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Features of ultrastructural restructuring of hemocapillaries of the submandibular gland in rats in case of diabetes mellitus

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Keywords: diabetes mellitus; submandibular gland; hemocapillaries.

Introduction. According to the International Diabetes Federation the number of patients with diabetes mellitus (DM) continues to grow rapidly. It has been established that already in the early stages of DM dysfunction of the vascular endothelium occurs leading to the development of diabetic microangiopathy that, in turn, results in dysfunction of salivary glands. The submandibular gland is considered to be the main source for basal secretion of saliva and its dysfunction in case of DM results in the development of xerostomia. However, the features of morphological changes of its hemocapillaries in this pathology have been insufficiently studied.
The aim is to establish the features of ultrastructural restructuring of hemocapillaries of the submandibular gland in case of experimental DM.
Materials and methods. In the Wistar rats DM was induced with streptozotocin (6 mg / g body weight). Submandibular gland's capillaries were investigated on the ultrastructural level and their morphometric parameters were measured.
Results. During experiment were observed structural changes in the basement membrane and morphometrically its thickening was increasing. Since 42nd day destructive changes of endothelial cells have developed manifested, shape factor of the lumen of capillaries was decreasing that indicating pronounced diabetic microangiopathy of the submandibular gland. In later stages (56 – 70 days) destructive changes have increased, morphometrically were confirmed the thinning of transport area of endothelial cells and greater decreasing of shape factor of hemocapillaries lumen.

Conclusions. Hemocapillaries of the submandibular gland are sensitive to pathogenetic factors developing in DM and dependent on its duration.

1. Introduction.

According to the International Diabetes Federation the number of patients with diabetes mellitus (DM) continues to grow rapidly. [8]. It has been established that already in the early stages of DM dysfunction of the vascular endothelium occurs [6; 7] leading to the development of diabetic macro- and microangiopathy [17] that, in turn, results in dysfunction of various organs including salivary glands [11]. The submandibular gland is known to be the main source for basal secretion of saliva and its dysfunction in case of DM results in the development of hyposalivation and xerostomia [5]. However, the features of morphological changes of its hemocapillaries in this pathology have been insufficiently studied [2].

The objective of our research is to establish the features of ultrastructural restructuring of hemocapillaries of the submandibular gland in case of experimental DM.

2. Materials and methods

2.1. Animals and experimental model

Experiment was performed according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The study was carried out on 1-year-old mature male Wistar rats which were divided into three groups: the intact group (norm), the experimental group and the control group. In animals of the experimental group DM was induced by an intraperitoneal administration of streptozotocin (60 mg/kg body weight) dissolved in 0.1 M citrate buffer. Animals of the control group were injected with citrate buffer only. The samples were collected on the 14^{th}, 28^{th}, 42^{nd}, 56^{th} and 70^{th} days of the experiment (5 rats were used for one time period). The control of the development of DM was performed monitoring the levels of glucose and glycated hemoglobin (HbA_{1C}). Animals in the experimental group having blood glucose concentration not less than 12 mmol/l were considered diabetic. Electron microscopic study was used to investigate hemocapillaries of the submandibular gland (SMG) [15].

2.2. Morphometry

Morphometry of the blood vessels was performed using an our original method [9] in ImageJ v. 1.47 (NIH, USA, http://imagej.nih.gov/ij) [16]. In hemocapillaries of the SMG on electron diffraction patterns the lumen diameter, shape factor (SF) of the inner perimeter which is the ratio of the luminal surface of the blood vessel to the square of its parameter and tends to “0” if “scallop pattern” of the luminal surface increases [14], thickness of transport zone of endotheliocyte and basement membrane thickness were determined.

2.3. Statistics

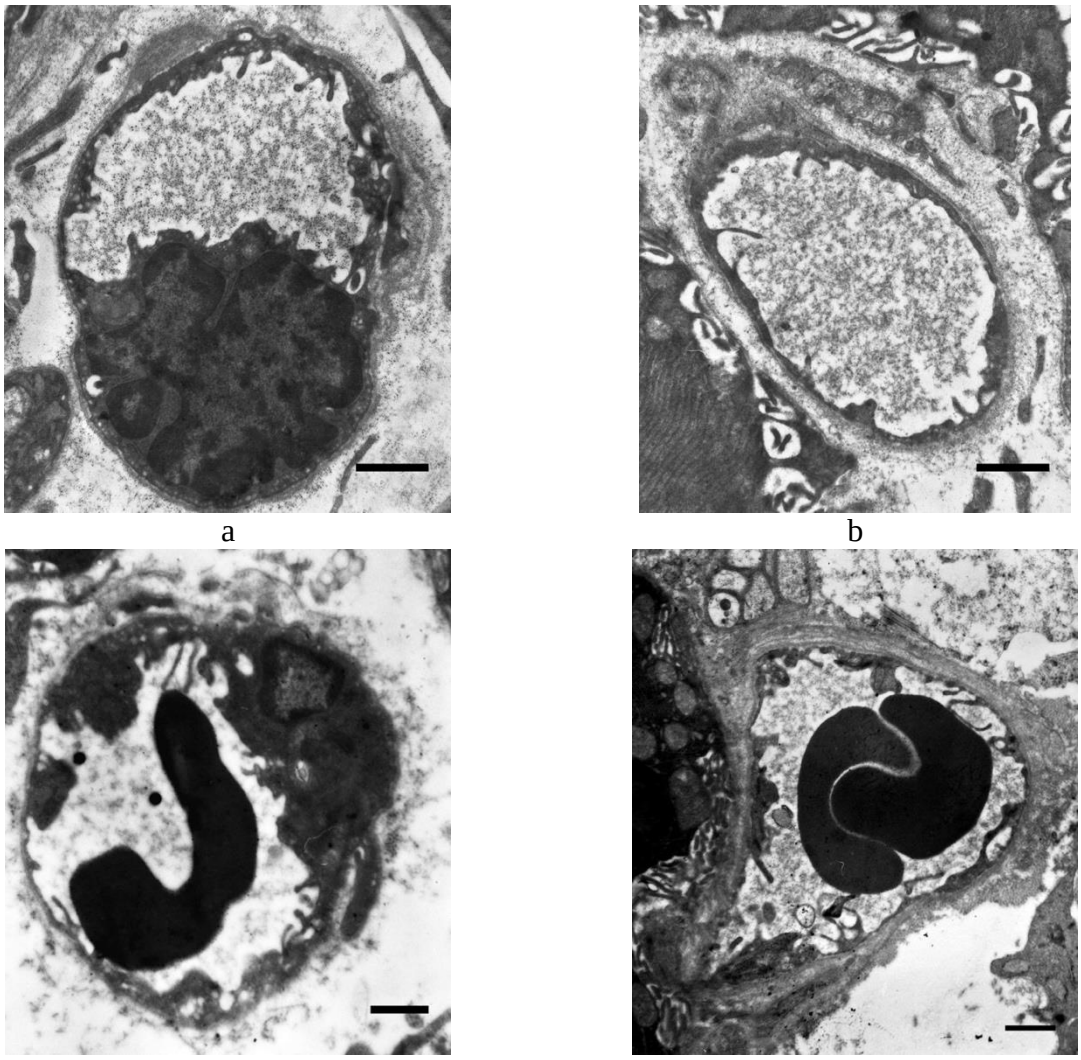
Statistical processing of the obtained data was performed in the R v. 3.0 [12] using nonparametric statistical methods (Wilcoxon-Mann-Whitney test).

3. Results and discussion

Hemocapillaries of the SMG in intact animals (diameter – 3,79 ± 0,82 μm) were usually formed by one endotheliocyte (Figure 1) divided into four separate areas, namely, nuclear, perinuclear, peripheral and contact. The cytoplasm of most endotheliocytes was of moderate electron density.

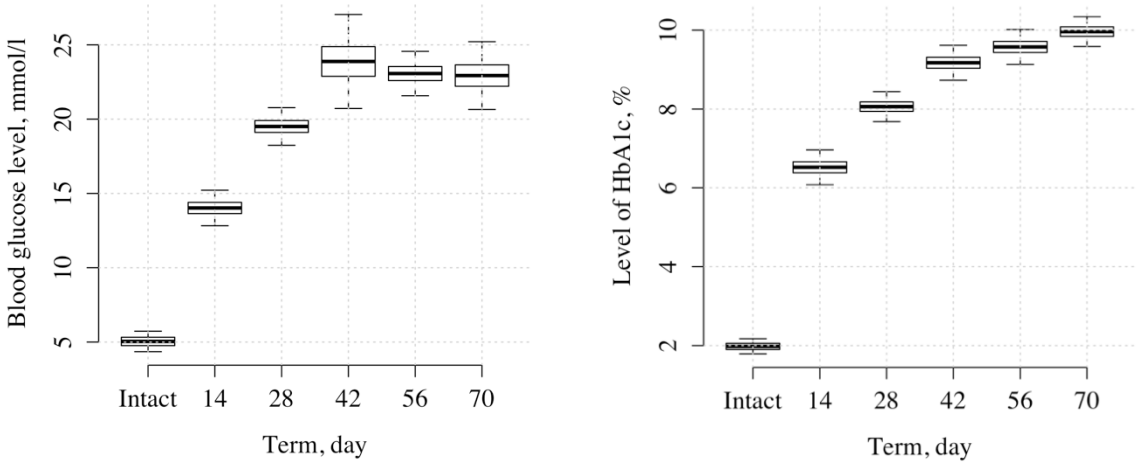
The Golgi apparatus, granular endoplasmic reticulum, ribosomes and polysomes were located in the perinuclear space. There were several mitochondria with enlightened matrix containing a few cristae. Some fenestrae and a large number of micropinocytosis vesicles were detected in the peripheral zone (width – 0,30 ± 0,08 μm) indicating an active transendothelial transport. The plasmalemma of endotheliocytes had clear contours and its luminal surface appeared as wavy forming short microprojections (SF – 0,65 ± 0,14) and basal surface was similar to the basement membrane (width – 66,84 ± 14,72 nm).

Considering the fact that DM is mainly characterized by the development of stable hyperglycemia [4], we present the results of biochemical studies. Changes in biochemical parameters in animals of the experimental group have already been found during early observation periods. Thus, on the 14^{th} day after the administration of streptozotocin the blood glucose level increased by 2.78 times ($p < 0.01$) compared to normal level and constituted 14.02 ± 1.02 mmol/l (Figure 2). On the 28^{th} and 42^{nd} days this parameter increased by 3.87 and 4.74 times compared to intact animals ($p < 0.001$). On the 56^{th} and 70^{th} days of the experiment the blood glucose level decreased slightly ($p > 0.05$), however, it was 4.57 and 4.55 times higher than in intact animals ($p < 0.001$). At the same time, the level of glycated hemoglobin increased by 3.29 – 5.02 times ($p < 0.01 – 0.001$) during the experiment. The obtained results are consistent with the results obtained by N.S. Tokaruk [18] and indicate the development of stable DM. In animals of the control group changes in these parameters during each observation period were similar to the intact group ($p > 0.05$).



a b c d

Figure 1. Ultrastructure of hemocapillaries of the SMG in intact animals a), on the 14th b), 42nd c) and 70th d) days of the development of streptozotocin-induced diabetes. Transmission electron microscope images. Scale bar 1μm.



a b

Figure 2. Dynamics of changes in the blood glucose (a) and glycated hemoglobin (b) levels at different stages of the experiment

On the background of indicated biochemical parameters on the 14th and 28th days of the experiment we did not identify any visible ultrastructural changes in endotheliocytes (Figure 1b). There were also no changes in the thickness of transport zone of these cells and SF of the inner parameter of the capillary ($p > 0.05$). However, the lumen diameter of hemocapillary was 1.14 times larger than that in intact animals ($p < 0.05$) on the 14th day of the experiment, and on the 28th day and during other observation periods it did not differ from that in the intact group ($p > 0.05$). In addition, capillary basement membrane thickened by 2.09 and 2.64 times ($p < 0.001$) compared to intact animals and in some capillaries its area was loosened. Moreover, on the 28th day of the experiment perivascular edema was identified. Such changes in the basement membrane indicated the development of diabetic microangiopathy [1]. It should be noted that E. C. Carlson et al. [3] based on the morphometric analysis of electron diffraction patterns of hemocapillaries of different organs in 1-year-old diabetic rats OVE26 also identified the thickness of the basement membrane of hemocapillaries of renal glomeruli, retina, alveoli of the lungs and diaphragm. However, we have found reports on such changes in the SMG only in the research work of Swedish researchers [13], who investigated exchange vessels in female rats in the 4th and 12th months of the development of streptozotocin-induced diabetes.

On the 42nd day of the experiment the cytoplasm of endothelial cells became more electron dense, microvesicles were hardly detectable (Figure 1c). In transport zone, the average thickness of which did not differ from that in intact animals ($p > 0.05$), cytoplasmic islands alternated with areas of significant thinning of the cytoplasm appeared. The contours of the luminal surface of their plasmalemma were occasionally not clear. The luminal surface itself contained numerous microprojections and caveolas leading to a decrease in capillary SF by 1.22 times compared to intact animals ($p < 0.05$). Microclasmatosis occurred. These changes indicate micro-circulatory hypoxia and decreased activity of transcellular processes [2]. The basement membrane was uniformly thickened, disorganized, and loosened, however, its thickness did not differ from the previous observation period ($p > 0.05$) and remained 2.64 times larger ($p < 0.001$) than that in intact animals. Significant plasmorrhagia occurred. Similar changes in hemocapillaries of the eyeball in rats in streptozotocin-induced diabetes were also observed by Kh.A. Kyryk [10], however they occurred earlier – in the fourth week.

On the 56th and 70th days of the experiment pathological changes intensified more significantly (Figure 1d) that, as compared to intact animals, was morphometrically confirmed by a decrease in SF of their inner perimeter by 1.29 and 1.40 times, respectively ($p < 0.001$), thickening of capillary basement membrane by 2.98 and 3.82 times ($p < 0.001$) and thinning of transport zone of endothelial cells during the last observation period ($p < 0.05$).

4. Conclusions

1. Hemocapillaries of the submandibular gland are sensitive to pathogenetic factors developing in diabetes mellitus and dependent on its duration.
2. The first signs of the development of diabetic microangiopathy include structural changes in the basement membrane which develop on the 14th day and manifest themselves as loosening, disorganization and can be morphometrically confirmed by its thickening increasing by the end of the experiment.
3. Since the 28th day plasma impregnation has occurred and since 42nd day destructive changes of endothelial cells have developed manifested themselves as microclasmatosis, an increase in the amount of microprojections, caveolas and morphometrically confirmed by a decrease in shape factor of the internal diameter of hemocapillaries indicating pronounced diabetic microangiopathy of the submandibular gland.

4. In later stages of the observation destructive changes have increased that was morphometrically confirmed by the thinning of transport area of endothelial cells, greater decrease in shape factor of hemocapillaries indicating the progression of microcirculatory hypoxia and a reduction in activity of transendothelial processes.

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