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Original research

Pathomorphological changes in the periodontal complex tissues in the period of experimental bacterial-immune periodontitis chronization

A. Ye. Demkovych, Yu. I. Bondarenko, T. K. Holovata

I. Horbachevsky Ternopil State Medical University, Ukraine

Objective – to investigate pathomorphological changes in the periodontal complex on the 30th day of the experimental bacterial-immune periodontitis development.

Materials and methods. The study was conducted with the use of non-breeding clinically healthy male rats. Experimental bacterial-immune periodontitis in experimental animals was induced by injection of complex microbial mixture diluted with ovalbumin into periodontal tissue. Morphological analysis was used for estimation of the structural changes degree in the maxillofacial tissues. The mandible-related tissue fragments, in particular the periodontal complex material was embedded in the paraffin blocks. The cross sections of 5–6 microns thickness were made on a microtome. The preparations obtained were stained with hematoxylin and eosin.

Results. The high intensity of inflammatory infiltration was noted, although the progression of inflammatory and destructive processes was significantly slowed down. The histological examination showed that inflammatory process was characterized by signs of disorganization and destruction of the connective tissue and walls of the dental alveoli, the structural reconstruction of the gingival epithelium and its lamina propria. Inflammatory infiltration was accompanied by expressive signs of the connective tissue disorganization. At the same time there were morphological signs of reparative processes, proliferation and collagen fibers thickening. In this period granulation tissue was formed. At this stage inflammatory infiltration extended the crista of the cellular bone and penetrated the epithelium of dento-gingival junction and gingival sulcus.

Conclusions. The revealed pathomorphological changes in the periodontal complex tissues in the long-term period of the experimental bacterial-immune periodontitis are indicative of the inflammatory process chronization.

Key words: periodontitis, inflammation, periodontium, cell proliferation, collagen.

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E-mail: demkovushae@tdmu.edu.ua

Патоморфологічні зміни у тканинах пародонтального комплексу в період хронізації експериментального бактеріально-імунного пародонтиту

А. Є. Демкович, Ю. І. Бондаренко, Т. К. Головата

Мета роботи – дослідити патоморфологічні зміни в пародонтальному комплексі на 30 добу розвитку експериментального бактеріально-імунного пародонтиту.

Матеріали та методи. Дослідження здійснили на білих безпородних клінічно здорових щурах-самцях. Експериментальний бактеріально-імунний пародонтит у дослідних тварин викликали шляхом ін'єкції у тканини пародонтального комплексу суміші мікроорганізмів, що розведена яєчним протеїном. Для оцінювання ступеня структурних змін у тканинах щелепно-лицевої ділянки застосували морфологічне дослідження. Фрагменти тканин нижньої щелепи, зокрема пародонтального комплексу заливали в парафінові блоки. На мікротомі виготовляли поперечні зрізи товщиною 5–6 мкм. Препарати, що одержали, забарвлювали гематоксиліном та еозином.

Результати. Гістологічне дослідження показало, що на цей період перебігу запального процесу відбувалась дезорганізація, деструкція сполучної тканини та стінок зубних альвеол, структурна перебудова епітеліальної вистилки ясен та її власної пластинки. Визначили високу інтенсивність запальної інфільтрації, однак прогресування запальних і деструктивних процесів істотно сповільнювалося. Запальна інфільтрація супроводжувалася виразними ознаками дезорганізації сполучної тканини. Виявили морфологічні ознаки репаративних процесів, відбувалася проліферація та потовщення колагенових волокон. Поряд із переформатуванням клітинного складу інфільтратів наростали патологічні зміни мікроциркуляторного річища. Стінки судин потовщувалися внаслідок проліферації в них колагенових волокон і круглоклітинної інфільтрації. У цей період формувалась грануляційна тканина. На цьому етапі дослідження запальна інфільтрація досягала гребеня коміркової кістки та проникала в епітелій зубоясенного прикріплення, ясенної борозни. Паралельно з деструктивними змінами визначили морфологічні ознаки репаративних процесів, проліферацію та потовщення колагенових волокон. Формувалась грануляційна тканина. За таких морфологічних змін у м'яких тканинах пародонта виявляли суттєві зміни кісткової тканини. У пластинчастій кістці альвеолярного відростка спостерігали нерівномірне стоншення кісткових балок. Таке явище забезпечувалось остеокластичною лакунарною резорбцією кістки, подекуди – гладкою. Остеоцити набули різних розмірів, часто з пікнотичними ядрами. Траплялися порожні лакуни.

Висновки. Виявлені патоморфологічні зміни у тканинах пародонтального комплексу в пізній період вказують на ознаки хронізації запального процесу.

Ключові слова: пародонтит, запалення, періодонт, проліферація, колагенові волокна.

Запорізький медичний журнал. – 2018. – Т. 20, № 6(111). – С. 827–831

Патоморфологические изменения в тканях пародонтального комплекса в период хронизации экспериментального бактериально-иммунного пародонтита

А. Е. Демкович, Ю. И. Бондаренко, Т. К. Головата

Цель работы – исследовать патоморфологические изменения в пародонтальном комплексе на 30 сутки развития экспериментального бактериально-иммунного пародонтита.

Ключевые слова: пародонтит, воспаление, периодонт, пролиферация, коллагеновые волокна.

Запорожский медицинский журнал. – 2018. – Т. 20, № 6(111). – С. 827–831

Материалы и методы. Исследование проведено на белых беспородных клинически здоровых крысах-самцах. Экспериментальный бактериально-иммунный пародонтит у опытных животных вызвали путем инъекции в ткани пародонтального комплекса смеси микроорганизмов, разбавленной яичным протеином. Для оценки степени структурных изменений в тканях челюстно-лицевой области провели морфологическое исследование. Фрагменты тканей нижней челюсти, в частности пародонтального комплекса заливали в парафиновые блоки. На микротоме изготавливали поперечные срезы толщиной 5–6 мкм. Полученные препараты окрашивали гематоксилином и эозином.

Результаты. Гистологическое исследование показало, что на данный период течения воспалительного процесса имела место дезорганизация и деструкция соединительной ткани и стенок зубных альвеол, структурная перестройка эпителиальной выстилки десны и ее собственной пластинки. Отмечена высокая интенсивность воспалительной инфильтрации, однако прогрессирование воспалительных и деструктивных процессов значительно замедлялось. Воспалительная инфильтрация сопровождалась отчетливыми признаками дезорганизации соединительной ткани. Отмечены морфологические признаки репаративных процессов, происходила пролиферация и утолщение коллагеновых волокон. Вместе с переформатированием клеточного состава инфильтратов нарастали патологические изменения микроциркуляторного русла. Стенки сосудов утолщались за счет пролиферации в них коллагеновых волокон и круглоклеточных инфильтраций. В данный период формировалась грануляционная ткань. На этом этапе исследования воспалительная инфильтрация достигала гребня воротниковой кости и проникала в эпителий зубодесневого прикрепления и десневой борозды. Параллельно с деструктивными изменениями наблюдали морфологические признаки репаративных процессов. Отмечена пролиферация и утолщение коллагеновых волокон. Формировалась грануляционная ткань. При такой картине морфологических изменений в мягких тканях пародонта происходили существенные изменения костной ткани. В пластинчатой кости альвеолярного отростка наблюдали неравномерное истончение костных балок. Такое явление обеспечивалось остеокластической лакунарной резорбцией кости, иногда – гладкой. Остеоциты приобрели разные размеры, часто с пикнотичными ядрами. Определяли пустые лакуны.

Выводы. Обнаруженные патоморфологические изменения в тканях пародонтального комплекса в поздний период указывают на признаки хронизации воспалительного процесса.

Introduction

Inflammatory periodontal disease is the initial stage of the destructive process, which leads to teeth loss, impairment in the performance of communicative social skills, decrease in the quality of life and result in social problems [1,2].

An important feature of the oral cavity organs and tissue functioning is that pathological processes development is accompanied by constant presence of various microbial associations, which are an etiological factor and at the same time are the trigger mechanisms for their further development [3,4].

The manifestation and progression of the periodontitis signs depends on many reasons, including presence of somatic diseases, social, behavioral, systemic, genetic factors, microbial plaque and other risk factors [5,6]. Among chronic periodontal diseases, generalized chronic periodontitis is in the first place [7]. The factors that induce prolonged inflammation and periodontal tissues destruction usually include exo- and endotoxins of parodontopathogenic bacteria. Diseases of the periodontal complex represent an important medical and social problem and are characterized by constant growth and widespread not only among the elderly population, but also among young persons. There is an increasing trend towards periodontitis prevalence among young people [8].

The exact sequence of events triggering the bacterial-immune periodontitis remains unclear, but it is certainly that the destruction of tissues, rapid progress of alveolar processus destruction and teeth loss are resulted from pathologic reaction of the organism to periodontopathogens invasion [9].

A clinical diagnosis of periodontitis is based on the clinical constellation and complaints, patient's age, clinical examination; in particular, determination of periodontal pockets depth and subgingival region state, as well as X-ray evaluated alveolar bone destruction [10,11].

The problems and issues of inflammatory-dystrophic periodontal diseases treatment remain unresolved at the present time and are the subject of scientific researches of many specialists and collectives. It is associated with widespread pathology and significant occurrence of severe forms of periodontal disease, as well as insufficient effectiveness of the proposed medicines [12,13].

The purpose

The purpose of this study was to determine the nature of pathomorphological changes in the periodontal complex and assess them in the chronic course of experimental bacterial-immune periodontitis.

Materials and methods

The study was conducted with use of non-breeding clinically healthy male rats (10 animals) weighing 150–200 g in vivarium conditions in accordance with sanitary-hygienic norms and GLP requirements. The animals were in a standard diet balanced for the main elements of nutrition. Experiments were carried out in accordance with the general rules and provisions of the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments" (Kyiv, 2001). The rats were randomly selected and divided into groups: the I – intact animals, control (n = 5); the II – animals with experimental periodontitis on the 30th day of the study (n = 5). Experimental bacterial-immune periodontitis in experimental animals was induced by injection of complex microbial mixture diluted with ovalbumin into periodontal tissue [14]. Simultaneously with the microbial pathogen injections, complete Freund's adjuvant was injected into the rat's paw to enhance the immune response. Healthy rats of the same age were used as control. The pathogen

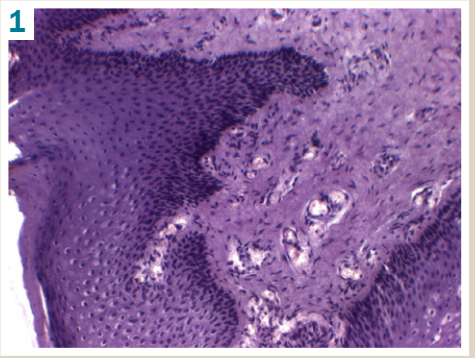

Fig. 1. Structural organization of the rat's gingival mucosa after 30 days of the experiment. The image demonstrates expressed papillary epithelial hyperplasia, acanthosis. Hematoxylin and eosin staining, ×100.

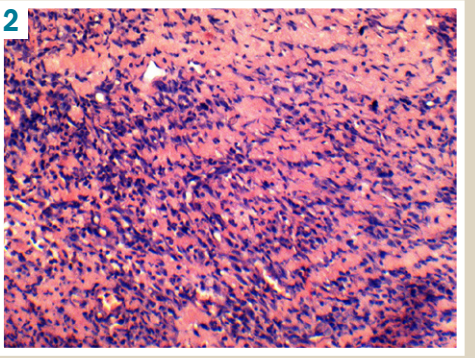

Fig. 2. Histological structure of the rat's lamina propria of gingival mucous after 30 days of the experiment. The image demonstrates fibroblasts, histiocytes, lymphocytes prevalence in the cellular infiltrate. Proliferation of collagen fibers. Hematoxylin and eosin staining, ×200.

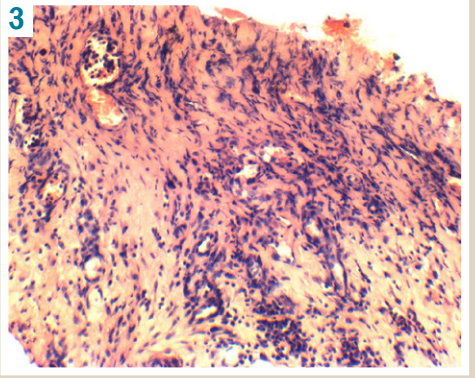

Fig. 3. Histological structure of the rat's lamina propria of gingival mucous after 30 days of the experiment. The image demonstrates leukostasis and mural aggregation of erythrocytes with mural thrombi formation. Hematoxylin and eosin staining, ×400.

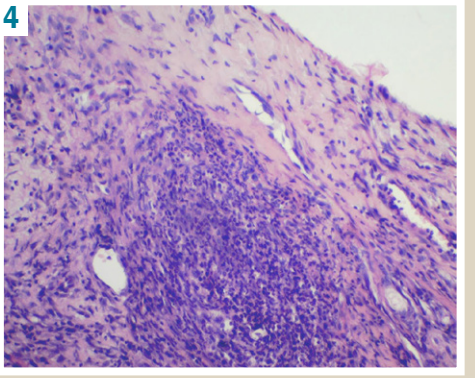

Fig. 4. Histological structure of the rat's lamina propria of gingival mucous after 30 days of the experiment. The image demonstrates polymorphocellular infiltration of the lamina propria with granuloma formation. Hematoxylin and eosin staining, ×400.

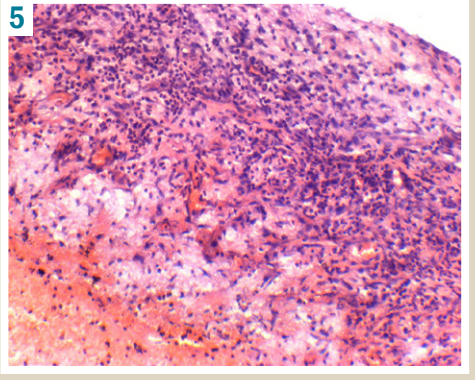

Fig. 5. The fragment of periodontium with polymorphocellular infiltration, degraded collagen fibers, edema, vascular hyperemia after 30 days of the experiment. Hematoxylin and eosin staining, ×200.

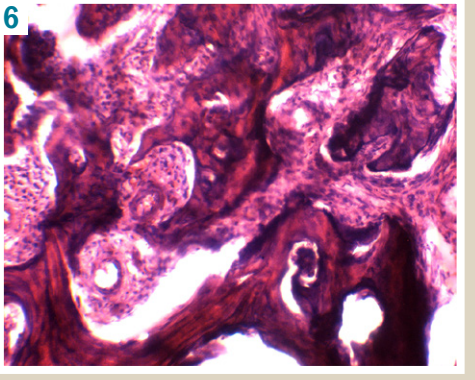

Fig. 6. Histological structure of the rat's bone alveolar process after 30 days of the experiment. The image demonstrates destruction and resorption of bone trabeculae. Disorganization of mineralized matrix, osteocytes are absent or slightly contoured. There are inflammatory cells and proliferation of collagen fibers in the mesenchyma. Hematoxylin and eosin staining, ×200.

and adjuvant were injected repeatedly on the 14th day of the experiment.

Morphological analysis was used for estimation of the structural changes degree in the maxillofacial tissues. The experimental animals were sacrificed on the 30th day via decapitation being anesthetized with thiopental. The mandible-related tissues fragments, in particular the periodontal complex, were removed, rinsed from the blood in saline and fixed in 10 % neutral formalin solution, then subjected to a graded series of alcohols for dehydration, and then embedded in paraffin wax. Paraffin blocks were cut into 5–6-μm-thick sections using a sledge microtome MC 2. The tissue slices were then stained with hematoxylin (acid Mayer's solution) and eosin for microscopic examination [15], which was performed with the help of Granum microscope. Microphotographing of images was done using a digital video-camera Delta Optical DLT – Cam Basic 2 MP. Photos were processed on computer Intel (R) Celeron (R) 2.7 GHz with the help of Toup View program.

Results and discussion

Microscopic examination of periodontal tissues at this experimental stage showed their significant structural rearrangement. The inflammatory infiltration of high intensity was noted, although the progression of inflammatory and destructive processes was significantly slowed down. Despite the positive changes in the dynamics of the inflammatory process, destructive-proliferative changes in all structural components of periodontium were increased.

In this case, the vertical orientation of epithelial lining and stratified squamous gingival epithelium were disturbed. The number of the squamous cells layers was increased with the reduction number of the squamous cells layers in the areas of connective tissue papilla invasion into the epithelium of gingiva. The parakeratosis, hyperkeratosis and acanthosis, balloon dystrophy of epithelial cells phenomena were regularly observed *(Fig. 1)*. The basal cell activity was found in places of erosion.

In addition, neoangiogenesis was observed after 30 days, the number of vessels was increased, there were changes in pre-existing blood vessels: they became larger, their walls were thickened, sclerotized and enlarged lumen.

The dense inflammatory infiltrates in the lamina propria of gingival mucous and adjacent tissues changed their structure. The cellular composition of infiltrates, consisting mainly of lymphocytes, histiocytes, monocytes, also was increased quantity of plasmatic cells. Along with these cells, polynuclear cells also were observed regularly extending the adjacent bone tissue. Swelling of tissues remained (*Fig. 2*).

The microcirculatory flow pathological changes were worsened together with the cellular composition of infiltrates transformation. The vessels walls were thickened due to collagen fibers proliferation and round cellular infiltration. The endothelial cells were desquamated, stratified, stripping the vessels basement membrane, and the remaining cells were swollen, having a light foamy cytoplasm. These changes contributed to increased transudation of plasma into perivascular space and worsened hypoxia in the periodontal tissues. Blood flow was uneven, venous hyperemia prevailed. The usual phenomenon was mural aggregation of erythrocytes, leukostasis with formation of thrombotic masses (*Fig. 3*).

The inflammatory infiltration of the lamina propria of gingival mucous was accompanied by clear signs of connective tissue disorganization – mucoid and fibrinoid swelling of collagen fibers with subsequent fragmentation and lysis. The morphological signs of reparative processes were observed along with destructive changes. There were proliferation and thickening of collagen fibers. The granulation tissue was formed.

The inflammatory infiltration extended the crista of the cellular bone and penetrated the epithelium of dento-gingival junction and gingival sulcus. The polymorphic inflammatory infiltrates were often localized with the formation of granuloma (*Fig. 4*).

Expressed microstructural reorganization of the periodontal connective tissue occurred in this case. The lamina propria of gingival mucous was sclerosed, and in some places it was hyalinized. The collagen bundles showed an irregular pattern of arrangement, often with cross-linking. Lymphocyte, plasma cell, histiocyte, macrophage diffuse and cellular infiltration in various ratio of these cells was observed. Such round cell infiltration was significant in some places, and combined with collagen fibers degradation (*Fig. 5*).

Significant changes in bone tissue were observed upon such morphological changes in periodontal soft tissues. Uneven thinning of bone trabeculae was observed in the cancellous bone of the alveolar process. Such phenomenon was provided by osteoclastic lacunar bone and sometimes smooth resorption (*Fig. 6*). Simultaneously the structure of mineralized intercellular spaces was changed. It became heterogeneous, with resorption and porous spaces, osseous fibers were interrupted and not always visualized. Osteocytes had various sizes, often with picnotic nuclei. There were empty lacunae. The cellular proliferation was observed in the mesenchyma. There were also lymphocytes, occasionally neutrophils and basophils. The collagen fibers were clearly proliferated. The osteoblasts were arranged irregularly, but osteoid layer thickening was observed in places of their localization. There were vessels walls thickening and uneven blood flow. The lymphocytes were observed perivascularly and at a distance from them neutrophils and basophils were revealed occasionally.

It should be noted that the severity and nature of the inflammatory process in different animals at this stage ranged from mild to severe manifestation of the periodontitis. In this regard, it is reasonable to assume, that this pathomorphological picture reflects the state of tissue-vascular and immune processes in the animals, which determine the dynamics of degenerative-dystrophic disorders from the onset of tissue damage to their final stage.

Conclusions

1. Development of the inflammatory process in the periodontal complex tissues includes a regular sequence of tissue-vascular and cellular changes with predominance in the late period (on the 30th day of observation) of the structural reconstruction of the gingival epithelium, its lamina propria, disorganization and destruction of the connective tissue and walls of the dental alveoli, the bone of the alveolar processus, degenerative-dystrophic and proliferative phenomena with granulation tissue and granulomas formation.

2. The nature of pathomorphological changes in the periodontal complex tissues in the late period of the experimental bacterial-immune periodontitis development: slow down and stabilization of tissue-vascular and cellular changes, progression of sclerotic processes and bone resorption testify about chronization of the inflammatory process.

The prospect of further scientific research in this regard is to study the best methods of pathomorphological changes pharmacological corrections in the periodontal tissue in experimental bacterial-immune periodontitis.

Conflicts of interest: authors have no conflict of interest to declare.
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Information about authors:
Demkovych A. Ye., MD, PhD, Associate Professor of the Prosthetic Dentistry Department, I. Horbachevsky Ternopil State Medical University, Ukraine.
Bondarenko Yu. I., MD, PhD, DSci, Professor, Head of the Pathophysiology Department, I. Horbachevsky Ternopil State Medical University, Ukraine.
Holovata T. K., MD, PhD, Associate Professor of the Pathologic Anatomy with Sectional Course and Forensic Medicine Department, I. Horbachevsky Ternopil State Medical University, Ukraine.

Відомості про авторів:
Демкович А. Є., канд. мед. наук, доцент каф. ортопедичної стоматології, ДВНЗ «Тернопільський державний медичний університет імені І. Я. Горбачевського МОЗ України», м. Тернопіль.
Бондаренко Ю. І., д-р. мед. наук, професор, зав. каф. патологічної фізіології, ДВНЗ «Тернопільський державний медичний університет імені І. Я. Горбачевського МОЗ України», м. Тернопіль.
Головата Т. К., канд. мед. наук, доцент каф. патологічної анатомії з секційним курсом та судовою медициною, ДВНЗ «Тернопільський державний медичний університет імені І. Я. Горбачевського МОЗ України», м. Тернопіль.

Сведения об авторах:
Демкович А. Е., канд. мед. наук, доцент каф. ортопедической стоматологии, ГВУЗ «Тернопольский государственный медицинский университет имени И. Я. Горбачевского МЗ Украины», г. Тернополь.
Бондаренко Ю. И., д-р. мед. наук, профессор, зав. каф. патологической физиологии, ГВУЗ «Тернопольский государственный медицинский университет имени И. Я. Горбачевского МЗ Украины», г. Тернополь.
Головата Т. К., канд. мед. наук, доцент каф. патологической анатомии с секционным курсом и судебной медициной, ГВУЗ «Тернопольский государственный медицинский университет имени И. Я. Горбачевского МЗ Украины», г. Тернополь.

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