Electronic microscopic research on periodont in experimental two-weight opioid action and after its over for four weeks

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Introduction

The tissues and organs of the oral cavity are especially responsive to any harmful stimuli in the body [9, 18, 26]. Under the influence of factors of exogenous origin disturbances of gums resistance arise, which causes pathological changes in periodontium tissues [14, 19]. It should be noted that periodontium diseases and gums bleeding are more common in drug abusers compared to the general population [10, 20, 21, 25]. Important are the issues of therapeutic tactics of inflammatory periodontal diseases in drug addicts, as well as the abolition of the drug, which is necessary for both local and general action on the tissues of the periodontal complex [5, 6, 16, 21, 23]. However, the mapping of the pathomorphological pattern in periodontal tissues is a rather complicated process and
changes in this case occur over a period of time with the involvement of different mechanisms of pathogenesis [3, 7]. Therefore, experimental studies related to the study of the ultrastructural organization of the periodontium and the correction of pathological changes that occur during the action of opioids are particularly important today and will help to develop an adequate scheme of corrective effects to stabilize the morphofunctional condition of periodontium tissues.

The aim of the study was to determine the features of the submicroscopic organization of the structural components of the periodontium under the action of an opioid analgesic for two weeks and its four-week withdrawal in the experiment.

Materials and methods
The study was conducted on 22 adult rats-males of the Wistar line, weighing 160 g, aged 4.5-6 months. Animals were divided into 2 groups. The first group included intact rats (n=10). The second group included 12 animals, which were administered intramuscularly opioid analgesic nalbuphine daily for the first two weeks, based on the mean therapeutic dose for the rat, and considering the mean weight of the experimental group (0.212 mg/kg) and its subsequent 4 weeks. Controls were 3 male rats administered intramuscularly saline. Sampling was performed after 6 weeks of the experiment.

All animals were kept under standard vivarium conditions and all experiments were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985). Before sampling the biopsy material, the animals were primed with intraperitoneal administration of sodium thiopental (25 mg/kg).

For electron microscopic examination, pieces of soft periodontal tissue were used in the gums papilla. The tissue fragments were fixed in a 2.5 % solution of glutaraldehyde and in a 1 % solution of osmium tetroxide on phosphate buffer pH = 7.2-7.4, dehydrated in alcohols and propylene oxide and poured into a mixture of epoxy resins with araldite [24]. Ultra-thin sections were made on a UMPT3m ultramicrotome, which was counterstained with uranyl acetate and lead citrate and studied in a PEM-100-01 electron microscope.

Results
Electron microscopic studies have shown that the structural changes in the epithelium of the different sites studied were similar in nature, but not as significant as in the subgroup of animals without opioid withdrawal. The cells of the basal layer of the epithelial plate of its free part have an enlarged nucleus area, the karyoplasm of which includes the osmiophilic regions of heterochromatin, but they are located mainly near the nuclear envelope. Small, compact nucleolus are available. The karyolemma has shallow invaginations, and its perinuclear spaces are enlarged only in some places. In the cytoplasm there is a normalization of organelles, damaged ultrastructures are small in number. Part of the mitochondria has a focal electron-light matrix and partially damaged cristae. In the hyaloplasm, areas with poorly contoured tonofilaments were detected, some of them homogenized. Thick areas with fuzzy intercellular contacts (Fig. 1) are noted between plasmalemmas. The epitheliocytes of the spinosum and granulosum layers are also less altered, the structure of the nucleus and cytoplasm normalizing.

The nuclei have a shallow invagination of the karyolemma, euchromatin predominates in the karyoplasm. There are different sizes of cells in the granular layer, sometimes large clumps of keratohyalin. The desmosomal contacts are sometimes damaged. The
Ultrastructural organization of the gums epithelium of this group is also unchanged. There is no swelling of the cytoplasm of the epitheliocytes of the basal and spinosum layers. Only the nuclei of the superficial layers of cells have karyolemma invaginations, and the cytoplasm is virtually unchanged. Clear contours of plasmolemma and desmosomal contacts (Fig. 2). Submicroscopically, the epithelium of the attached part of the gums of the animals is found to be unaltered. Epitheliocytes include the oblong-shaped nucleus with shallow invasions of the karyolemma, and euchromatin is noted in the karyoplasm. In the cytoplasm, there are small mitochondria, a few cristae. The clear contours of the plasmolemma, the intercellular contacts are osmiophilic, appear enlarged (Fig. 3).

Electron microscopic studies of the periodontium of animals have found that its structural components are unchanged. Collagen fibers are characterized by a tufted arrangement of fibrils, only in the superficial area they are partially stratified and moderate swelling of the amorphous component of the intercellular substance of the connective tissue is present. Fibroblasts of the usual structure, and some fibrocytes have irregular shape with invaginations of karyolemma of the nucleus and placement of heterochromatin on the periphery. The cytoplasm contains ribosomes, enlarged tubules of the granular endoplasmic reticulum, part of the mitochondria with the enlightened matrix (Fig. 4). Lymphocytes, neutrophils and macrophages are observed in connective tissue surrounding the periodontium. Ultrastructurally, the cytoplasm of the macrophage reveals lysosomes, a little phagosome, indicating little damage to the structures. Plasmolemma forms protrusion in the form of outgrowths and has the invaginations required for phagocytosis of damaged structures (Fig. 5).

Submicroscopic studies of the mucous membrane of the rats gums of this group found that moderate reactive changes are characteristic of the hemomicrocirculatory bed of the gums. The lumps of the blood capillaries are small, in them the formed elements of blood, mainly erythrocytes are found. The nucleus and cytoplasm of endothelial cells have their own structural organization. In the wide cytoplasmic areas of the cells, there are many foam pinocytosis vesicles, caveolae.

In the perinuclear part of the cytoplasm organelles are small in number. The tubules of the endoplasmic reticulum are moderately dilated, mitochondria with electron-light matrix and small cristae. The elongated nucleus is well contoured, the perinuclear spaces are small, and...
Discussion

In the modern professional literature there are more and more studies devoted to the study of the effect of opioid drugs on various organs and systems [1, 8, 12, 17, 22], but their effect on the structural organization of the oral cavity and periodontium remains unknown [6, 10, 20, 21]. Scientific research is mainly aimed at studying the changes in the internal organs that develop as a result of the introduction of such classic opioids as morphine, tramadol and codeine using significantly high doses [4, 5, 8, 15]. In addition, studies on the effects of the opioid analgesic nalbuphine on tissues and organs of the oral cavity are virtually absent in the literature. Preferably, such studies have been performed to study the characteristics of pharmacodynamics and pharmacokinetics both in experiment and in volunteers [2, 11, 13].

As a result of our studies of the experimental effect of opioid for two weeks and its 4-week cancellation, we found no significant destructive changes in periodontium. However, the positive dynamics of regeneration of periodontal components at the ultrastructural level were also not observed. Submicroscopically, there are reactive changes, which are manifested by focal sites of expansion of perinuclear spaces, fuzzy intercellular contacts, partial damage of the cristae in the mitochondria of epitheliocytes and endothelial cells, as well as swelling of the intercellular substance and minor constituents of periodontium.

In the available medical literature there are no results of studies of the ultrastructural organization of periodontal tissues in dynamics at different terms of opioid exposure and after its abolition, which makes it impossible to perform a comparative analysis of the data obtained. Given the relevance of this problem, the study of periodontal tissues in drug addicts is insufficient and requires further scientific research [5-7, 16, 21, 25]. Therefore, we believe that the method of creating a biological experimental model of opioid exposure will help to find out the peculiarities of submicroscopic organization in periodontal tissues, which is relevant and necessary in modern dentistry and periodontology.

Prospects for further research are to study the features of ultrastructural reorganization in periodontal tissues when exposed to opioid, after its abolition, and at the application of medical correction at an early date.

Conclusions

1. At the end of week 6 of the experiment, no irreversible changes in the ultrastructural organization of periodontal components were detected in the short-term effect of the opioid for two weeks and its subsequent cancellation of.

2. Submicroscopically, it was found that the abolition of opioid analgesic at longer periods (3-6 weeks) does not lead to complete restoration of the structural components of the periodontium, regenerative processes are slow, there are signs of reactive changes.

euchromatin prevails in the karyoplasm. The basal membrane is wide, wavy and has clear contours. Perivascular edema is insignificant; collagen fibers and an amorphous component are present in the loose connective tissue of the advent (Fig. 6). Venules have moderately blood-filled lumps, the cytoplasm of endothelial cells is preserved, however, here and there destructively altered mitochondria are present. Separate areas of the cytoplasm protrude into the lumen, which increases the area of interaction of endothelial cells with the internal contents of the vessel. The basal membrane is thickened, but is well contoured, the perivascular spaces are slightly enlarged (Fig. 7).
Зважаючи на шкідливий вплив опіоїдних речовин при неконтрольованому їх вживанні, неможливо легковажити ранніми

**References**


В работе исследованы особенности субмикроскопической организации структурных компонентов пародонта при действии опиоидного анальгетика в течение двух недель и после его 4-недельной отмены в эксперименте. Исследование проведено на 22 половозрелых крысах-самцах линии Wistar, массой 160 г, в возрасте 4,5-6 месяцев. Животным в течение первых двух недель вводили ежедневно внутримышечно однократно опиоидный анальгетик налбуфин в перерасчете средней терапевтической дозы для крысы, а также с учетом среднего веса подопытной группы (0,212 мг/кг) и его последующей отменой в течение следующих 4 недель. Для электронно-микроскопического исследования использовали фрагменты мягких тканей пародонта. Субмикроскопически выраженных деструктивных изменений в тканях пародонта выявлено не было. Положительной динамики регенерации компонентов пародонта на ультраструктурном уровне также не выявлено. В цитоплазме клеток эпителия свободной части десны наблюдали деструкцию органелл, частично поврежденные кристы митохондрий, плохо контурировались тонофиламенты, установлены неглубокие инвагинации кариялеммы, между плазмolemмами наблюдали утолщенные участки и поврежденные десмосомальные контакты. В поверхностных участках периодонта коллагеновые волокна были частично расслоенными, наблюдали умеренный отек межклеточного вещества соединительной ткани, у части фиброцитов установлены инвагинации кариялеммы и наличие гетерохроматина на периферии. Ультраструктурно в цитоплазме макрофага отмечены лизосомы, фагосом немного, что свидетельствует о незначительном повреждении структур. В просветах кровеносных капилляров выявляются фармакологические изменения, преимущественно эритроциты, в перинуклеарной части цитоплазмы органелл немного, митохондрий с электронно-светлым матриксом и небольшими кристами, периваскулярный отек незначительный, в цитоплазме эндотелиоцитов венул наблюдается некоторое повышение плотности митохондрий, базальная мембрана утолщена, периваскулярные пространства увеличены. Таким образом, в конце 6 недели эксперимента при кратковременном действии опиоида в течение двух недель и его дальнейшей 4-недельной отмены, глубоких необратимых изменений ультраструктурной организации компонентов пародонта не наблюдалось, в наличии яркие признаки реактивных изменений и замедленные репаративные процессы.

Ключевые слова: пародонт, опиоид, отмена опиоида, ультраструктура, эксперимент.