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MORPHOFUNCTIONAL PECULIARITIES OF THE PERIODONTAL TISSUE UNDER CONDITIONS OF SIMULATED EIGHT-WEEK OPIOID EFFECT

Abstract. *The objective of the work is to examine morphofunctional peculiarities of the periodontal tissue in case of eight-week opioid effect in small doses in the experiment. To achieve the aim light optic examination of the periodontal tissue was used. The tissue was stained with hematoxylin, eosin and azan. Single everyday opioid introduction for rats during eight weeks was found to result in the development of dystrophic-inflammatory changes in the periodontal tissue. The data obtained can be used to make differential characteristics of pathomorphological changes on the microstructural level observed in the dynamics with increasing opioid dose. The above will enable to elaborate methods of probable correcting effect in case of development of generalized periodontitis against the ground of opioid intoxication.*

Key words: *opioid, light optic examination, periodontal tissue, rats.*

Introduction. Numerous literary sources, data of official medical statistics indicate that many pharmaceutical agents in addition to useful therapeutic action can cause complications of different degree of severity [1, 2]. A considerable amount of scientific publications deal with destructive opioid effect on the organs, tissues and body systems, although dental pathology of this contingent of patients is not sufficiently studied [3 - 5]. Considering this aspect it should be noted that today the problem of morphogenesis of chronic generalized periodontitis and chronic periodontitis remains not solved completely [5-7]. In this respect various methods of modeling periodontal injury of inflammatory-dystrophic character are applied [7-13]. Experimental models simulating inflammatory process in animals will enable to determine chronology of changes in the periodontal tissue and parodontium on the whole, which is impossible to be made under clinical conditions in the man in case of development of the similar disease [9,13-15].

The objective of the work is to examine morphofunctional peculiarities of the periodontal tissue in case of eight-week opioid effect in small doses in the experiment.

Materials and methods. The study was

performed on 16 outbred mature male rats with the body weight of 255 g, aged 4,5 months. The animals were given a single intramuscular injection of the drug "Nalbuphine" every day during 8 days. The dose of the opioid analgesic was increased to the maximal single dose from 0,212 mg/kg to 0,283 mg/kg at the end of the 8th week. The animals were divided into 2 groups in the experiment. In the first group rats were given the drug "Nalbuphine" during 56 days followed by taking material (at the end of the 8th week). The second control group during 8 weeks was given intramuscular injections of physiological solution at the same period of time. The animals were kept in vivarium; everything concerning their keeping, care, marking and other manipulations were conducted according to the principles of "The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985), the Law of Ukraine № 3447 – IV «On Protection of Animals against Cruel Treatment». The Bioethics Committee of Danylo Halytsky Lviv National Medical University determined that the scientific study corresponds to the ethic requirements according to the Order of the Ministry of Public Health of Ukraine № 231 dated 01. 11. 2000 (minutes № 10 dated 26.12. 2011), (minutes №2

dated 20.02.2012). Before taking material for biopsy the animals were put to sleep by means of intraperitoneal thiopental introduction (25 mg/kg). Amputated upper and ex-articulated lower jaws were used for light optic examination, considering topographic correlation of the dental tissue on histological specimen 5-7 mcm thick. The tissue specimens were prepared according to the common method with previous decalcification [16] stained with hematoxylin, eosin and azan by Heidenhain's method. The specimens were microscopically examined and photos taken by means of the microscope Meiji MT4300 LED and digital camera Canon EOS 550D.

Results and discussion. Everyday injection of opioid analgesic in small doses to rats during 56 days was found to result in the development of dystrophic-inflammatory changes in the periodontal tissue. Morphological changes found under light optic microscope can be compared with clinical signs of generalized periodontitis.

Tissue injuries first of all refer to the gingival structures. Keratosis, the signs of hyperkeratosis and hypertrophy of the stratified squamous keratinized epithelium were found in the adjacent and free parts of the gums. In all the cases desquamation was intensified with exfoliation of destructed epithelial cells. At the same time, slow regeneration of the epithelial layer and progress of desquamation process results in increasing gradual atrophy of the gingival structure. It is manifested by thinning of the oral and sulcus epithelial portions of the free gingival part. Moderate acanthosis of the epithelium was found in the free and adjacent parts of the gums. Epithelial papillae were mainly of irregular size, smoothed, moderately developed or absent, as it is seen on Figures 1 and 2.

In the result of progressing of dystrophic-inflammatory process against the ground of long opioid intoxication, the rate of regeneration of sulcus and connective portions of the epithelium of the free gingival part was determined. As a result, generalized injury of the gingival structures leads to the destruction of the epithelial attachment and formation of periodontal pocket.

The changes found were associated with diffuse erosion of the sulcus and connective epithelium with perforation of the periodontal pocket bottom, which is the place for entrance of

pathogenic microorganisms into the periodontal tissue. Hyperplasia and signs of desquamation of the periodontal pocket epithelium were determined, as it is seen on fig. 3.

Changes of dystrophic-inflammatory character prevailed in the spongy fibrous and dense shapeless connective tissues, where pronounced swelling was found with exfoliation and destruction of the collagen fibers. Fibrinoid swelling with exfoliation of the spongy connective tissue was found in the papillary layer of the proper lamina of the gingival mucous membrane. Single signs of moderate fibrosis with diffuse round cellular infiltration were determined in the

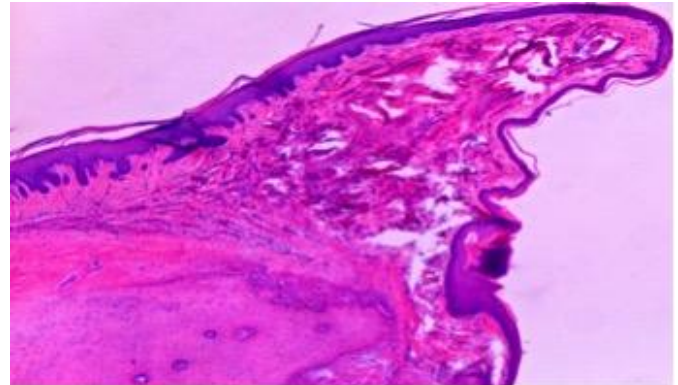


Fig. 1. Periodontal tissue of a rat 8 weeks after opioid injection. Stained with hematoxylin and eosin. Magnified: x 100.

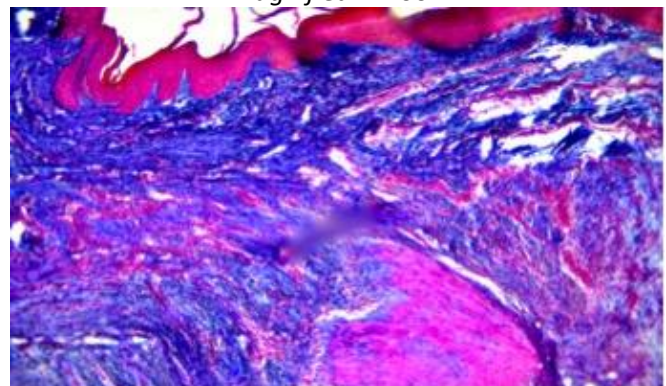


Fig. 2. Periodontal tissue of a rat 8 weeks after opioid injection. Stained with azan. Magnified: x 200.

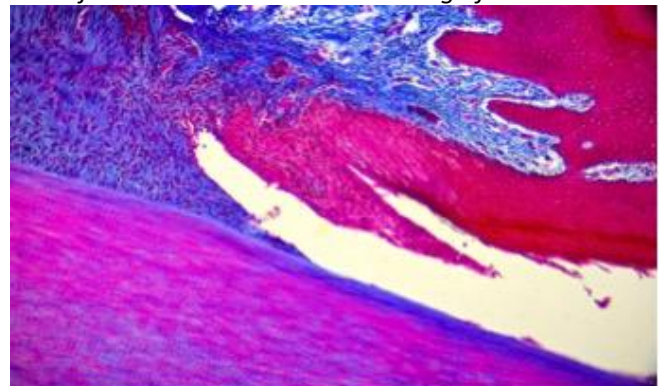


Fig. 3. Periodontal tissue of a rat 8 weeks after opioid injection. Stained with azan. Magnified.: x 200.

subepithelial layer. The connective tissue papillae were moderately outlined and smoothed. In certain cases in the area of the formed periodontal pocket acanthotic cords were found with proliferation of the connective tissue into hypertrophic epithelium of the gingival papilla. In the reticular layer of the proper lamina venostasis was detected, focal hemorrhages of diapedesis character and insignificant intensification of trophism resulted from the opening of a reserved capillary group of the afflicted area, as it is seen on fig. 1, fig. 2 and fig. 3.

Hyperplasia with exfoliation of fibers of the dense formed connective tissue was found within the borders of marginal periodontal tissue. Progressing of the diffuse injury of tissue in the area of the periodontal pocket was directly associated with destruction of collagen fibers of the circular ligament. Spongy fibrous shapeless connective tissue of the apical periodontal tissue was mainly thinned, where by the morphological peculiarities of the fibrous direction peri-radical and periosteal layers were determined. Diffuse round cellular infiltration without vessels filled with blood was determined in the periradical layer, which differed by more compact and longitudinal direction of fibers. The signs of mucoid swelling were found in the periosteal layer, where collagen fibers were of a bundle structure. The vessels filled with blood with the signs of venostasis were also determined as it is seen on fig. 2 and fig. 4.

The osseous tissue of the dental cell is mainly non-homogenous which is caused by local portions of the resorptive process and metaplasia. The signs of lacunar resorption were determined in the area of the intercellular septum apex. The signs of substitution of the osseous tissue by the connective one along the external margin of the intercellular septum were found as a sign of inconsiderable processes of compensation. Numerous Haversian canals with the signs of osteofibrosis were found, as well as hyperplasia of the periosteum with proliferation of osteoblasts in the compact layer of the periosteum, as it is seen on fig.1; fig. 2 and fig. 4. Primary and secondary dental cement were characterized by hypercementosis, found in the area of the upper third and within the borders of the dental root apex, as it is seen on fig. 3 and fig. 4.

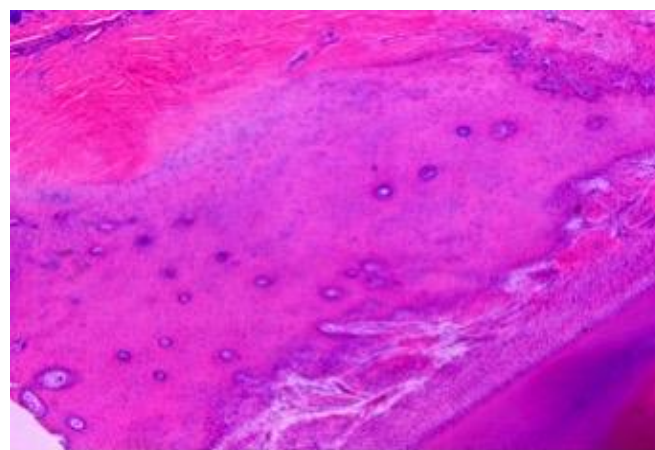


Fig. 4. Periodontal tissue of a rat 8 weeks after opioid injection. Stained with hematoxylin and eosin.

Magnified: x100.

Conclusions. Opioid analgesic effect in small doses during 8 weeks resulted in diffuse injury of the periodontal tissue on the microstructural level. Intensification of desquamation process was indicative of the integrity damage of the epithelial barrier and at the same time, it is one of the signs of protective reaction of the oral mucosa. As a result, pronounced thinning off the epithelium and deterioration of blood supply of the proper lamina promoted slow gingival atrophy. The signs of fibrinoid swelling with incomplete opening of a reserved group of capillaries, disturbed permeability of the blood vessels and rheological blood properties in the connective tissue against the ground of development of resorptive process in the osseous tissue Як наслідок, виражене стоншення епітелію та погіршення кровопостачання власної пластинки обумовлювало уповільнену атрофію ясен. Ознаки фібриноїдного набрякання з неповним розкриттям резервної групи капілярів, порушення проникності судин і реологічних властивостей крові у сполучній тканині, на фоні розвитку , were indicative of progressing of dystrophic-inflammatory changes in the periodontal tissues.

Prospects of further studies. The determined pathomorphological changes can be used for differential characteristics of morphological signs in the periodontal tissues with increasing dose of opioid analgesic effect in small doses at different terms. The results obtained can be used in future to initiate possible correcting influence in case of development of simulated generalized periodontitis occurring due to chronic opioid effect.

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