типу та при застосуванні N-ацетилцистеїну як антиоксиданта. Проведене дослідження показало, що можливе, ушкодження тонких механізмів синтезу та секреції передсердного натрійуретичного пептиду може бути причиною виникнення серцево-судинної патології за умов цукрового діабету. Застосуванням препаратів антиоксидантної дії було встановлено, що N-ацетилцистеїн частково зменшує набряк кардіоміоцитів, підвищує кількість передсердних гранул, що містять передсердний натрійуретичний пептид та стимулює автофагію. Базуючись на проведених ультраструктурних дослідженнях передсердних кардіоміоцитах при цукровому діабеті першого типу, встановлено, що ушкодження білкового синтезу та накопичення і трансформація аномальних білків відбувається елімінацією їх двома шляхах: через убіквитин-протеасомальну та автофаго-лізосомальну системи. Автофагосоми трапляються як з подвійною мембраною тобто новоутворені, так і з одинарною після контакту з лізосомами. Ці процеси здійснюються для підтримки гомеостазу у кардіоміоцитах та у міокарді в цілому. При порушенні цих систем можливий розвиток діабетичної кардіоміопатії.

Ключові слова: кардіоміоцити правого передсердя, цукровий діабет у щурів, автофагія

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типа и в условиях применении N-ацетилцистеїна, как антиоксиданта. Проведенное исследование показало, что возможное, повреждение тонких механизмов синтеза и секреции предсердного натрійуретического пептида может быть причиной развития сердечно-сосудистой патологии в условиях сахарного диабета. Применение препарата антиоксидантного действия показало, что N- ацетилцистеїн частично уменьшает отек кардиомиоцитов, повышает количество предсердных гранул, содержащих предсердний натрийуретический пептид и стимулирует автофагию. Основываясь на проведенных ультраструктурных исследованиях предсердних кардиомиоцитов при сахарном диабете первого типа, установлено, что повреждение белкового синтеза, накопление и трансформация аномальных белков осуществляется элиминацией их двумя путями: через убиквитин-протеасомальний и автофаго-лизосомальную системы. Автофагосоми обнаруживаются как с двойной мембраной, то есть новообразованные, так и с одинарной после контакта с лизосомами. Эти процессы осуществляются для поддержки гомеостаза у кардиомиоцитах и в миокарде в целом. При нарушение этих систем возможное развитие диабетической кардиомиопатии.

Ключевые слова: сахарный диабет 1 типа у крыс, кардиомиоциты правого предсердия, предсердные гранулы, автофагия.

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SUBMICROSCOPIC CHANGES IN PERIODONTIC TISSUES UNDER EXPERIMENTAL OPIOID ACTION WITHIN TEN WEEKS

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This paper presents the results of soft periodontal tissues sub-microscopic examination under the experimental effect of an opioid analgesic within ten weeks. The study was carried out on 22 male rats of reproductive age. The animals were administered opioid analgesic in multiple ascending dose from 0.212 mg / kg to 0.283 mg / kg. At the ultrastructural level, it has been established that the prolonged action of opioid for ten weeks leads to marked heterogeneity and reorganization of the periodontal cell components against the background of the chronic inflammatory process development, which is exacerbated by sclerotic changes.

Key words: electron microscopic examination, periodontal tissues, opioid analgesic, rats.

The work is a fragment of the research project "Morpho-functional features of organs in pre- and postnatal periods of ontogeny, under the influence of opioids, nutritional supplements, reconstructive operations and obesity", state registration No 0120U002129.

Opioid analgesics, due to their pronounced analgesic effect, are indispensable medicines in medical practice [2]. Conversely, long-term and not always controlled use of drugs causes formation of tolerance, physical and psychological symptoms of withdrawal, which often leads to overdose and death [7, 12]. The urgency of this issue is determined by the fact that the negative impact of psychoactive substances, in particular, opioids leads to the destruction of almost all organs and systems of the body, especially the mouth, periodontal tissues [3, 5, 8, 10]. However, many issues regarding structural changes in tissues and organs in the use of opioid drugs remain unresolved [11, 13]. It should be noted that comprehensive information on the features of the periodontal components structural organization is extremely important in view of the recent data on formation of a new model of periodontitis pathogenesis [1, 4, 6, 9, 15]. Given the above, we believe that the peresent study is necessary and relevant in terms of both experimental and practical dentistry and periodontology.

The purpose of the work was to study the depth and dynamics of the of submicroscopic changes growth in the periodontium after ten weeks of opioid exposure.

Materials and methods. The study was carried out on 22 white male rats of reproductive age, Wistar line, weighing 160 - 255 g, 4.5 - 7 months of age. The experimental animals were divided into 2

groups. The first group consisted of intact animals (10). In the second group, rats (12) were injected daily with a single intramuscular injection of the nalbuphine opioid analgesic for ten weeks. The starting dose was 0.212 mg / kg. In the second group, the animals were increased the dose up to 0.283 mg / kg over 9 and 10 weeks. According to the drug instructions, initially an average single therapeutic dose was used, which was increased during the experiment, depending on the mean body weight of rats. The animals were weighed once every two weeks. All adult rats were kept under vivarium conditions and the work on maintenance, care, and other manipulations was performed in compliance with provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes". Before sampling of the material, the animal was euthanized by intraperitoneal administration of sodium thiopental (25 mg / 1 kg). For electron microscopic examination, fragments of periodontal soft tissue were used in the area of the gum margin. The tissue pieces were fixed in 2.5% glutaraldehyde solution and in 1% osmium tetraoxide solution in the phosphate buffer with pH 7.2-7.4, dehydrated in alcohols and propylene oxide and embedded into a mixture of epoxy resins with araldite. Using UMPT3m ultramicrotome ultrathin sections were made and counterstained with uranyl acetate and lead citrate to be studied with PEM-100-01 electron microscope.

Results of the study and their discussion. Submicroscopically, it was established that the gums epithelium in the inact rats was characterized by the layered location of cells, in the cytoplasm organelles, nuclei with even contours and perinuclear space were visualized. Intercellular contacts were clearly traced. The periodontium consisted of collagen fiber bundles that were restricted by layers of loose connective tissue where fibroblasts, basophils, and lymphocytes were present. The gum hemocapillaries were of the somatic type, their wall was formed by endothelial cells and the basement membrane.

Electron microscopic studies of the animal gums, performed after ten weeks with the use of opioids, revealed pronounced submicroscopic changes in the structural components. In the epithelial plate of the gums' spare part, most cells of the basal layer exhibited karyorexis and nucleus segregation. Intercellular contacts were not clearly structured, having a look of osmophilic lumps, sometimes partially reduced. Plasmolemmas in part of sites were indistinct, intercellular spaces uneven, sometimes expanded, which may indicate the progression of the edema process (fig. 1). In the epitheliocytes' cytoplasm of the spinous layer, tonofilaments were with fuzzy contours, some of them sticking together and forming dense bundles.

The swollen mitochondria were round-shaped, had an electron-lucent matrix, and the cristas in them were significantly damaged. The granular layer epitheliocytes' cytoplasm contained a large number of electron-dense inclusions of keratogialin. In the gum epitheliocytes' nuclei numerous invaginations of karyolema were formed, signs of nucleoli segregation in the presence of electron-lucent karyoplasms were observed. In the cytoplasm of the basal layer cells, organelles were mostly damaged, mitochondria were vacuolated, with reduced crystas, tonofilaments were mostly fragmented. There were signs of acantholysis, where intercellular contacts were predominantly reduced. Intercellular spaces were significantly enlarged, which was an evidence of spongiosis.

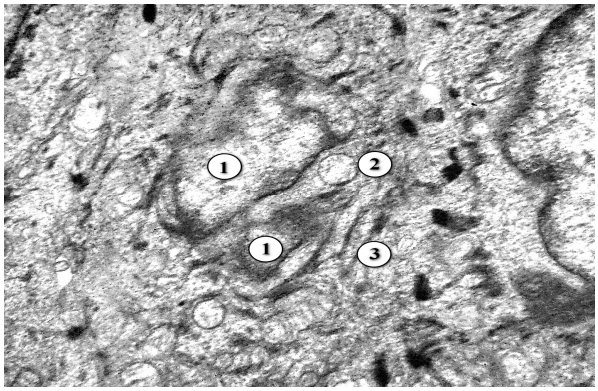

Fig. 1. Epitheliocyte of the basal layer in the spare part of the rat gingiva epithelium ten weeks after opioid administration. 1 - karyorexis, 2 - mitochondria, 3 - bundles of tonofilaments. Electronogram. Approx. 14,000.

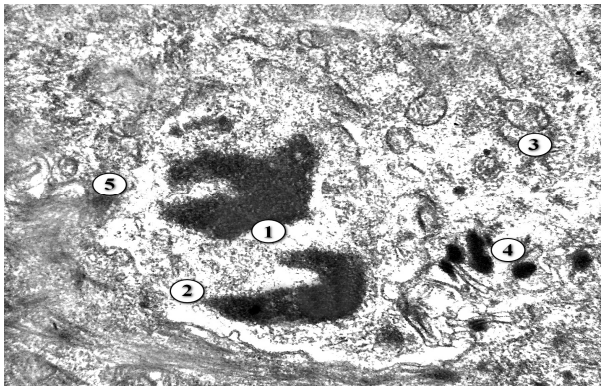

Fig. 2. The epithelium of the attached part of the rat gums after ten weeks with opioid use. 1 - karyorexis, 2 - electron-lucent area of the cytoplasm, 3 - mitochondria, 4 - lysosomes, 5 - lysis of tonofilaments. Electronogram. Approx. 14,000.

In separate epithelium cells of the attached rat gums part, signs of apoptosis, destruction of karyoplasm, karyorexis were revealed, manifested by osmiophilic fragments of the nucleus. In their cytoplasm there were electron-lucent structureless areas, lysosomes. Necrotic processes lead to the destruction of organelles, fragmentation and lysis of tonofilaments (fig. 2).

Submicroscopically, significant changes in its structural components were established in the periodontium. The ultrastructure of fibroblasts was disturbed.

Part of the cells had karyopicosis and karyorexis, deep invasions of karyolema divide the nucleus into fragments. Nuclear membranes were indistinct, perinuclear space was undetermined, karyoplasm was osmiophilic, where heterochromatin predominates. In the cytoplasm, organelles were damaged, vacuole-like structures and lysosomes were observed. Collagen fibers were destructively altered, there was swelling of the intercellular substance (fig. 3).

Electron microscopic studies of the animal gum mucous membrane had established pronounced changes in the vessels of the microcirculatory bed. In the lumen of the blood capillaries, the blood formed elements were visualized; in some areas a sludge-effect was observed. The nuclei of the endothelial cells changed significantly, there were signs of karyopicnosis, due to the deep invaginations of the karyolema, the nuclei had an irregular shape. Heterochromatin occupied a considerable area in their karyoplasm. The endothelial cells cytoplasm had thickened and narrow areas, they lacked organelles and pinocytic vesicles, vacuole-like structures were present. Basal membrane was uneven, fuzzy contoured. In perivascular space, coarse bundles of collagen fibers were observed, indicating the development of sclerotic changes (fig. 4).

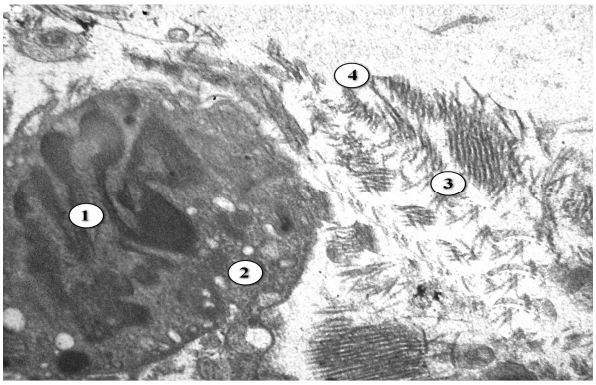

Fig. 3. Rat periodontium ten-weeks after the opioid administration. 1 - the phenomena of karyopicnosis and karyorexis of fibroblast, 2 - vacuolation of the fibroblast cytoplasm, 3 - damaged collagen fibers, 4 - swollen connective tissue. Electronogram. Approx. 12,000.

Fig. 4. Rat gums hemocapillary after ten weeks of opioid administration. 1 - lumen with erythrocytes, 2 - karyorexis, 3 - edema of the endotheliocyte cytoplasm, 4 - collagen fibers in the perivascular space. Electronogram. Approx. 12,000.

Ultrastructurally observed significantly expanded, blood-filled lumens of venules, sludge effect was observed. In part of endotheliocytes, nuclei were significantly altered; they had an elongated shape, a wavy karyolema,, and an osmiophilic karyoplasm. Destruction of organelles and the cytoplasm edema were noted. Separate areas of the endotheliocytes' adjunctive surface protrude into the lumen of the vessels. Uneven perinuclear space, different sizes vacuoles and electron-dense inclusions were observed in the cytoplasm. The basal membrane was thickened, poorly contoured, perivascular spaces were significantly enlarged.

The obtained data about the ultrastructural reorganization of periodontal tissues under the long-term opioid action permit to compare it to the results of the studies performed in patients with chronic gingivitis and the initial degree of generalized periodontitis. Researchers have found that during the inflammatory process in the periodontium patients developed signs of intracellular reorganization of epitheliocytes, impaired desmosomal contacts, showed intercellular edema [1, 14]. In patients with generalized periodontitis with concomitant arterial hypertension, the authors noted hydropic degeneration of epitheliocytes, foci of cell necrosis and the cytoplasm compaction [6].

In our observations, in addition to these changes, we also noted the reduction of intercellular contacts and the fragmentation or compaction of tonofilaments. In chronic cadmium intoxication, the researchers found pronounced changes in the organelles and nuclear apparatus of epitheliocytes [4]. The above data are compared to the data obtained by us under opioid exposure, where the destruction of organelles, segregation of the nuclei and invagination of the karyolema were observed, which showed irreversible changes in the structure of the nucleus and the cell as a whole.

In patients with gingivitis and periodontitis, the authors noted the obstruction of the microvessels, changes in the of endotheliocyte plasmolemma, the basement membrane integrity disruption [1, 3, 14]. With long-term opioid action, we observed in the endothelial cells thickening of the basement membrane, expansion of perivascular spaces and signs of a sludge effect. Necrotic processes in the endothelium under these conditions led to the destruction of organelles, fragmentation and lysis of tonofilaments, which testified to the signs of hypoxia development.

Conclusion

The ultrastructural study of soft periodontal tissues has established that prolonged opioid action within ten weeks leads to pronounced heterogeneity and reorganization of cellular components against the background of chronic inflammatory process, which is exacerbated by sclerotic changes.

The prospect of further research is to perform an ultrastructural study at later terms of opioid exposure in order to study in depth the dynamics of submicroscopic changes in soft periodontal tissues.

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Реферати

СУБМІКРОСКОПІЧНІ ЗМІНИ В ТКАНИНАХ ПАРОДОНТА ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ДІЇ ОПІОЇДІВ ПРОТЯГОМ ДЕСЯТИ ТИЖНІВ

Фік В.Б., Пальтов Є.В., Кривко Ю.Я.

В роботі представлено результати ультраструктурного дослідження м'яких тканин пародонту при експериментальному впливі опіоїдного анальгетика протягом десяти тижнів. При дослідженні використано 18 щурів-самців репродуктивного віку. Тваринам вводили опіоїдний анальгетик у зростаючих дозах від 0,212 мг / кг до 0,283 мг / кг. На ультраструктурному рівні було встановлено, що тривалий вплив опіоїдів протягом десяти тижнів призводила до вираженої гетерогенності і реорганізації клітинних компонентів пародонту на тлі розвитку хронічного запального процесу, який поглиблювався склеротичними змінами.

Ключові слова: електронномікроскопічні дослідження, тканини пародонту, опіоїдний анальгетик, щури.

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СУБМИКРОСКОПИЧЕСКИЕ ИЗМЕНЕНИЯ В ТКАНЯХ ПАРОДОНТА ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ВОЗДЕЙСТВИИ ОПИОИДА В ТЕЧЕНИЕ ДЕСЯТИ НЕДЕЛЬ

Фик В.Б., Пальтов Е.В., Крывко Ю.Я.

В работе представлены результаты ультраструктурного исследования мягких тканей пародонта при экспериментальном воздействии опиоидного анальгетика в течение десяти недель. При исследовании использовано 18 крыс-самцов репродуктивного возраста. Животным вводили опиоидный анальгетик в возрастающих дозах от 0,212 мг / кг до 0,283 мг / кг. На ультраструктурном уровне установлено, что длительное воздействие опиоида в течение десяти недель предопределяло прогрессирование выраженной гетерогенности и реорганизации клеточных компонентов пародонта на фоне развития хронического воспалительного процесса, который углублялся склеротическими изменениями.

Ключевые слова: электронномикроскопические исследования**,** ткани пародонта, опиоидный анальгетик, крысы.

Рецензент Єрошенко Г.А.