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MORPHOLOGICAL SIGNS OF DESTRUCTION AND REGENERATION OF THE PERIODONTAL TISSUE OF RATS IN EXPERIMENT

Abstract. *80 albino outbred mature male rats were used in the experiment distributed into five groups (16 animals each): two control groups – IC (negative control) and IIC (conditioned positive control) and three experimental ones: IE, IIE, IIIE. The experiment was conducted in two stages. At the 1st stage every day in addition to the main forage rats from the IIC group and experimental groups were introduced per os with 0,04% ammonium chloride solution (400 mg/kg) – acidotic periodontitis, IC group – per os isotonic salt solution (400 mg/kg). At the 2nd stage rats of both control groups were introduced with isotonic salt solution (400 mg/kg), IE group – i/m 5% meldonium dehydrate solution – drug of metabolic action, IIE – per os calcium glycerophosphate – 133 mg/kg; IIIE – i/m 5% meldonium dehydrate solution and calcium glycerophosphate per os simultaneously. After the experiment was completed the blocks of lower jaws with teeth were isolated, the tissues were fixed during 48 hours in 10% neutral formalin solution. Paraffin sections 5-7 microns thick were stained with hematoxylin and eosin according to van Gieson staining method. Purified from paraffin sections after being treated with aniline alcohol were stained with Heidenhain azan stain. Pictures were taken of separate areas of the specimens by means of the digital photo-adapter under the microscope Leica DFC 420. A comparative histological examination of the mandibular cellular processes fragments stained with hematoxylin and eosin according to Heidenhain azan stain was conducted. Hyperemic, swollen and smoothed dome-shaped interdental papillae were found on the acidotic model of periodontitis (28 days) in rats of IIC group contrary to the animals from IC group. Due to intensified desquamation of cells from the corneal and granular epithelial layers the gingival epithelial layer is partially preserved, erosive areas are covered with granulation tissue. Intercellular spaces of polymorphic cells of the acanthocyte and basal layers are dilated, and on certain areas contacts between cells are absent. Endothelial swelling is found in the blood capillaries of the gums. Resorption of the collagen fibers and osseous tissue of the cellular part in the lower jaw of rats occurred, leukocyte infiltrations were found as the main signs of inflammatory-dystrophic damage.Metabolic correction on the 42nd day of the experiment resulted in an incomplete regeneration of the gingival epithelium and completed reconstruction of the osseous tissue, consolidation of intercellular contacts and basal membrane of the gingival epithelium, appearance of newly formed thin-walled vessels, areas of replacing sclerosis in the sub-epithelial connective tissue, homogeneous structure of the cellular part osseous tissue of the lower jaw in rats.*
Key words: *periodontal pattern, gingival epithelium, connective tissue, cellular bone.*

Introduction. In spite of considerable achievements of modern clinical medicine and newest biotechnological results the problem of restoration and retention of the structural and functional adequacy of the periodontal tissues still remains topical [4, 9, 14]. Thus, according to the data submitted by Komarevtseva I.A. et al (2009) [5] a comprehensive response of the body connective tissue as an integral structural-functional system performing plastic and trophic functions is an effective natural means to eliminate pathogenic factors and regenerate the damaged tissues [5, 16].

Crucial factors of damage and morphological signs of periodontal restoration are studied at the cellular, sub-cellular and molecular levels [8, 18, 12]. Thus, for example, while simulating periodontitis association of dystrophic and

reparative changes were found in the alternative and proliferative phases of inflammatory process [2, 19, 20]. Generally morphological signs of damage of the mucous and sub-mucous membranes of the gums occur earlier and are more pronounced than those in the osseous tissue of the cellular process [6, 7, 10]. In case or periodontitis collagen protein content decreases in the connective tissue of gums due to prevailing processes of collagen decay over the processes of its synthesis [13, 15], and under conditions of chronic nitrate intoxication in particular [1]. Considering the presented data the condition of the cellular and fibrous elements of the connective tissue attracts special attention while studying the structure of the periodontal tissue in rats with induced periodontitis and after metabolic correction.

Objective: to determine morphological signs of damage and peculiarities of restoration of the mandibular periodontal complex of rats on metabolic pattern of periodontitis.

Materials and methods. The experiment lasted 42 days and was conducted in two stages (the first one – 28 days and the second one – 14 days) on 80 albino outbred male rats aged 2-4 months with the body weight of 170-240 g kept on standard vivarium diet at Danylo Halytskyi Lviv National Medical University. The animals were distributed into five groups (16 animals each): two control groups – IC (negative control) and IIC (conditioned positive control) and three experimental ones: IE, IIE, IIIE. At the 1st stage every day in addition to the main forage rats from the IIC group and experimental groups were introduced per os with 0,04% ammonium chloride solution (400 mg/kg) – acidotic periodontitis, IC group – per os isotonic salt solution (400 mg/kg). At the 2nd stage rats of both control groups were introduced with isotonic salt solution (400 mg/kg), IE group – i/m 5% meldonium dehydrate solution (the drug "Vasonat", Olainpharm, Latvia) – 0,25 mg/kg; IIE – per os calcium glycerophosphate (PTC "Lugansk Chemical-Pharmaceutical Plant") – 133 mg/kg; IIIE – i/m 5% meldonium dehydrate solution and calcium glycerophosphate per os simultaneously.

The study was conducted keeping to the requirements of the European Convention of Bioethics (1997), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Studies, general ethical principles of experiments on animals, approved by the First National Congress of Ukraine on Bioethics (2001) [3].

Animals were taken out from the experiment under ether narcosis. The blocks of lower jaws with teeth were isolated, and the tissues were fixed during 48 hours in 10% neutral formalin solution. On completion of fixation with the purpose of decalcification mandibular bones were treated during 4 days in changed solutions of 7% nitric acid, dehydrated in the ascending graded alcohols and filled with paraffin (D.S. Sarkisova, Yu.L. Perova, 1996). Paraffin sections 5-7 microns thick were stained with hematoxylin and eosin according to van Gieson staining method (BlikMedPrep set, RF). Purified from paraffin sections after being treated with aniline alcohol were stained with Heidenhain azan stain (BioVitrum set, RF). Pictures were taken of separate areas of the specimens by means of the digital photo-adapter under the microscope Leica DFC 420 (Germany) using the eyepiece x 10; 20 and object glass x4; 8.

Results of the study. In rats from IC group a free part of the gums delimited from the neck of the tooth by the gingival slit forms the gingival margin and interdental papillae of an elongated shape (Fig. 1).

The corneal epithelial layer of the gums is formed by nuclear-free flat squamosal cells, the

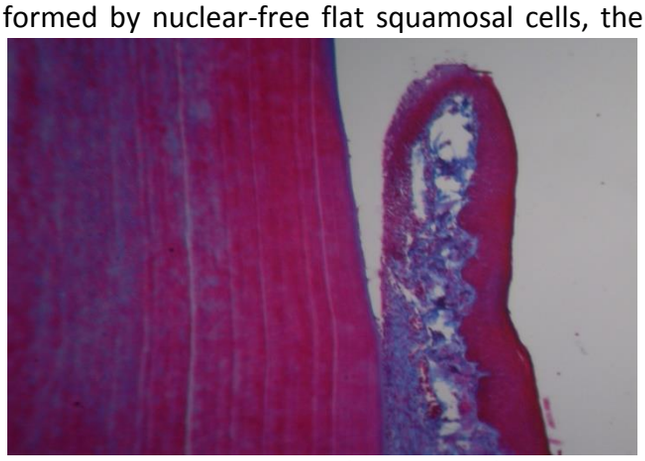

Fig. 1. Mucous membrane of the gums of a rat in the norm (IC). Stained with Heidenhain azan stain. Magnification: oc. 10, ob. 8.

granular layer – by the layers of flat cells. Acanthocyte layer consists of several (2-3) layers of large cells of a polygonal shape smoothed close to the granular layer, which numerous acanthocyte processes are connected between themselves by means of desmosomes. The basal layer is formed by cubic cells located on the basal membranes. The plate of the gingival mucous membrane proper where single capillaries are located is formed by fibrous connective tissue penetrating in the form of papillae to the epithelial layer (Fig.1).

The cellular process is of a typical plate-like architectonics with homogeneous by the density of location and size beams of the spongy bone. Bundles of the collagen fibers of the periodontal tissue are fixed to the cement of the dental root from one side, and from another – connected with the periosteum (Fig2.).

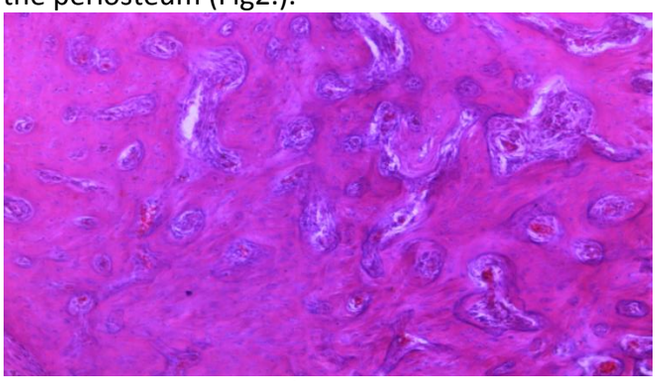

Fig. 2. Osseous tissue of the mandibular cellular part of a rat with ossesous trabecula and vascularized cells in the norm. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4.

Hyperemic, swollen and smoothed dome-shaped interdental papillae are determined in rats from IIC group. Intercellular spaces of polymorphic cells of the acanthocyte and basal layers are dilated as well, on certain areas contacts between cells are absent and only desmosomes are found.

In the blood capillaries of gums of a somatic type endothelial swelling is seen, in the pericapillary space and in the gingival connective tissue layer proper – growth of collagen fibers of the connective tissue. In the sub-epithelial connective tissue vessels are sclerosed, surrounded by leukocyte infiltration, microabscesses are found filled with neutrophils, and the areas of the granulation tissue spread deep to the cellular bone. The beams of the spongy bone are different by size, density of location and shape of the inter-beam cells (Fig.3). A free part of the gums in rats from IE group is infiltrated with lymphocytes. Collagen fibrils of the epithelial layer of the gums which are closely connected with the cement of the dental root in the norm and attach gums to the periosteum of the cellular process and form the base of the plate of the sub-mucous layer proper are inhomogeneous by the electron density and diameter, twisted between themselves. Beams of the spongy bone are homogeneous by the shape and size with a regular density of location. Inter-beam spaces contain thin-walled vessels and bone marrow (Fig.4).

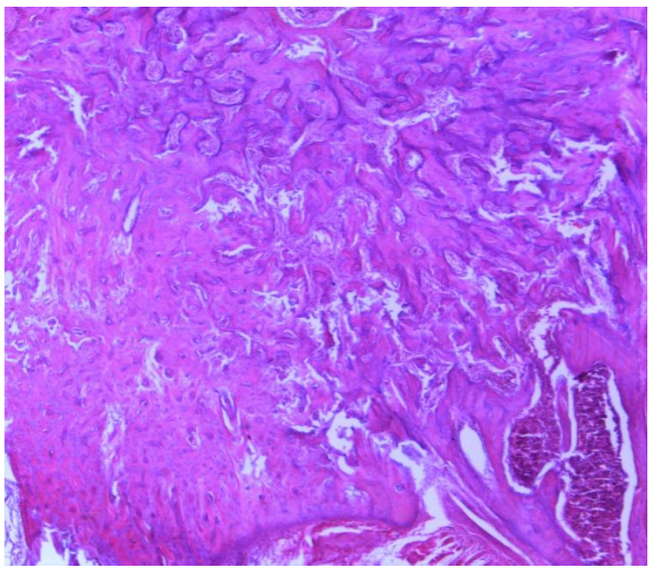

Fig. 3. Microphotograph of a fragment of the mandibular periodontal tissue of a rat from IIC group. фрагменту пародонту нижньої щелепи щура IIК групи. Staining with hematoxylin and eosin. Magnification: oc. 10, ob. 4

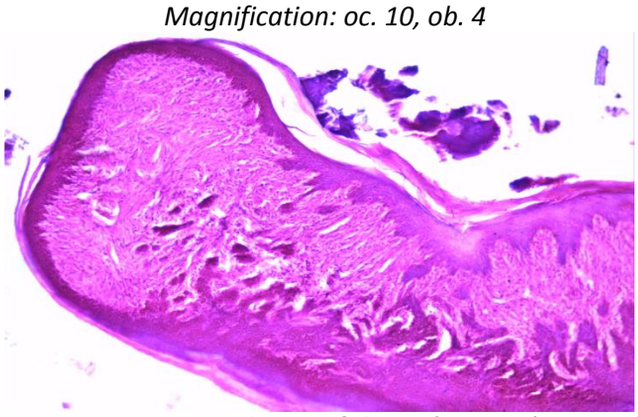

Fig. 4. Mucous membrane of the rat's gums (IE group) with signs of regeneration of the connective tissue and residual signs of inflammatory alteration. Staining with hematoxylin and eosin. Magnification: oc. 10, ob. 4

The connective tissue papillae in animals from IIE group are thickened, deepened into the own plate, acanthosis signs are absent, single intra-epithelial leukocytes are found, basal membrane is preserved. Infiltration and focal sclerosis in the vascular wall are found. Collagen bundles are homogeneous by the shape and size, although they are disorganized in the areas of mixed-cellular infiltration. Beams of the osseous tissue are of a usual structure, similar by the shape and size, although with different degree of density and numerous foci of irregular excessive calcification (Fig. 5).

Epithelial layer in rats from IIIE group is thickened on separate areas, intercellular spaces are narrow, basal membrane is formed by a network of thin reticular fibers. Collagen bundles of the sub-epithelial connective tissue are located in order, without signs of fragmentation, residual

signs of inflammatory process, and single areas of substitutive sclerosis are found. Beams of the spongy bone are similar by the shape and size with regular density of a compact plate. Small inter-beam cells, in addition to vessels, are filled with focally sclerosed connective tissue and bone marrow (Fig. 6).

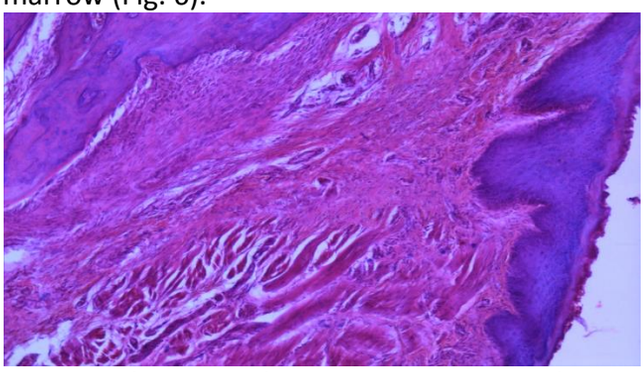

Fig. 5. A fragment of the periodontal tissue of a rat from IIE group. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4

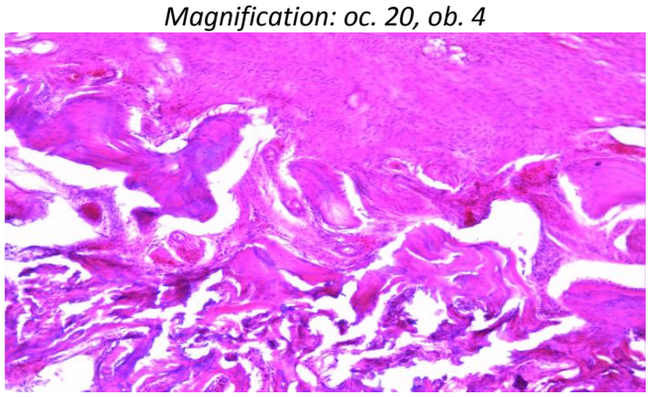

Fig. 6. A fragment of the osseous tissue of the mandibular cellular part of a rat from IIIE group with regular degree of density. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4

Discussion. In rats from the group of a negative control the mucous membrane of gums adhered to the periosteum of the cellular processes is covered with integral laminated epithelium. Numerous papillae of the own plate of the gingival mucous membrane enlarge the square of contact and strengthen connection between the epithelium and adherent connective tissue. Fibrillar proteins are known to be the base of the structure of the basal capillary layer, basal membrane of the epithelium, and the connective tissue of the gingival mucous membrane proper and intercellular substance of the osseous tissue of the cellular process [16].

Integrity of the intercellular connections in rats from the group of a positive control is disturbed due to intensified desquamation of the corneal and granular epithelium. Cornification of the superficial layer of the epithelium is slow, the gingival epithelial layer is partially preserved, and the areas of erosions are covered with the granulation tissue. Shortened, partially fragmented collagen bundles on separate areas of the pericapillary space as well as in the proper layer of the connective tissue of gums are indicative of an active process of proteolytic resorption. Resorption of collagen fibers according to the data presented by Kolesova N.A., et al (2008) [4] occurs with participation of primary and secondary lysosomes released in case of destruction of leukocytes and fibroblasts. Lacunar resorption of the osseous tissue of the cellular process is associated with formation of periodontal pockets.

In rats of IE group initial signs of perivascular sclerosis of a free part of the gums were seen and erosive defects absent. At the same time, the initial stage of the osseous tissue regeneration was characterized by inter-beam spaces with thin-walled vessels and cells of the bone marrow. Due to hyperplasia of the basal cells and accumulation of keratohyalin in the cells of the granular layer in animals of IIE group the epithelial layer of gums thickened.

In rats of IIIE group the epithelial layer on certain areas was also thickened, dense intercellular contacts, in formation of which belongs to fibronectin structural protein – a product of fibroblast secretion of the connective tissue [8]. Collagen bundles of the subepithelial connective tissue are located in order, the structure of inter-beam cells is indicative of completion of reconstruction of the mandibular osseous tissue.

Conclusions.

1. Conditions of cells and fibrous structures of the connective tissue of gums and cellular process of the lower jaw of rats in the norm are crucial to assess regenerative ability of the periodontal complex in the experiment.
2. Acidotic pattern of periodontitis (28 days) determined the main signs of inflammatory-dystrophic damages such as erosions of the epithelium, disturbed integrity of the intercellular connections, resoprtion of collagen fibers and osseous tissue of the cellular part of the lower jaw of a rat, leukocyte infiltrations.
3. Metabolic correction resulted in an incomplete regeneration of the gingival epithelium and completed reconstruction of the osseous tissue, consolidation of intercellular contacts and basal membrane of the gingival epithelium, appearance of newly formed thin-walled vessels, areas of replacing sclerosis in the sub-epithelial connective tissue, homogeneous

structure of the cellular part osseous tissue of the lower jaw in rats.

Prospects of further studies. Presented results of the study are a part of the scientific-research work dealing with investigation of peculiarities of inflammatory diseases of the periodontal tissue against the ground of metabolic disorders. The results obtained in the study can be used to search and analyze effective methods of treatment of periodontitis in patients with metabolic disorders.

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