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Pathological changes in mucous membrane and microcirculatory bed vessels of rats oral cavity at modeling of diabetes mellitus

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Abstract

At experimental modeling of diabetes mellitus type II in rats, ischemic and dystrophic processes develop in periodontal tissues. It leads to emergence of destructive-inflammatory processes in animals and one of their pathogenic factors is apoptosis activation and compensatory activation of angiogenesis as evidenced by 1.81 times increase of monoclone BAX and 2.89 times increase of VEGF. The pathological processes mentioned should be taken into account in the development of therapeutic and prophylactic complexes of accompanying orthopedic treatment for diabetes mellitus patients.

Key words: experimental animal; oral mucosal membrane; microcirculatory bed vessels.

Diabetes mellitus (DM) and its components lead to the development of many pathological processes in the body. In this case, there is violation of fat and carbohydrate metabolism, bone metabolism, vascular disorders, which leads to inflammatory-dystrophic processes, including those in oral cavity, which can significantly affect the process of orthopedic treatment of patients when implants [1-6] are used.

The aim: to evaluate morphological disorders in the oral cavity of rats when modeling DM type II. **Materials and methods.** Wistar female white rats of herd breeding aged 10 months and mass of 230 ± 38 g were used in the experiment. Intact group comprised 6 animals and 6 animals had DM type II. Reproduction of DM type 2 in rats was carried out by intramuscular injection of protamine sulfate (Merck, Germany) at a dose of 18 mg / kg twice a day for 5 days and after a two-day break for another 5 days.

The animals were taken out of the experiment under thiopental anesthesia (40 mg / kg), then morphological studies were carried out.

In groups of animals under study the peculiarities of the microvasculature bed, the state of the bone tissue, the peculiarities of the course of reparative processes in the tissues of gums and bone tissues were invetigated [7].

The dissected tissues were fixed in 10% formalin solution with further routine methods of degreasing and dehydration. In the course of the further stage of the experiment, sections with a thickness of $5x10^{-6}$ m were prepared and several types of staining were applied to identify the features of the tissues of the oral cavity by microscopy.

In this case, staining with hematoxylin and eosin was used to study the state of the structures of the oral cavity, microarchitectonics of tissues, determine the peculiarities of the cellular composition, the interposition of various cells, fibers, and tissue vascularization. In addition, this method of staining helps to identify not only the quantitative composition of cells, features of their location, but also their quality. Van Gieson staining was used to identify the structural features of connective tissue elements, to determine the characteristics of the pathological process. For the same purpose, a modified Mallory stain was used, which made it possible to determine the interposition of collagen, elastic and reticular fibers and intercellular substance. Rego staining was performed to identify ischemic zones, which is important for understanding the features of vascularization.

Immunohistochemical study was carried out by staging a non-direct immunoperoxidase reaction with monoclonal antibodies to VEGF (monoclone, vascular endothelial growth factor), BAX (monoclone, apoptosis activator; firm Thermo scientific). The reaction was visualized using the UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen kit (Thermo scientific) with counterstaining of nuclei with Mayer's hematoxylin.

The slides were studied using an Olympus BX-41 microscope with x4, x100, x200, x400 objectives; the results were further processed with the Olympus DP-soft version 3.2

software. For each micro-preparation, morphometric processing was carried out in 30 fields of view.

The degree of expression of VEGF and BAX receptors was assessed by a semiquantitative method, considering the reaction as 1 point negative, 2 points - weakly positive (1% <n <10%), 3 points - moderately positive (11% <n <20%) and 4 points - strongly positive (n> 21%).

Results and discussion. Intact animals' mucous membrane (MM) was pale pink, moist, clean, without hemorrhage; it did not bleed when touched with a spatula. The oral cavity in its greater space was covered with multilayer squamous non-keratinizing epithelium, and only in the area of the marginal and alveolar surfaces of the gums the areas of keratinizing epithelium were determined. All layers of the epithelial layer are sufficiently expressed, the boundaries between them are clear, well visualized. Keratinization processes are insignificant. Signs of submerged growth of the epithelium were not observed in the animals of the group under study. The lamina propria was represented by elastic fibers without any signs of their destructive changes. There were practically no inflammatory infiltrates. A weakly positive reaction to the BAX apoptosis activator was an important indicator of the absence of a damaging factor. It should be noted that all cellular elements were located singly, did not form accumulations and infiltrates. There was a plethora of blood vessels in the microvasculature. The diameter of arterioles in this subgroup was $22.68 \pm 0.54 \times 10^{-6}$ m, precapillary arterioles $13.03 \pm 1.02 \times 10^{-6}$ m, capillaries $7.65 \pm 0.47 \times 10^{-6}$ m, postcapillary venules 27 , $44 \pm 1.63 \times 10^{-6}$ m, venule $47.03 \pm 0.31 \times 10^{-6}$ m (in the intact group $38.80 \pm 1.88 \times 10^{-6}$ m). When staging a peroxidase reaction to VEGF, it was found that the pattern of expression of the receptors for this protein is the cytoplasm, the cell membrane and the components of the extracellular matrix. Bone-destructive processes were not expressed.

In the study of a group of animals with simulated DM type II, pathological changes were revealed in comparison with the group of intact rats from the side of the oral mucosa, submucosa and vessels of the microvasculature bed. The mucosa was thinned, dry, covered with stratified squamous non-keratinizing epithelium, in the gum region - keratinizing. In the study of histological preparations stained with hematoxylin and eosin, uneven thickening of the epithelial layer was determined. The cells of the granular layer were of normal size and contained an increased amount of keratohyalin grains in their cytoplasm. The cells of the spinous layer were flattened. Vacuoles were visualized in the cytoplasm, reaching large sizes, pushing the nucleus to the periphery. The cell cytoplasm was basophilic, the nuclei were hyperchromic. A weakly positive PAS response was more pronounced in the spiny layer. The

revealed signs are characteristic of degenerative and proliferative processes. Dystrophic processes were observed practically throughout the entire thickness of the epithelium with the involvement of the prickly and granular layers. Inflammatory infiltrates with the presence of lymphocytes, leukocytes, macrophages, and plasmocytes were diffuse throughout the entire mucosal lamina propria. The basement membrane was thickened and homogenous. Also, single fibroblasts were detected in the perivascular space. Collagen fibers were arranged in bundles, fuchsinophilic in places there were single lysed fragmented elements. The reticular fibers of the mesh layer were coarse, branching, rarely anastomosed with each other, with a small amount of lysed fibers. In the zone of the periodontal junction, periodontal pockets are formed (Fig. 1).

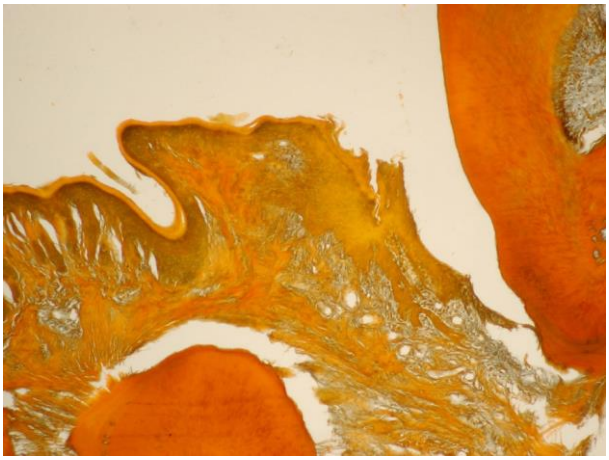

Fig. 1. Formation of a periodontal pocket in the area of the tooth-gingival junction. Modified Mallory staining, x100 magnification.

The vessels of the microvasculature bed were thickened, uneven blood filling with the presence of thrombotic formations, anastomosed among themselves (Fig. 2)

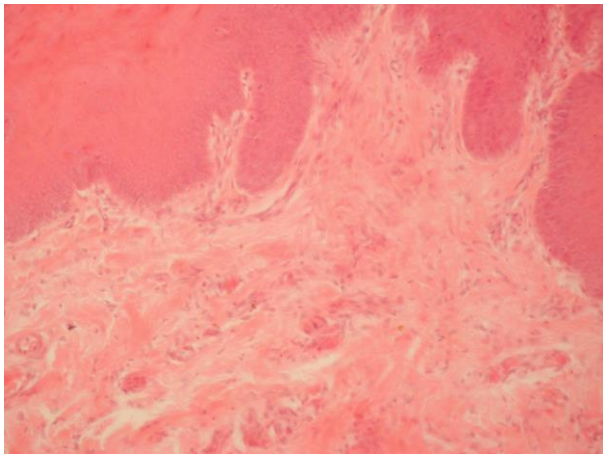

Fig. 2. Thickening of the vessels of the microcirculatory bed, their uneven blood filling, focal inflammatory infiltrates. Staining with hematoc-silin and eosin. x200.

In some places, newly formed capillaries forming a network were determined. At the same time, narrowed vessels were noted in combination with paretic dilated venules with increased blood filling. In the lumen of these vessels, the aggregation of blood corpuscles with the formation of thrombotic masses was determined, and in the perivascular space - diapedesic hemorrhages.

When analyzing the results of the peroxidase reaction with MCA to VEGF, its activation was observed from 0.93 ± 0.15 conv. units in the intact group up to 1.69 ± 0.11 conv. units after modeling DM type II.

The pathological processes caused by the modeling of DM were also expressed in the submucosal layer. Inflammatory infiltrates were located in the perivascular space; the vessels were characterized by signs of fibrinoid swelling.

Activation of apoptosis processes was noted, as evidenced by the results of the peroxidase reaction to BAX, which increased from 0.75 ± 0.47 conv. units in the intact group of rats to 2.17 ± 0.16 conv. units in the group with DM type II. Pathological processes were also expressed in the underlying tissues, in particular, in smooth muscle tissue. These changes were characterized by partial destruction of smooth muscle fibers, reduction of nuclei.

When carrying out morphometric studies, it was found that the diameter of arterioles in this subgroup was 17.14 ± 0.93 x 10^{-6} m, precapillary arterioles 11.54 ± 0.39 x 10^{-6} m, capillaries 6.90 ± 0.29 x 10^{-6} m, postcapillary venules 29.52 ± 0.73 x 10^{-6} m, venules 43.21 ± 1.67 x 10^{-6} m.

Destructive processes were observed in the bone tissue of animals. Trabeculae of cancellous bone tissue were sparse, in some places they were anastomosed to each other. Osteoclasts were functionally active, located on the surface of the trabecular meshwork (Fig. 3).

Osteoblasts were located in the periosteum, functionally inactive, osteocytes were located in lacunae, often dilated. The number of cytoplasmic processes was reduced, which indicates that metabolic processes are slower than in animals of the intact group. The intertrabecular substance was represented by a loose fibrous connective tissue with an abundance of cellular elements that form inflammatory infiltrates. Collagen fibers were partially lysed, single reticular fibers, less crimped than in animals of the intact group, were connected to each other, forming a network.

Osteons of lamellar tissue were also well expressed; in the central part, the Haversian canal, they contained a neurovascular bundle. They were found against the background of sharply spasmodic, sharply paretic dilated vessels with thrombotic masses.

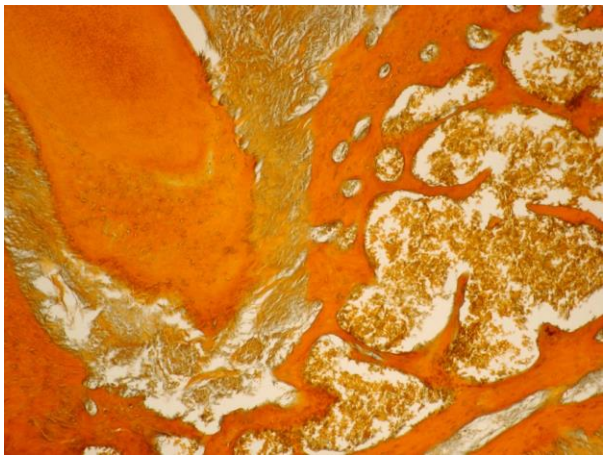

Fig. 3. Periodantal space with the presence of ligaments and bone tissue. Functionally active osteoclasts on the surface of the trabecular meshwork. Modified Mallory staining, x100 magnification.

Conclusions. It was shown that at DM type II, ischemic and dystrophic processes develop in the periodontal tissues of rats, which leads to the development of destructive inflammatory processes in animals, one of the pathogenesis factors of which is the activation of apoptosis, compensatory activation of angiogenesis, as evidenced by 1.81 times of BAX increase, and 2.89 times of VEGF increase.

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