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Reconstruction Hemo-Microcirculatory Bed Links Myocardium Of Rat Under Experimental Streptozotocin-Induced Diabetes

Dzhalilova E. A.

Abstract:-The research on the dynamics of ultrastructural changes in the hemo-microcirculatory bed at different stages of experimental streptozotocin-induced diabetes has been conducted. There has been made a comparison of the results at the end of the 6th, 8th and 10th weeks of the experiment with the initial norm indices in order to obtain data on the beginning of the morphological changes in the area of the left heart vascularization and the beginning of the critical period of changes and the subsequent rearrangements in the rat hemo-microcirculatory bed links.

Key words:- *Diabetes mellitus, heart,* hemo-microcirculatory *bed, rat, streptozotocin*.

I. INTRODUCTION

According to the official statistics in Ukraine diabetes is diagnosed in about one million people, around 10-15% of them are with insulin-dependent diabetes mellitus [1,2]. Thus the prevalence of diabetes in Ukraine is about 1,700 people per 100,000 of population, and it is much higher in the central and eastern industrialized regions, while in western Ukraine this figure is 1221 people per 100,000 of population [3]. However, these data do not reflect the true situation. In confirmation of this some epidemiological studies data may be provided clearly showing that along with every registered patient there are 2 – 2.5 undiagnosed diabetes patients [3,4].

At the present time one of the most significant challenges for the world diabetology is the prevalence of cardiovascular pathology among the patients with diabetes [5,6]. In fact, in Ukraine the question of the cardiovascular death rate growth in the general population is possibly one of the most burning in Europe. According to Ukrainian (SYNADIAB) and European (EVRODIAB) registers, 25-35% of the patients with diabetes die of cardiovascular disease. One of the most important tasks of modern diabetology is to reduce the cardiovascular morbidity and mortality of patients with diabetes [7].

II. MATERIALS AND METHODS

The research is carried on 38 sexually mature Wistar rats - males weighing 100 – 130g, 4.5 – 7.5 months old. The insulin-dependent form of the experimental streptozotocin-induced diabetes in rats, the one similar in humans, is caused by a single 7 mg injection of streptozotocin of "Sigma" company per 100 g of body weight (prepared on 0.1 M citrate buffer, pH = 4, 5). The development of diabetes is monitored for 90 days with the increase of glucose level in blood, measured by the glucose oxidase method. Immediately after the streptozotocin injection the rats are provided with the unlimited amount of liquid (in order to stabilize the water balance and prevent dehydration). The crucial period after the streptozotocin injection is at the end of the 3rd and the 4th day. The study is conducted on animals with the glucose level of above 13.48 mmol / 1 l. The terms of the experimental model of the streptozotocin-induced insulin-dependent diabetes mellitus are 6, 8 and 10 weeks. The intact animals of the appropriate age form the controlling groups.

The research material was examined by the members of the Bioethics Commission of Danylo Halytsky Lviv National Medical University, the transaction № 20 from 15.05.2006, the commissioners reached consensus that the materials provided for the examination are science-based. There were foreseen measures concerning the moral and ethical standards in accordance with the principles of the Helsinki Declaration of Human Rights, the European Convention on Human Rights and Biomedicine and the pertinent bills of Ukraine.

While conducting the experiments on the laboratory animals (rats) the requirements of "Guidelines for the Use of Experimental Animals", approved by the Ministry of Health order № 1045-73 from 06.04.1973, the USSR Ministry of Higher Education order № 742 from 13.11.1984, were met as well as the "Preclinical studies of drugs" methodological recommendations (Kyiv, 2001).

Before the research area material sampling the animal is put to sleep by the prenatal anesthesia using thiopental (estimated 25mg / kg). A small part of rat left ventricular heart is cut with a blade and placed immediately in a large drop of 2% osmium tetroxide solution on 0.1 M phosphate buffer (pH 7.36) with sucrose for the fixation.The ultrathin cuts are prepared on the ultramicrotome UMTP-3M using glass knives made by SSN-1 apparatus. The silver or pale lemon yellow cuts are selected for the study. The sections are contrasted first in 2 % uranyl acetate solution [8], and then in lead citrate [9]. The study and the material photographing are

carried out using a microscope UEMV - 100 K (Ukraine) at accelerating voltage of 75 kV and microscope magnification of 1500 x - 30000 x.

III. RESULTS

As a result of the ultrastructural study of the rat myocardium at the end of the 6 – 8th weeks of streptozotocin-induced experimental diabetes the changes in the component links of hemo-microcirculatory bed are identified. In the blood capillaries (arterial part) the attenuatous, of high electron density, light and of medium thickness or locally broadened areas of cytoplasm and endothelial cells around medium or narrow lumens are observed. There are found hemocytes and separate structure elements (of damaged or desquamated endothelial cells).The vague destroyed areas of these cells plasmolemma, significant changes in their cytoplasm witness of this. In the electron light parts the organelles are missing, pinosomes and small pits are in small numbers. The microvilli are rare, occur in the places of endothelial cells contacts. Organelles, vesicles and small pits are not revealed in the attenuatous dark areas of endothelial cells cytoplasm. The basement membrane is significantly damaged, in some areas it is uncontoured and has tight contacts with fibrillar structures of interstitium.The perivascular spaces in some areas are rather big with lymphocytes, damaged fibroblasts, adventitial cells, remnants of destroyed cells. In fibroblasts the irregularly shaped nuclei with invaginations, large heterochromatin areas and vague karyolemma are observed. The cytoplasm consists of damaged organelles, vague plasmalemma, however, near it and in the intercellular substance of connective tissue there are clusters and separate fibrils 'Fig. 1'.In the interstitium occur hemocytes - red cells, platelets, due to the destruction of certain parts of the blood capillary walls.

For the venous section of the microcirculatory bed at these phases of the experiment (6-8 weeks) capillaries and mainly venules with especially wide lumens, filled with erythrocytes and plasma, are typical. It is submicroscopically seen that the part of endothelial cells enclosing nucleus evaginates into lumen, sometimes significantly. The nuclei are rather various: thickened, with osmiophilic karyoplasm, medium invaginations, irregular contours, considerable invaginations, also small with electron light karyoplasm and uneven karyolemma.The endothelial cells cytoplasmic space is extensive, narrow with few organelles. The basement membrane is thin, uneven, badly contoured. The perivascular spaces in some areas are wide and electron light.

Submicroscopically the following changes in venules are marked. Their lumens are very broad with numerous erythrocytes. The nuclear part of endothelial part evaginates into lumen. The basement membrane is irregular and badly contoured. The perivascular space with fibrous structures, fibroblasts and adventitial cells fragments is enlarged 'Fig. 2'.

The ultramicroscopic study of myocardium at the end of the 10th week of experimental streptozotocin-induced diabetes made it possible to state significant changes in the components of hemo-microcirculatory bed links. In the lumens of the arterial capillaries the clusters of the altered erythrocytes and platelets are found.

The coated capillary lumens and altered erythrocytes (violation of their plasticity and increased ability to aggregatedion) contribute to blood flow difficulty, increasing the pressure on the walls of the vessels. The capillary wall components are also significantly damaged.

The clearness of the basement membrane is lost, in some areas its contour disappears. The cytoplasmic space of endothelial cells is attenuatous, homogenized, without organelles. They consist of osmiophilic and some light, swollen areas. Pinosomes and small pits are absent as well as microvilli on the surface of luminal endothelial cells. In the wide perivascular space there are many fibrils arranged loosely or in clusters, they contact and replace the damaged basement membrane of capillaries. The fibrotic basal membrane and clusters of fibrous structures are a manifestation of interstitial syndrome. There are damaged adventitial cells, fibroblasts, and lymphocytes in the interstitium 'Fig. 3'.

Some blood capillaries are even more damaged. The deep endothelial cells destruction is evident in the destroyed plasmalemma areas, injured organelles and cytoplasmic spaces homogenization.

The nuclei are with osmiophilic karyoplasm and vague nuclear membrane. The basement membrane in considerable areas is fibrotic. Thick broad clusters of fibrils replace the basement membrane of such blood vessels.The lymphocytes are also found in the widened interstitial spaces. Their structure is significantly changed; the electron dense nuclei are surrounded by a narrow rim of cytoplasm, which has no organelles only few ribosomes.The blood capillaries of venous section are significantly altered. The accumulation of erythrocytes and platelets is observed in the wide lumens. The violation of the endothelial cells structure declares itself in the karyoplasm osmiophily of the endothelial cells having oblong shape and small area. The cytoplasmic spaces of such cells are highly attenuatous, electron dense, lacking organelles, pinosomes and small pits.The hemocapillary basement membrane is heterogeneous, having thin and thickened areas with fibrous structures, and is not clearly contoured on the side of the perivascular spaces.

The interstitial widened spaces include fibrillar structures, damaged adventitial cells and fibroblasts.

IV. CONCLUSION

In the experimental streptozotocin-induced diabetes the symptoms of microcirculation disturbance in myocardium are noticed. The changes in the vessel walls permeability, in the first place hemocapillaries, are caused by the violation of the endothelial cells transport function, complicated by transcapillary exchange, oxygen delivery to cardiomyocytes and metabolites excretion. Edema, growth of perivascular spaces and formation of deposits in it is associated with the beginning of angiopathy formation.In the later phases of diabetes development in the myocardium the destructive changes of histohematogenous barrier are established in all areas (in all components) of hemo-microcirculatory bed. The expressive obstruction of blood vessel lumens by erythrocytes, their spasms, thickening and fibrotizatin of the basement membrane, or its significant thinning with the integrity violation are typical. The sclerotic interstitium changes – the sclerosis with the formation of fibrous structures clusters (collagen fibrils), fibrocytes and adventitial cells destruction are present.

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[10] Fig. 1. Submicroscopic changes of myocardial blood capillary at the end of the 6th week of diabetes. 6000 x.

[11] 1 - thickened interstitial space; 2 – fibroblast; 3 - capillary lumen with erythrocyte, 4 - thin cytoplasmic area of endothelial cell; 5 - vague basement membrane.

[12] Fig. 2. Venule submicroscopic condition of myocardium at the end of the 8th week of diabetes. 3000 x.

[13] 1 - wide lumen with erythrocytes; 2 - thin cytoplasmic area of endothelial cell; 3 – nucleus; 4 - structurally altered basement membrane; 5 - interstitial space.

[14] Fig. 3. Myocardium hemocapillar ultrastructure of animals with diabetes at the end of the 10th week. 7000 x.

[15] 1 - capillary lumen with erythrocytes; 2 - platelets in capillary lumen; 3 - attenuatous endothelial cellcytoplasm,4-fibroblastininterstitium; 5 - collagen fibers.