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PECULIARITIES OF MORPHOLOGICAL MANIFESTATION OF THE PERIODONTAL TISSUE IN EXPERIMENTAL ANIMALS AGAINST THE GROUND OF A SHORT-TERM EFFECT OF OPIOID ANALGESIC

Abstract. *Pathomorphological changes in the periodontal tissues on early terms of opioid effect in experiment have been studied. 16 mature outbred male rats with the body weight of 200 g and age of 4,5 months were used as the material of the study. The animals were subjected to opioid analgesic injections i/m every day once a day during 14 days. The initial dose of Nalbuphine was 0,212 mg/kg during the first week and 0,225 mg/kg during the second week. Thus, conditions of chronic opioid effect were created. Amputated upper and exarticulated lower jaws were used for microstructural examination. Histological specimens were prepared with preliminary made decalcination using hematoxylin, eosin and azan by Heidenhain's method. The results of the study obtained were indicative of changes available possessing an inflammatory character and present in all the soft periodontal tissues, although within the borders of the hard tissues those changes were not considerable without visible signs of pathologic lesion.*

Key words: *periodontium, opioid, microscopic examination, rats.*

Introduction. A long-term and not always controlled administration of psychotropic drugs, opioid analgesics, tranquilizers, certain stimulators and pain relieving medicines caused a problem of pharmacological addiction similar to that of drug addiction [18]. According to the results of the studies opioid-addicted individuals in addition to severe changes of the internal organs present considerable lesions of the dentoalveolar system, which complicates to make differential diagnostics and treatment of oral pathology including diseases of the periodontium. Therefore this problem is not only of a medical but social importance as well [2-4, 6-8, 11, 13, 16, 19]. Dental pathology in drug addicted persons is not studied considerably on clinical material, and thus it requires special scientific studies including experimental ones [4, 10].

The published literary sources present data concerning experimental reproduction of pathological changes in the periodontal tissues of laboratory rats with modeling acute and chronic morphine intoxication using high doses of morphine hydrochloride – from 10 to 30 mg/kg of the body weight [1, 4]. There are also findings concerning general influence of drug pathology in rats with modeling of morphine intoxication injecting maximal doses from 5 to 70 mg/kg of the

body weight [5, 12, 17].

Although, the literary sources available do not present data concerning peculiarities of morphological periodontal changes in experimental animals against the ground of a gradual increase of small doses of an opioid analgesic.

Objective: to study the dynamics of morphological changes in the periodontal tissues on early terms of opioid effect.

Materials and methods. 16 mature outbred male rats with the body weight of 200 g and age of 4,5 months were used as the material of the study. The animals were subjected to opioid analgesic injections i/m every day once a day at the same period of time (10-11 a.m.) during 14 days. The initial dose of Nalbuphine was 0,212 mg/kg during the first week and 0,225 mg/kg during the second week. Thus, conditions of chronic opioid effect were created [14].

The animals were distributed into two groups. The 1st group of animals received Nalbuphine during 14 days with the following material sampling (the end of the 2nd week of experimental opioid effect); the 2nd group, as the control one, received injections of physiological solution i/m at the same period of time (10-11 a.m.) during 14 days. All the animals were kept under conditions

of vivarium, and the work concerned the issues of their keeping, care, labeling and other manipulations were conducted according to the regulations of "European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], "General Ethical Principles of Experiments on Animals" approved by the First National Congress in Bioethics [Kyiv, 2001], the Law of Ukraine № 3447 – IV «On Protection of Animals against Cruel Treatment». The Bioethics Committee of Danylo Halytskyi Lviv National Medical University found that conducted scientific studies correspond to ethical 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 the material was taken for biopsy examination the animals were put to sleep by means of intra-abdominal injection of thiopental (25 mg/1 kg). Amputated upper and exarticulated lower jaws were used for microstructural examination considering further preservation of topographic ratio of the dental organ making histological sections 5-7 mcm thick. Histological specimens were prepared according to common method with preliminary made decalcination [15] using hematoxylin, eosin and azan by Heidenhain's method [9]. Microscopic examinations and photos of specimens were made by means of the microscope Meiji MT4300 LED and digital camera Canon EOS 550D.

Results and discussion. Morphological changes found at the end of the 2nd week of the experiment appeared to be moderately pronounced in the soft tissues and practically absent in the hard tissues of the periodontal tissues. The changes found were of inflammatory character peculiar for catarrhal gingivitis. Inflammatory signs were found in the layer of the gingival epithelium manifested by hyperkeratosis of the stratified squamous keratinous epithelium of the oral portion of the free gingival part. Epithelial buds are of mainly common size, a round shape with signs of inconsiderable acanthosis, manifested by proliferation of the epithelial layer into the depth of the connective tissue of its proper plate presented on Fig. 1, 2.

There are signs of a moderate swelling and inconsiderable polymorphic-cellular infiltrate in the proper plate of the mucous membrane illustrated on Fig. 1, 2, 3, 4. Small aggregations of lymphocytes and single neutrophils, numerous

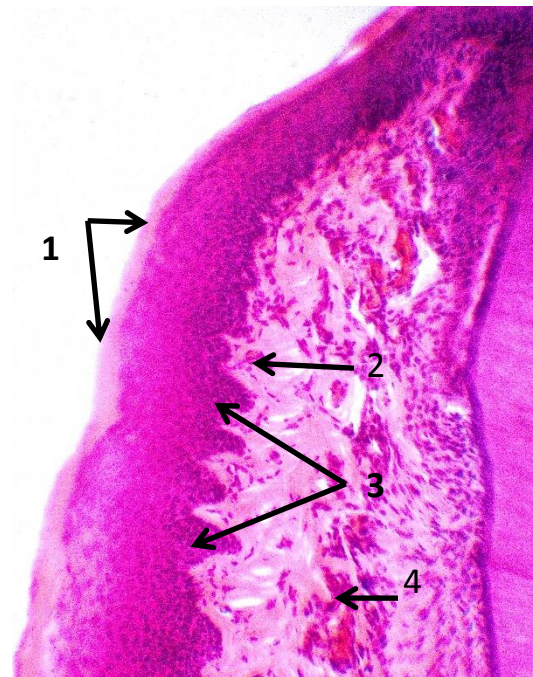


Fig. 1. 2nd week, the 1st group (hematoxylin and eosin, x 200). 1 – hyperkeratosis of the epithelium in the free gingival part; 2 – swelling of the proper plate of the gingival mucosa with moderately pronounced polymorphic-cellular infiltrate; 3 – inconsiderable acanthosis of the epithelium; 4 – blood filling of hemomicrocirculatory flow.

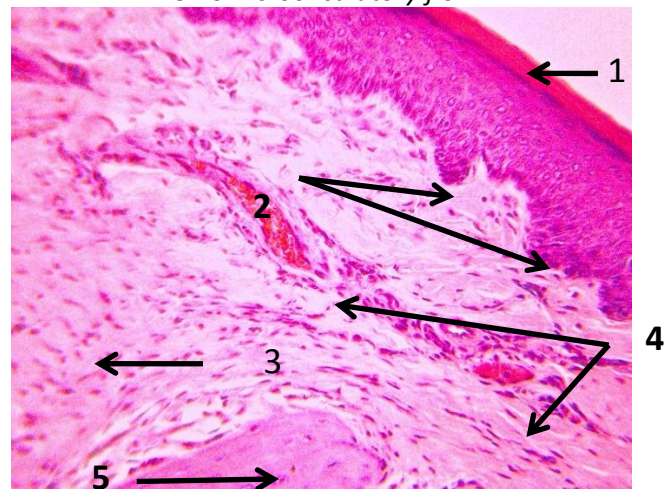


Fig. 2. 2nd week, the 1st group (hematoxylin and eosin, x 400). 1 – hyperkeratosis of the stratified squamous keratinous epithelium; 2 – round epithelial buds; 3 – moderate swelling of the gingival mucosa proper plate; 4 – vessels of the hemomicrocirculatory flow filled with blood; 5 – signs of mineralization disorders in the apical place of intercellular septum

fibroblasts and fibrocytes are visualized as it is presented on Fig. 5.

Granulation tissue appears in the gingival tissue from the vestibular site at the end of the 2nd week of opioid effect as it is seen on Fig. 4. The vessels of the hmeomicrocirculatory flow were filled with blood, which resulted from hypoxia.

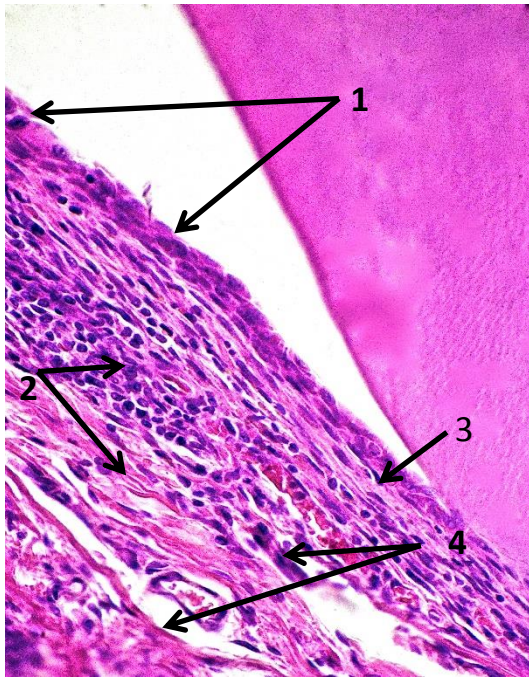


Fig. 3. 2nd week, the 1st group (hematoxylin and eosin, x400). 1 – thinning of the stratified squamous keratinous epithelium of the dentogingival furrow; 2 – moderate swelling with signs of polymorphic-cellular infiltration; 3 – preserved intact epithelial attachment; 4 – hyperemia of the hemomicrocirculatory flow.

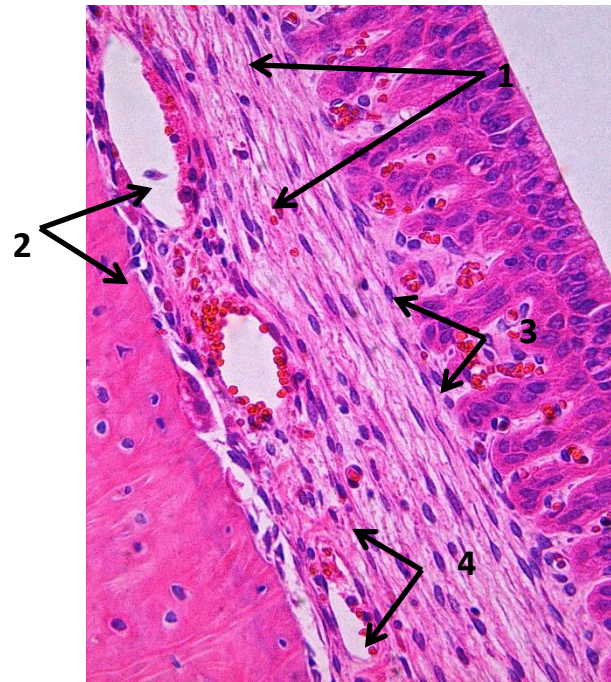


Fig. 5. 2nd week, the 1st group (hematoxylin and eosin, x 400). 1 – acanthotic teniae of the epithelium on the dentogingival furrow; 2 – inconsiderable aggregations of lymphocytes and single neutrophils in the proper plate of the mucous membrane; 3 – hyperemia signs in the links of hemomicrocirculatory flow in the place of the dentogingival furrow; 4 – numerous fibroblasts and fibrocytes.

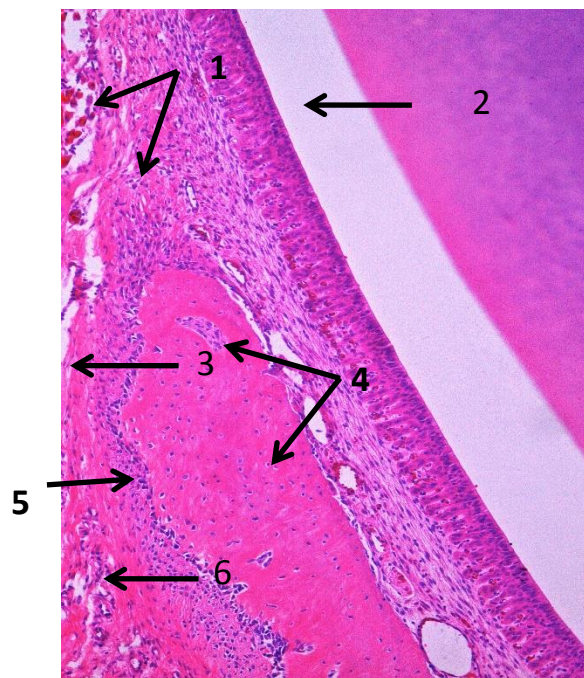


Fig. 4. 2nd week, the 1st group (hematoxylin and eosin, x 200). 1 – moderate swelling of the proper plate of the gingival mucosa; 2 – dentogingival furrow; 3 – granulation tissue in the gums from the vestibular site; 4 – numerous lymphoangiectasias of the proper plate of the gingival mucosa; 5 – apex of the cellular process of the jaw without changes; 6 – pronounced proliferation of osteoblasts.

Moreover, numerous lymphoangiectasias were found as it is illustrated on Fig. 1, 2, 3, 4, 5.

The lumen of the dentogingival furrow is clearly visualized as it is seen on Fig.3 and 4. A characteristic feature for the experimental animals of this group was thinning of the stratified squamous keratinous epithelium and regular outlines of the epithelial attachment with preserved integrity in the place of the dentogingival furrow as it is illustrated on Fig.3. In half of the cases the place of epithelial attachment coincides with the level of the enamel-cement border (within the norm in 75% higher than this level). The signs of hyperemia were found in the portions of hemomicrocirculatory flow, between acanthotic teniae of the stratified squamous keratinous epithelium of the dentogingival furrow as it is illustrated on Fig.4 and 5.

Epithelial buds in the area of the furrow are rather high with signs of pronounced acanthosis, which is atypical for this area, since within the norm bud are absolutely absent or considerably lower than in other areas of the gums. These changes are characteristic signs of primary inflammatory-destructive signs occurring in the

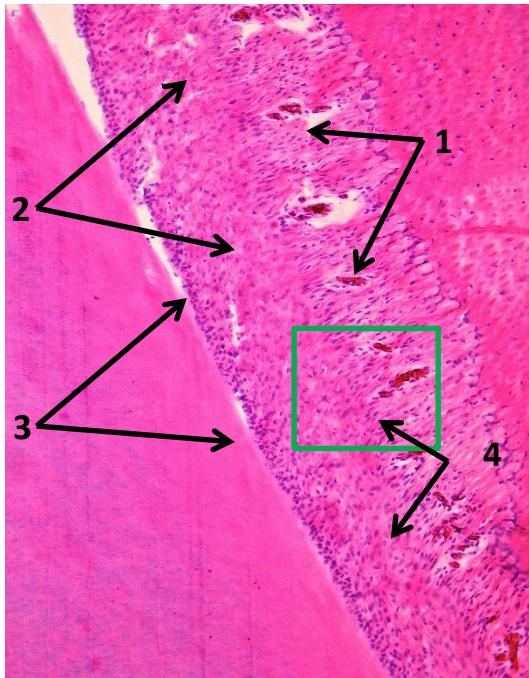


Fig. 6. 2nd week, the 1st group (hematoxylin and eosin, x 200). 1 – area of fixation of the collagen fibers of the periosteal layer into the compact plate of the cellular process of the jaw; 2 – angiomas with signs of erythrocyte aggregation; 3 – signs of fibroblast cytotis and swelling of fibers of the periradical periodontal layer; 4 – hyperemia of the links of hemomicrocirculatory flow of the periodontium.

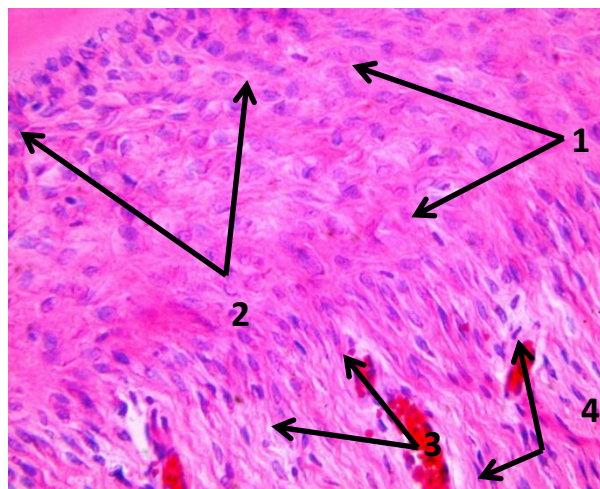


Fig. 7. Fragment of Fig. 6. 2nd week (hematoxylin and eosin, x400) 1 – more "cytosoled" periradical periodontal layer at the expense of fibroblasts; 2 – mitosis in fibroblasts; 3 – more fibrous periosteal periodontal layer at the expense of fibrocytes and collagen fibers; 4 – hyperemia with aggregation of erythrocytes in hemomicrocirculatory flow of the periodontium.

soft periodontal tissues under the action of small doses of opioid analgesic as it is illustrated on Fig. 4 and 5. The periodontal tissue at this term of opioid effect was characterized by available

longitudinal and transverse oriented fibers of the connective tissue. Thus, the fibers of the periosteal layer were transverse, the fiber of the periradical layer – longitudinal. At the same time, the periosteal periodontal layer was more fibrous and presented by fibrocytes and collagen fibers of I type. In its turn, the periradical layer was more "cytosoled" at the expense of fibroblast mitosis as it is seen on Fig.6 and 7.

Collagen fibers of the periosteal periodontal layer are atypically combined with the compact plate of the cellular process of the jaw in the form of "cellular line" as it is demonstrated on Fig. 6 and 7. Within the borders of loose sufficiently vascularized connective periodontal tissue hyperemic vessels were found with erythrocyte aggregation, which was indicative of exertion of the links of the hemomicrocirculatory flow as it is seen on Fig. 6 and 7. The osseous tissue of the cellular process of the jaw was evenly mineralized as it is shown on Fig. 4, 5 and 6, with available single areas with signs of mild mineralization. It was mainly found in the area of the apical intercellular septum as it is illustrated on Fig. 2.

Conclusions. Pathomorphological changes found at the end of the 2nd week of the experiment in case of a short-term effect of opioid appeared to be inflammatory and moderately pronounced in the soft tissues and practically absent in the hard tissues of the periodontal tissues.

Prospects of further studies. The conducted studies concerning opioid effect during 14 days will enable to observe gradually the appearance of pathomorphological changes in the dynamics and their growth at later terms. In the long term possessing the clinical manifestation and growth of pathomorphological changes in the periodontal tissues will enable to determine correcting effect at early and late terms of opioid impact.

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