilnitska LA, Mankovskiy BM. EndotelIalna disfunktsIya pri tsukrovomu dIabetI 2 tipu. Endokrinologlya. 2001;(1):95-103.

5. Pshenichnyiy NF, Pogorelyiy VV. Modelirovanie krovoobrascheniya v yaichkah v norme i pri varikotsele. VIsnik morfologIyi. 2005;(11):60-2.

6. Saltyikov BB, Paukov VS. Diabeticheskaya mikroangiopatiya. Moskva; 2002. P. 23-5.

7. Anigi S, Romanello MG, Domeneghini C. Ultrastructure of the epithelium that lines the ductuli efferentes testis in domestic eguidae, with particular reference to spermatophagy. Acta Anat. 1994;149(3):174-84.

8. Setchell BP. Human reproduction. The missing parts of the puzzle. Adv Exp Med Biol. 1997;424:1-15.