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Реферати

МОРФОГЕНЕЗ ЩИТОПОДІБНОЇ ЗАЛОЗИ ЩУРІВ МОЛОЧНОГО ПЕРІОДУ ПІСЛЯ ПРЕНАТАЛЬНОЇ ДІЇ СТАФІЛОКОКОВОГО АНАТОКСИНУ

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Одержано результати щодо темпів морфогенезу щитоподібної залози після внутрішньоплідної антигенної дії стафілококового анатоксину. Пренатальне введення стафілококового анатоксину призвело до формування більш вираженого структуроутворення елементів паренхіми і стромы, але вони мали ознаки функціональної незрілості, що призвело до наявності морфологічної картини гіпотиреозу вже після народження (щури 1-7 доби постнатального онтогенезу). З початком середнього молочного періоду (7-21 доба життя) з'являється лімфоцитарна інфільтрація в окремих ділянках щитоподібної залози, відбувається пребудова синтетичного апарату та процесу резорбції колоїду, тобто починається функціональне «дозрівання» вже морфологічно сформованих структур. Такі скачкоподібні зміни в щитоподібній залозі експериментальних тварин обумовлені системним пренатальним антигенним навантаженням організму в цілому та носить пристосувально-компенсаторний характер.

Ключові слова: щитоподібна залоза, антиген, стафілококовий анатоксин, морфогенез, експеримент.

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МОРФОГЕНЕЗ ЩИТОВИДНОЇ ЖЕЛЕЗЫ КРЫС МОЛОЧНОГО ПЕРИОДА ПОСЛЕ ПРЕНАТАЛЬНОГО ДЕЙСТВИЯ СТАФИЛОКОКОВОГО АНАТОКСИНА

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Получены результаты о темпах морфогенеза щитовидной железы после внутриплодного антигенного воздействия стафилококкового анатоксина. Пренатальное введение стафилококкового анатоксина привело к формированию более выраженного структурообразования элементов паренхимы и стромы, которые имели признаки функциональной незрелости, что привело к наличию морфологической картины гипотиреоза уже после рождения (крысы 1-7 суток постнатального онтогенеза). С началом среднего молочного периода (7-21 сутки жизни) появляется лимфоцитарная инфильтрация в отдельных участках щитовидной железы, происходит перестройка синтетического аппарата и процесса резорбции коллоида, то есть начинается функциональное «созревание» уже морфологически сформированных структур. Такие скачкообразные изменения в щитовидной железе экспериментальных животных обусловлены системным пренатальным антигенным воздействием на организм в целом, и носит приспособительные-компенсаторный характер.

Ключевые слова: щитовидная железа, антиген, стафилококковый анатоксин, морфогенез, эксперимент.

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ULTRASTRUCTURAL ORGANIZATION FEATURES OF PERIODONTAL TISSUES AFTER TWELVE WEEKS OF OPIOID INFLUENCE

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In the experiment on white rats, ultrastructural studies of periodontal tissues were carried out against the background of twelve-weeks opioid action. Animals were subjected to intramuscular injections of an opioid analgesic with a gradual increase in the mean single dose every two weeks. The initial dose was 0.212 mg/kg, and within 11–12 weeks the dose was increased up to 0.3 mg/kg. The results of submicroscopic studies have revealed the progression of chronic sclerosing inflammation in the periodontium and the development of regenerative-plastic deficiency of epitheliocytes, endothelial cells and the periodontium structural components, which was caused by a long-term opioid action.

Key words: periodontium, rat, opioid, ultrastructure.

The work is a fragment of the research project “Morpho-functional features of organs in the pre – and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity”, state registration No. 0120U002129.

Periodontal diseases of a dystrophic-inflammatory nature are quite widespread in practically most age groups with more than 75% of the population affected worldwide, which is an important socio-medical problem [1, 3, 6, 12, 13]. In particular, a number of researchers consider generalized periodontitis a polyetiological disease with various mechanisms of pathogenesis [5, 6, 13]. In numerous disorders of metabolism that cause the development of morphological manifestations in periodontal tissues, complications occurring under the influence of narcotic intoxication play a significant role [4, 8]. It should be noted that the pathology of organs and tissues in the oral cavity of opioid-dependent persons has not been sufficiently

studied, which prompts the need for scientific research, including experimental models [9, 14, 15]. Due to the ethical aspects of human material use, animal models are widely applied in experimental medicine, so that the necessary information can be obtained and analysis of the tissues and organs structural organization can be performed with a gradual worsening of the pathological process [10, 7, 11, 14].

However, questions about the beginning of the pathomorphological changes development in the structural components of the periodontal complex, the dynamics of the opioid analgesic effect assuming the mean single therapeutic dose on the worsening of these changes in terms of hemodynamic disorders, as well as on the emergence of oral cavity pathogenic microflora against the background of the prolonged opioid action. In future, this will permit to solve the problem of figuring out the scheme for these changes correction at the early and late stages of chronic opioid exposure.

The purpose of the work was to study the features of periodontal tissues ultrastructural organization against the background of chronic exposure to opioid analgesics for twelve weeks under experimental conditions.

Materials and methods. The studies were performed on 22 white adult 4.5 – 7.5 months old Wistar male rats weighing 160 – 270 g. In the experiment, the animals were divided into two groups. The first group was intact rats (10). In the second group, animals (12) were daily administered nalbuphine opioid analgesic intramuscularly, belonging to the group of opiate receptor antagonist agonists, for 84 days. The starting dose was 0.212 mg/kg. During the 11 and 12 weeks, the mean single therapeutic dose was increased up to 0.3 mg/kg, taking into account the mean weight of the study group. Controls were 3 rats, intramuscularly injected with normal saline. The experimental animals were kept under vivarium conditions and the material was collected according to generally accepted rules. Before sampling, the animals were euthanized by intraperitoneal administration of sodium thiopental (25 mg/kg). For ultrastructural examination, pieces of soft periodontal tissue were used in the area of the maxillary and mandibular gingival papilla. The obtained tissue fragments were immediately placed for fixation into 2.5% solution of glutaraldehyde and into 1% solution of osmium tetroxide in phosphate buffer with pH 7.2 – 7.4. Subsequently, dehydration of tissue fragments in alcohols and propylene oxide was carried out and the samples were embedded into a mixture of epoxy resins with araldite [2]. Ultra-thin sections were made using the UMTP3m ultramicrotome, followed by contrasting with uranyl acetate and lead citrate and studied in detail with PEM-100-01 electron microscope.

Results of the study and their discussion. Electron microscopic studies have shown that in the control group rats, the ultrastructural organization of periodontal tissues was preserved. The multilayered flat epithelium of the gingival mucosa had the usual layered structure of cells, nuclei were round shaped, nucleoli were dense, organelles were present. The periodontium components were clearly structured, intercellular substance interlayers, collagen fibers, fibroblasts, fibrocytes were visualized. Microcirculatory bed vessels had a well-defined structure of the endothelium and the basement membrane. Submicroscopic examination of the animal gums mucous membrane with long-term administration of opioid analgesics for twelve weeks revealed profound changes in all its cellular components.

In the basal layer of the epithelium in the spare part of gums, most of the epitheliocyte nuclei have uneven contours, karyolemma forms deep invaginations. Karyoplasm of electron light, nucleoli with segregated granular and fibrillar components. Intercellular contacts are damaged, reduced in some areas. Intercellular spaces are unevenly contoured and mostly enlarged, which is a sign of spongiosis. Cytoplasm of the spinous layer epitheliocytes includes diffusely arranged thickened bundles of tonofilaments. In the perinuclear zone, electron-lucent anhistic structures with formation of vacuole-like structures are present, indicating the development of partial necrosis. The karyolemma forms invaginations, nucleoli are segregated (fig. 1). In the cytoplasm of the granular layer epitheliocytes, many electron-dense keratogialin inclusions of different sizes are found.

Most nuclei of gingival crest epitheliocytes have uneven contours, the karyolemma forms invaginations. The karyoplasm is electron-lucent, nucleoli are absent. In the basal layer cells cytoplasm, most organelles are damaged. Mitochondria have reduced cristas, diffusely vacuolated. There are few tonofilaments, they are either fragmented or lysed. Intercellular contacts are indistinct, reduced; there are also unevenly enlarged intercellular spaces. In the spinous layer, the cytoplasm of the epitheliocytes includes many osmiophilic, irregularly shaped inclusions that were formed due to the increased keratinization.

In the attached part of the gums, significant changes in epitheliocytes, apoptosis phenomena, were found. In the nuclei there are signs of karyorexis and karyolysis, karyolemma is osmiophilic. Characteristic is the of subplasmolemmotic vacuolation of the cytoplasm and large-sized non-structural osmiophilic areas present in the epitheliocytes. Lysis and fragmentation of the tonofilaments, as well as the organelles destruction are caused by the progression of necrotic changes in the cell (fig. 2).

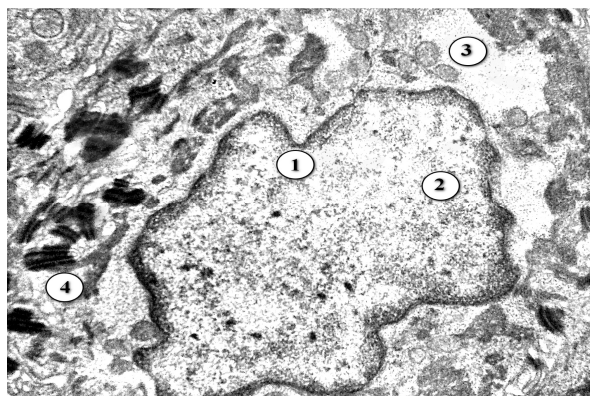


Fig. 1. The epitheliocyte ultrastructure in the epithelium spinous layer of the rat gingiva spare part after twelve weeks of opioid administration. 1 –karyolemma invaginations, 2 – nucleoli segregation, 3 – vacuole-like structures, 4 – tonofilament bundles. Electronogram. Approx. 12,000.

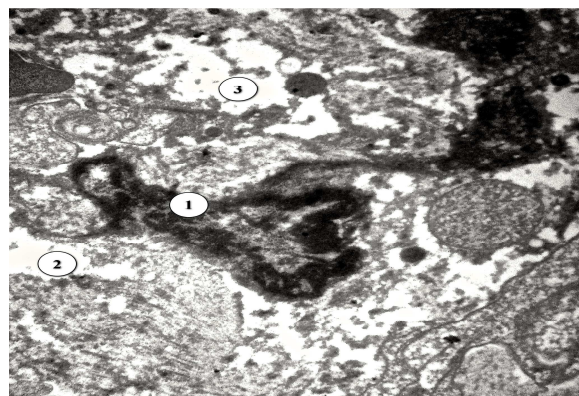


Fig. 2. Epithelium of the attached part of the rat gums after twelve weeks of opioid administration. 1 – karyolysis, 2 – vacuolation of the perinuclear zone, 3 – necrotic changes in the cytoplasm. Electronogram. Approx. 14,000.

Electron microscopic studies have also revealed pronounced changes in the structural components of the periodontium. The ultrastructure of fibroblasts, which showed signs of destruction in the nuclei, invagination of the karyolemma, is significantly disturbed. There are few organelles in the cytoplasm, they are destructively altered. Significant destruction of collagen fibers, their fragmentation and destruction have been found. There is a pronounced edema of the amorphous component of the intercellular substance of the connective tissue. The structure of fibrocytes is significantly altered, macrophages are activated and there are degranulated tissue basophils.

The performed ultrastructural studies have established profound changes in the vessels of the hemomicrocirculatory bed. During this period of experiment, the lumen of the blood capillaries is filled with blood cells, mainly erythrocytes, and there is a sludge effect. The of endotheliocyte nuclei are destructively altered, they are small, their pycnosis is present, with predominance of heterochromatin in the karyoplasm. The karyolemma produces deep invaginations. In the cytoplasm of endothelial cells, damaged organelles, a small number of pinocytotic vesicles were found. Sclerotic changes progress, as evidenced by the presence of collagen fibers in the perivascular space. The basement membrane is not distinctly contoured, it is thickened in some areas.



Fig. 3. Ultrastructure of the rat gingival mucosa venule after twelve weeks of opioid administration. 1 – wide lumen with erythrocytes, 2 – vacuole-like structures in the cytoplasm, 3 – bundles of tonofilaments. Approx. 9000.

Submicroscopically, dilated venule lumens with phenomena of congestion are observed in the late experimental period. In many endothelial cells, the nuclei are significantly altered, and the karyoplasm is osmophilic. The characteristic feature is numerous protrusions and focal lamination of the endothelial cell surface. In the cytoplasm, organelles are destructively altered, vacuole-like formations are of different size, phenomena of mitochondrial vacuolation are found, which is the first sign of cell autolysis. Perivascular spaces are significantly enlarged, the basement membrane is not distinctly contoured, and it is considerably thickened in some areas (fig. 3).

The experimental modeling results of long-term opioid exposure in rats correlate with the data of ultrastructural study in patients with gingivitis and generalized periodontitis [1, 4]. Twelve-weeks exposure to the opioid mediator revealed submicroscopically profound changes in the cellular components of the gingival epithelium, the periodontium connective tissue, and the components of the periodontium hemomicrocirculatory bed. With a long-term opioid effect, apoptosis phenomena occur, necrotic changes progress, vacuolation of the perinuclear zone is observed, indicating evidence of partial necrosis.

The obtained results also confirm the data on lesions of the hemomicrocirculatory bed components in the periodontium and accordingly the development of tissue ischemia and hypoxia, which is also one of the dominant signs in the periodontitis pathogenesis [5]. Since there are no results of studies on the periodontal cell components submicroscopic changes in dynamics at the late stages of experimental opioid exposure, it was not possible to compare them with the data of other researchers.

Conclusion

The performed ultrastructural study of soft periodontal tissues revealed the progression of chronic sclerosing inflammation and the development of regenerative-plastic deficiency of epitheliocytes, endotheliocytes and the periodontium structural components due to long-term opioid action.

Prospects for further research are to carry out a comparative analysis of pathomorphological changes in the periodontium at different times of opioid exposure and to develop a scheme of probable corrective effects.

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Реферати

**ОСОБЛИВОСТІ УЛЬТРАСТРУКТУРНОЇ
ОРГАНІЗАЦІЇ ТКАНИН ПАРОДОНТА
ЧЕРЕЗ ДВАНАДЦЯТЬ ТИЖНІВ ОПІОЇДНОГО
ВПЛИВУ**

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В експерименті проведено ультраструктурні дослідження пародонту при дії опіоїдів протягом дванадцяти тижнів. Тваринам проводили внутрішньом'язові ін'єкції опіоїдного анальгетика з підвищенням разової дози кожні 2 тижні. Початкова доза – 0,212 мг/кг, протягом 11 – 12 тижнів збільшили до 0,3 мг/кг. Одержані результати субмікроскопічних досліджень встановили прогресування хронічного склерозуючого запального процесу в пародонті і розвиток регенераторно-пластичної недостатності епітеліоцитів, ендотеліоцитів і структурних складових пародоннта, що обумовлено тривалою дією опіоїда.

Ключові слова: пародонт, щур, опіоїд, ультраструктура.

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**ОСОБЕННОСТИ УЛЬТРАСТРУКТУРНОЙ
ОРГАНИЗАЦИИ ТКАНЕЙ ПАРОДОНТА
ЧЕРЕЗ ДВАНАДЦАТЬ НЕДЕЛЬ ОПИОИДНОГО
ВЛИЯНИЯ**

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В эксперименте проведены ультраструктурные исследования пародонта при действия опииодов на протяжении двенадцати недель. Животным проводили внутримышечные инъекции опииодного анальгетика с повышением разовой дозы каждые 2 недели. Начальная доза – 0,212 мг/кг, в течение 11 – 12 недель увеличили до 0,3 мг/кг. Полученные результаты субмикроскопических исследований установили прогрессирование хронического склерозирующего воспалительного процесса в пародонте и развитие регенераторно-пластической недостаточности эпителиоцитов, эндотелиоцитов и структурных составляющих пародоннта, что обусловлено длительной действием опииода.

Ключевые слова: пародонт, крыса, опииод, ультраструктура

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