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Abstract

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PATHOMORPHOLOGICAL CHANGES OF THE PERIODONTAL COMPLEX IN EXPERIMENTAL BACTERIAL-IMMUNE PERIODONTITIS AND THEIR CORRECTION BY FLAVONOL

Introduction. One of the most urgent problems of modern dentistry is periodontitis; it concerns, first of all, modern treatments. Development of inflammation in the periodontal complex includes numerous links that lead to generalization and chronicity of it, loss of teeth and complications of other organs.

Purpose of this research was to investigate the pathomorphological changes in the periodontal complex in experimental bacterial-immune periodontitis formation and treatment effect of the flavonol quercetin.

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 caused by introducing complex mixture of microorganisms diluted with egg protein into periodontal tissue near the lower central incisors. Quercetin was used by intramuscular injection (100 mg/kg body weight) for 7 days. The transverse sections on a microtome were made in the thickness of 5-6 microns. The resulting preparations were stained with hematoxylin and eosin.

Discussion. The histological study showed that the experimental periodontitis course was characterized by increased inflammatory response by the 14^{th} day of experiment, and its spread throughout the periodontium with accumulation of sufficiently large number of different cells, including neutrophils, macrophages, fibroblasts. The inflammatory reaction was accompanied with distinct structural changes. The analysis of the microscopic examination of the periodontal tissues in the animal group with experimental periodontitis showed positive dynamics of structural organization as compared with animals that were not treated during this period by the quercetin. At the same time, there was improved structuring of the gingival epithelial plate, its vertical and horizontal differentiation. The linearity of the epithelial layer became clearer and the number of dystrophic altered cells was decreased. The use of the flavonol quercetin during this period of the experimental bacterial-immune periodontitis development improved to normalization of the morphological state of periodontal tissues.

Keywords: periodontitis, inflammation, periodontium, pathomorphologic, cementoblasts, cementocytes.

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Резюме

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ПАТОМОРФОЛОГІЧНІ ЗМІНИ В ПАРОДОНТАЛЬНОМУ КОМПЛЕКСІ У ПРОЦЕСІ РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО БАКТЕРІАЛЬНО-ІМУННОГО ПАРОДОНТИТУ ТА КОРЕКЦІЯ ЇХ ФЛАВОНОЛОМ

Однією із найбільш актуальних проблем сучасної стоматології є пародонтит, вона стосується, у першу чергу, розробки нових методів лікування. Розвиток запального процесу в пародонті включає низку складних процесів, що приводять до генералізації і хронізації його, втрати зубів та появи ускладнень з боку інших органів. З огляду на це метою даного дослідження було дослідити патоморфологічні зміни в пародонтальному комплексі в процесі розвитку експериментального бактеріально-імунного пародонтиту та вплив на них флавонолу кверцетину. Дослідження проведено на білих щурах Експериментальний бактеріально-імунний пародонтит у дослідних тварин викликали шляхом ін'єкції у тканини пародонтального комплексу в ділянці нижніх центральних різців суміші мікроорганізмів, розведеної яєчним протеїном. Застосовували кверцетин шляхом внутрішньом'язових ін'єкцій (100 мг/кг маси тварини) впродовж 7-ми діб (з 7-ої по 14-ту добу). Для оцінки ступеня структурних змін в тканинах щелепно-лицевої ділянки проводили морфологічне дослідження. На мікротомі виготовляли поперечні зрізи товщиною 5-6 мкм. Отримані препарати забарвлювали гематоксиліном та еозином. Гістологічне дослідження показало, що перебіг експериментального пародонтиту на 14-ту добу характеризувався як посиленням запальної реакції, так і поширенням її на весь пародонт із скупченням достатньо великої кількості різноманітних клітин, у тому числі нейтрофілів, макрофагів, фібробластів. Запальна реакція супроводжувалася виразними структурними змінами. Аналіз результатів мікроскопічного дослідження тканин пародонта у групі тварин з експериментальним пародонтитом за умови його корекції кверцетином показав позитивну динаміку структурної організації порівняно із тваринами, які не підлягали лікуванню протягом даного терміну. При цьому відбулося покращення структуризації епітеліальної пластинки слизової ясен, її вертикальної та горизонтальної диференціації. Більш чіткою стала рядність епітеліального шару та зменшувалася кількості дистрофічно змінених клітин. Використання флавонову кверцетину протягом даного періоду розвитку експериментального бактеріально-імунного пародонтиту сприяло нормалізації морфологічного стану тканин пародонта.

Ключові слова: пародонтит, запалення, періодонт, патоморфологія, цементобласти, цементоцити.

Резюме

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МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ПАРОДОНТАЛЬНОМ КОМПЛЕКСЕ В ПРОЦЕССЕ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО БАКТЕРИАЛЬНО-ИММУННОГО ПАРОДОНТИТА И КОРРЕКЦИЯ ИХ ФЛАВОНОЛОМ

Одной из наиболее актуальных проблем стоматологии является пародонтит, она касается, в первую очередь, разработки новых методов лечения. Развитие воспалительного процесса в пародонте включает ряд сложных процессов, приводящих к генерализации и хронизации его, потери зубов и появления осложнений со стороны

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других органов. Учитывая это, целью данного исследования было исследовать патоморфологические изменения в пародонтальном комплексе в процессе развития экспериментального бактериально-иммунного пародонтита и влияние на них флавонола кверцетина. Исследование проведено на белых крысах. Экспериментальный бактериально-иммунный пародонтит у опытных животных вызвали путем инъекции в ткани пародонтального комплекса в область нижних центральных резцов смеси микроорганизмов, разбавленной яичным протеином. Применяли кверцетин путем инъекций (100 мг/кг массы животного) в течение 7-ми суток (с 7-ой по 14-е сутки). Для оценки степени структурных изменений в тканях челюстно-лицевой области проводили морфологическое исследование. На микротоме изготавливали поперечные срезы толщиной 5-6 мкм. Полученные препараты окрашивали гематоксилином и эозином. Гистологическое исследование показало, что течение экспериментального пародонтита на 14-е сутки характеризовался как усилением воспалительной реакции, так и распространением ее на весь пародонт со скоплением достаточно большого количества различных клеток, в том числе нейтрофилов, макрофагов, фибробластов. Воспалительная реакция сопровождалась выразительными структурными изменениями. Анализ результатов микроскопического исследования тканей пародонта в группе животных с экспериментальным пародонтитом при его коррекции кверцетином показал положительную динамику структурной организации по сравнению с животными, которые не подлежали лечению в течение данного срока. При этом произошло улучшение структуризации эпителиальной пластинки слизистой десен, ее вертикальной и горизонтальной дифференциации. Более четкой стала рядность эпителиального слоя и уменьшалась количества дистрофически измененных клеток. Использование флавонола кверцетина в течение данного периода развития экспериментального бактериально-иммунного пародонтита способствовало нормализации морфологического состояния тканей пародонта.

Ключевые слова: пародонтит, воспаление, периодонт, патоморфология, цементобласты, цементоциты.

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Introduction

The cavity of the mouth represents an original complex system, which is closely connected with other internal systems of organism and external environment. The high frequency of its damage largely due to the peculiarities of the structural organization of its elements and their constant functional load [1, 2].

Among the most important problems in dentistry is periodontitis, which manifests itself in various forms, forming in the oral cavity the foci of chronic infection [3, 4]. At the present stage, advantage majority of the researchers regard chronic generalized periodontitis to polyethiological pathology with various pathogenetic links of its development [5].

Among the risk factors, the violation of microbiocynosis in the oral cavity, the inadequacy of the immune response, insufficient antioxidant defense system, microcirculation disorders and transcapillary metabolism in the periodontal tissues are the most principal [6]. Significant prevalence, progressive and chronic course with frequent exacerbations of inflammatory periodontal diseases lead to premature destruction of the support apparatus and loss of teeth, which get worse the general state of human health, affects the ability to work and psychoemotional activity [7, 8]. All of the abovementioned allows attributing this pathology of the

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tooth-jaw system not only to the medical, but also to an important social problem [9].

Improvement of the existing methods and modern creation of the periodontitis treatment is one of urgent tasks, it requires extraordinary approaches to their solve. In that sense, attract attention quercetin (corvitin), which refers to flavonols with antioxidant, anti-ischemic, membrane-stabilizing and immunomodulatory properties [10, 11]. It has great reducing potential and declares anti-inflammatory, anabolic, anti-apoptotic properties [12]. The antioxidant activity of the drug due to its ability to suppress lipid peroxidation, reduce the concentration of the free radicals and toxic peroxidation products, stimulate catalase and superoxide dismutase activity. Anti-inflammatory and antiallergic its effects are also related of the quercetin ability to suppress calcium ATPase and the synthesis of leukotrienes. This flavonol is able to suppress the activity of hyaluronidase, increase the content of the immune cells system (phagocytosis, T-lymphocytes, B-lymphocytes) in the blood, resulting in decreased manifestations of secondary immunosuppression [13, 14]. Determine of the character of pathomorphological changes will allow establish mechanisms of damage of the periodontal complex structures and their manifestations for the inflammatory process formation of various severity [15, 16].

The purpose of this research was to investigate pathomorphological changes in the periodontal complex for acute period development of experimental bacterial-immune periodontitis and treatment effect of the flavonol quercetin.

MATERIALS AND METHODS. The study was conducted with use of non-breeding clinically healthy male rats weighing 150-200 g in vivarium conditions in accordance with sanitary-hygienic norms and GLP requirements. The animals were in a standard diet balanced by the main elements of nutrition. Experiments were carried out in compliance 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). Experimental animals were randomly selected and divided into 3 groups: I – intact animals; II – animals with experimental periodontitis on the 14th day of the study; III – animals with experimental periodontitis on the 14th day of the study, which was administered quercetin (corvitin).

Experimental bacterial-immune periodontitis in experimental animals was caused by introducing complex mixture of microorganisms diluted with egg protein into periodontal tissue near the lower central incisors [17]. Simultaneously with the injections of the microbial pathogen, a complete Freund's adjuvant was injected in the rat's paw to enhance the immune response. Systematically healthy rats of the same age were used as controls.

Quercetin was used by intramuscular injection (100 mg/kg body weight) for 7 days (from the 7th to the 14th day) to the rats of the third group. For estimation of the degree structural changes in tissues of the maxillofacial area was used morphological investigation. The experimental animals were sacrificed on the 14th day through decapitation under thiopental anesthesia, removed fragments of tissues of the mandible, in particular of the periodontal complex, washed in saline from the blood and fixed in 10% neutral formalin solution. The material was poured in paraffin blocks. The transverse sections on a microtome were made in the thickness of 5-6 microns. The resulting preparations were stained with hematoxylin and eosin [18].

RESULTS AND DISCUSSION. These studies were performed in accordance with suggested and patented our pattern of experimental periodontitis [19], which reflects the role of bacterial and immune disorders in the mechanisms of inflammation development in the periodontal complex. Study of experimental periodontitis in that version and indices of bacterial-immune inflammation before were not investigated.

According to histological studies, the course of experimental periodontitis on the 14th day of the study was characterized as strengthening of the inflammatory response that its spread to the all periodontium with accumulation of sufficiently large number of different cells, including neutrophils, macrophages, fibroblasts. The inflammatory reaction was accompanied by clear structural changes. In particular, the gingival epithelium and its stratum layer have different thickness. The areas of surface desquamation, erosion and ulceration with perifocal hemorrhages, thick infiltration of surrounding tissues with neutrophils were observed. The epithelial cells were in a state of vacuolic dystrophy (Figure 1). Proliferating nucleolar keratinocytes appeared in the inflammation area. Increase in the number of cells in the growing layer, which led to its thickening and papillomatous enlargement to the depth of its own plate was observed in the places of preserved epithelial layer. The atrophic areas were found side by side with mentioned above changes.

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The most distinct structural violations were found in areas of marginal gum zone.

The connective tissue of own gingival plate also exposed significant structural changes. First of all, the disorder of hemocirculation attracted attention. For that number of dilated and pletoric capillaries and arterioles with a stasis and aggregation of erythrocytes in the form of slugs were increased. In accordance to those vessels of the venous stream was sharply dilated, venous congestion arose. However, in some cases or within one of them arteriolospasm was observed. Capillaries acquired the form band of connective tissue (Figure 2).

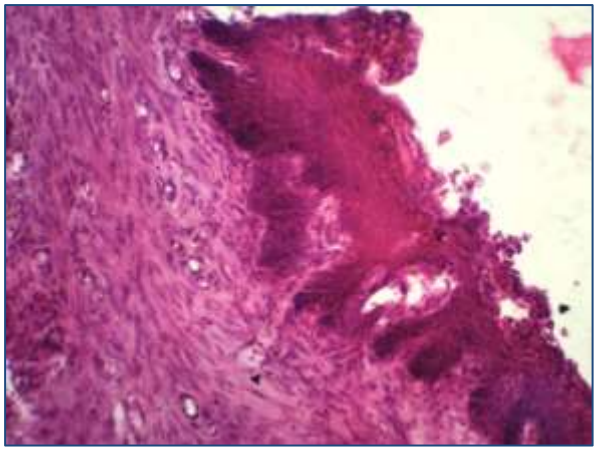

Figure 1 – Histological structure of the rat's gingiva on the 14th days of the experiment. Image shows erosion of epithelial lining. Cell proliferation of the basal layer. Staining with hematoxylin and eosin. × 200

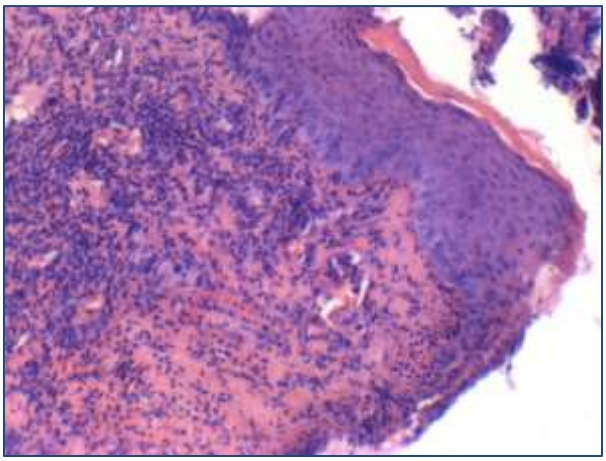

Figure 2 – Histological structure of the rat's gingiva on the 14th days of the experiment. Image shows epithelial dystrophy. Thick round cell infiltration of its own plate. Uneven arteriovenous blood flow. Staining with hematoxylin and eosin. × 100

Such hemodynamic disorders in that conditions cause deep hypoxic changes, which, in it turn, contributed to increased vascular permeability with subsequent edema of surrounding tissues and their degenerative-destructive changes.

At the same time, there was proliferation and thickening of collagen fibers. They lost a characteristic fibrillar structure, homogenized and disintegrated. Often, nonstructural homogeneous eosinophilic cells of fibrinoidal necrosis were found in the massif of the fibers.

Similar changes in the connective tissue were accompanied by significant clusters of cells. The composition of infiltrates included lymphocytes, plasmocytes, macrophages, and tissue basophiles. By constant and predominant components were neutrophils. Infiltrates were both local and diffuse character. The most expressed infiltrates were in the area of the gingival sulcus. Similar changes were found in the some parts of the periodontium. As for the types of infiltration, in our opinion, the mixed type prevails, which includes both the diffuse nature of cellular infiltration of periodontium with the transition to bone tissue, and microabsection.

Because inflammatory cellular infiltration reached the alveolar bone, it also exposed significant changes. In particular, the mesenchyma were revealed signs of disorganization of the main substance and cellular composition. Among of mesenchymal cells were revealed cells of hematogenous origin (Figure 3).

Hemocapillary filling located in the structure of alveolar bone and periodontium were uneven. Osteoblasts were arranged irregularly, therefore, the area of exposed calcified bone matrix was not infrequently visualized. The osteoid was thin and interrupted. Osteoclasts were detected more frequently as compared with previous observed period. In the places of their localization lacunars defects of the bone beams were formed. The thinness of the bone beams were also found regularly in the places where absented osteoclasts.

The peculiar sinuses with smooth walls were formed in the some areas. The contours of the bone plates began to lose clarity. The intercellular substance was unevenly enlightened; osteocytes had distinct picnotic nuclei and located in faint contoured gaps (Figure 4).

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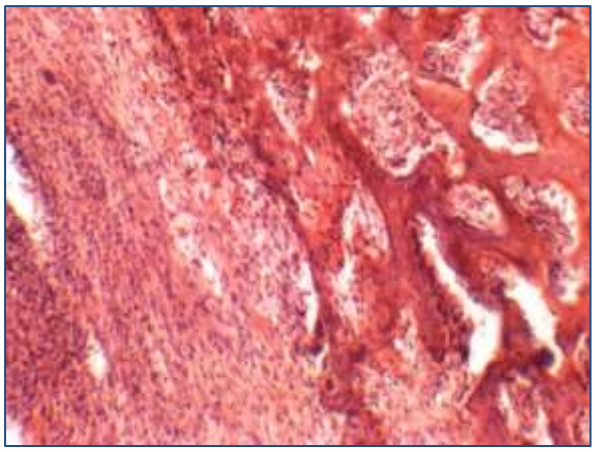

Figure 3 – Histological structure of the rat's alveolar bone and periodontium on the 14th days of the experiment. Image shows diffuse intensive leukocytic infiltration of the periodontium with transition to bone tissue. Staining with hematoxylin and eosin. × 200

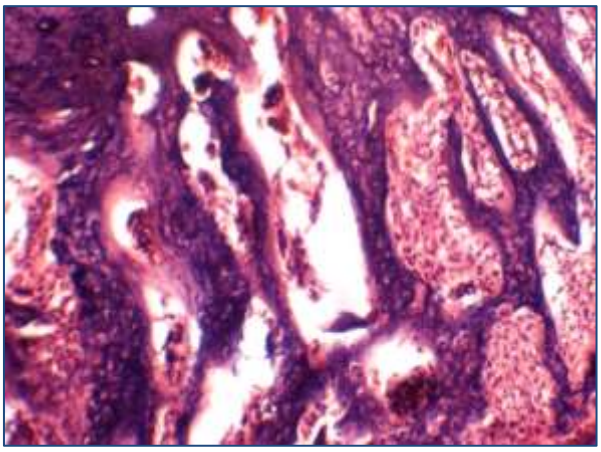

Figure 4 – Histological structure of the rat's bone alveolar processes on the 14th days of the experiment. Image shows Thinning of the bone plates, lacunar osteoclastic resorption. Staining with hematoxylin and eosin. × 200

The results of microscopic examination of periodontal tissues in the animals with experimental periodontitis, that was corrected with quercetin, the positive dynamics of structural organization was found as compared with the animals which were not treated for given period.

First of all, structuring of the epithelial plate of the gingival mucosa, in particular, its vertical and horizontal differentiation was improved. The linearity of the epithelial layer became more distinct and number of dystrophically altered cells was decreased.

In any case, there was not deep desquamation or ulceration. A similar trend was characteristic of all observed cases, but nevertheless it was uneven even within one line. The phenomena of parekeratosis, acanthosis, and increase homogeneous partly were preserved. The keratinous layer was thin and heterogeneous, but in the most cases it lay on the surface of the spinal layer with a solid band. Among of keratinocytes small lymphocytes were found in small quantities.

The transparency of amorphous substance somewhat was decreased in its own plate, the fibrillar structure of collagen fibers became clearer, basophilia was decreased. If during of the period between the 7^{th} and 14^{th} days in the periodontal tissues of animals with modeling periodontitis signs of disorganization of connective tissue were increased until their complete destruction, then these changes were stopped at the level of mucoid swelling after correction. It is attracting attention the features of cellular infiltration.

In addition to the fact that its intensity decreased, the trend of local infiltration formation with tissue destruction and granulomatosis disappeared. At the same time, the cellular composition of itself infiltrates also changed. The share of neutrophils, basophiles and plasmocytes significantly decreased. They were not infrequently visualized in structure of the infiltrate. Histiolymphocytic infiltration also become less intense, but it took place in the all specimens (Figure 5).

The vessels of the arterial and venous parts of the microcirculation stream had uneven blood-content. Swelling of tissues was localized perivasally and was poorly expressed, that evidenced about significant reduction of vascular permeability and associated with it transudaction of the plasma. Such phenomena were quite logical in conditions of reducing intensity of the inflammatory process.

The similar trend was observed in the periodontal connective tissue. The collagen fibers had the more compact location and their aslantoradial direction was observed. The cellular composition became similar to that as control group, although neutrophils rarely appeared.

Correction of the pathological process also contributed to better preservation of the structure of the alveolar bone. Among the bone beams amount of destructively altered significantly was decreased, especially in comparison with the histological picture on the 14^{th} day of the experiment. The number of resorptive gaps was decreased significantly. In part of cases, this phenomenon was absent.

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The mesenchyma was more homogeneous, the areas of disorganization were diminished and its manifestations weakened. The cellular component included ordinary for the mesenchyma cells, although lymphocytes not infrequently encountered or single neutrophils.

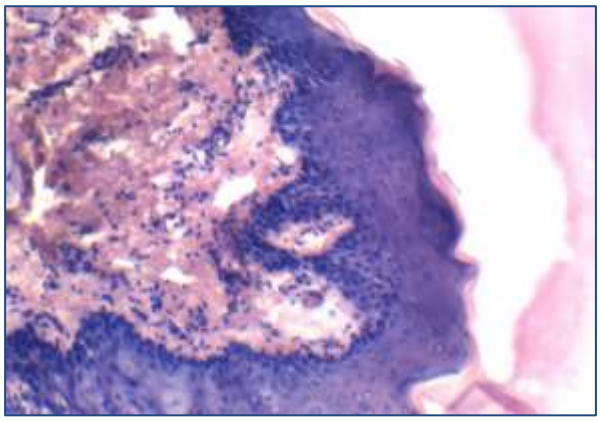

Figure 5 – Histological structure of rat's gingiva with experimental periodontitis after treatment with quercetin. Image shows moderate acanthosis of the gingival epithelium, edema and lymphocytic infiltration of the own plate mucous membrane. Staining with hematoxylin and eosin. × 200

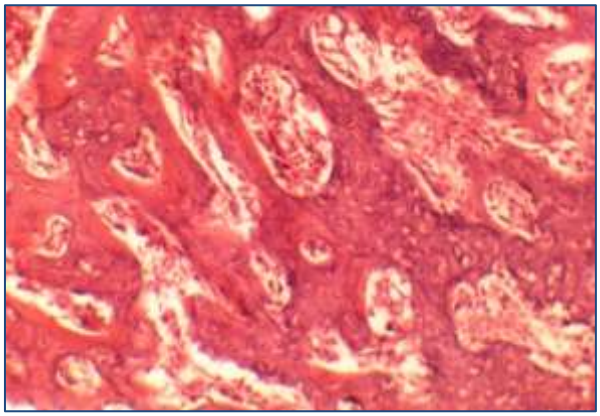

Figure 6 – Histological structure of the rat's processes alveolar with the experimental periodontitis after treatment with quercetin. Staining with hematoxylin and eosin. × 200

The unmineralized matrix – osteoid is intermittent, uneven thickness, located along bone plates marginally under several osteoblasts, the number of which became larger, and accordingly their activity were increased. Osteoclasts were found irregular. The calcified matrix became more homogeneous, the osseous fibers in the plates were clearly oriented. The walls of the lacunae were well contoured; the staining of the osteocytes nuclei was moderate intensity (Figure 6).

At the same time, vascular blood-content was decreased, and signs of disturbed reohemodynamics became less expressed. The uneven blood-content dominated, red blood cells were well contoured.

Conclusions

1. The progressing inflammatory changes include disorganization and destruction of the connective tissue, walls of the dental alveoli, gingival epithelial lining and its own plates in the experimental bacterial-immune periodontitis development. The structural disorders of the components animal's periodontal complex arise in the period of the greatest clinical manifestations of the inflammation (on the 14th day of the study).
2. The application with purpose treatment flavonol quercetin for the experimental bacterial-immune periodontitis formation reduces the manifestations of alterative changes in the connective tissue, decreases macro- and microphage infiltration, promotes to normalization of the periodontal tissues structure, which is a sign of limitation of the inflammatory process development.

References

1. Demkovych A, Bondarenko Yu, Hasiuk PA. Oxidative modification of proteins in the process of experimental periodontitis development. *Interventional Medicine and Applied Science.* 2017;9(4):218-221.
2. Sokos D, Everts V, de Vries TJ. Role of periodontal ligament fibroblasts in osteoclastogenesis: a review. *J Periodontal Res.* 2015;50(2):152-159.
3. Di Benedetto A, Gigante I, Colucci, S, Grano M. Periodontal disease: linking the primary inflammation to bone loss. *Clin Dev Immunol.* 2013;2013:503754.
4. Livingstone D, Murthy V, Reddy VK, Pillai A. Prosthodontic rehabilitation of a patient with aggressive periodontitis. *BMJ Case Rep.* 2015;5:2015.
5. Chapple IL, Van der Weijden F, Doerfer C, Herrera D, Shapira L, Polak D. Primary prevention of periodontitis: managing gingivitis. *J Clin Periodontol.* 2015;16:71-76.

DOI 10.21272/jcemr.2018.6(2):260–267

6. Arimatsu K, Yamada H, Miyazawa H, Minaqawa T, Nakajima M, Ryder MI, Gotoh K, Motooka D, Nakamura S, Iida T, Yamazaki K. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci Rep*. 2014;6(4):4828.
7. Ioannidou E. The Sex and Gender Intersection in Chronic Periodontitis. *Front Public Health.* 2017;5:189.
8. Slots J. Periodontitis: facts, fallacies and the future. *Periodontol* 2000. 2017;75(1):7-23.
9. Borisenko A. *Periodontal disease*. Kyiv: Medicine; 2013.
10. D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia*. 2015;106:256-271.
11. Miles SL, McFarland M, Niles RM. Molecular and physiological actions of quercetin: need for clinical trials to assess its benefits in human disease. *Nutrition Reviews*. 2014;**7**2(11):720-734.
12. Kravets BB, Regeda MS. The role of corvitin in the correction of violations of lipid peroxidation processes and the activity of the antioxidant system in guinea pigs blood in experimental bacterial keratitis against the background of bronchial asthma and pneumonia. *Medical and Clinical chemistry.* 2015;17(2):69-71.
13. Mikhailova YA, Kravchun PH, Petyunina OV, Lapshyna LA. The influence of cardioprotection on the state of hemodyncmics at treatment of cardiorenal syndrome in patients with chronic heart failure and chronic kidney disease. International *Medical Journal*. 2013;19(1):44-49.
14. Posokhova KA, Stechyshyn IP, Pidhirnyy VV. Influence of corvitin-containing compounds on myocardium status in diabetes mellitus type 2. *J Achievements of Clin and Exper Med.* 2014;2:17-21.
15. Bayani M, Pourali M, Keivan M. Possible interaction between visfatin, periodontal infection, and other systemic diseases: A brief review of literature. *Eur J Dent.* 2017;11(3):407-410.
16. Srivastava N, Nayak PA, Rana S. Point of Care – A Novel Approach to Periodontal Diagnosis – A Review. *J Clin Diagn Res.* 2017;11(8):ZE01-ZE06.
17. Demkovych AYe, Bondarenko YuI. Pathogenetic basis periodontitis modeling in rats. *Achiev of Clin and Exper Med.* 2015;1(22):54-57.
18. Chan JK. The wonderful colors of the hematoxylin-eosin stain in diagnostic surgical pathology. *Int J Surg Pathol.* 2014;22(1):12-32.
19. Demkovych AY, Bondarenko YI, inventor; I. Horbachevsky Ternopil State Medical Univ., assignee. Pathogenetic basis periodontitis modeling in rats. Ukraine patent 82388 u201303000. 2013 Jul 25.

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