Abstract

A. Ye. Demkovych, Yu. I. Bondarenko, V. R. Machogan,
I. Horbachevsky Ternopil State Medical University, Ukraine

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 14th 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.

Corresponding author: demkovushae@tdmu.edu.ua
Резюме
А. Є. Демкович, Ю. І. Бондаренко, В. Р. Мачоган,
ДВНЗ «Тернопільський державний медичний університет імені І. Я. Горбачевського МОЗ України»

ПАТОМОРФОЛОГІЧНІ ЗМІНИ В ПАРОДОНТАЛЬНОМУ КОМПЛЕКСІ У ПРОЦЕСІ РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО БАКТЕРИАЛЬНО-ІМУННОГО ПАРОДОНТИТУ ТА КОРРЕКЦІЯ ЇХ ФЛАВОНОЛОМ

Однією із найбільш актуальних проблем сучасної стоматології є пародонтит, вона стосується, у першу чергу, розробки нових методів лікування. Розвиток запального процесу в парадонти включає низку складних процесів, що приводять до генералізації і хронізації його, втрати зубів та появи ускладень 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 flavонолу кверцетину. Дослідження проведено на білих щурях Експериментальний бактеріально-імунний пародонтит у досліджених тварин викликали шляхом ін'єкції у тканини парадонтального комплексу в ділянці нижніх центральних різців суміші мікроорганізмів, розведеного 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 тканин парадонтального комплексу 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 посилення запальної реакції, так і поширенням її на весь парадонт із скупченням достатньо великої кількості різноманітних клітин, у тому числі нейтрофілів, макрофагів, фібробластів. Запальна реакція супроводжувалася виразними структурними змінами. Аналіз результатів мікроскопічного дослідження тканин парадонта 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 кількість дистрофічно змінених клітин. Використання flavонову кверцетину протягом даного періоду розвитку експериментального бактеріально-імунного пародонтиту сприяло нормалізації морфологічного стану тканин парадонта.

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

Резюме
A. E. Demkovych, Yu. I. Bondarenko, V. R. Machogan,
GВУЗ «Тернопільський государственный медицинский университет имени И. Я. Горбачевского МЗ Украины»

МОРОФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ПАРОДОНТАЛЬНОМ КОМПЛЕКСЕ В ПРОЦЕССЕ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО БАКТЕРИАЛЬНО-ИМУННОГО 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
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 psycho-emotional activity [7, 8]. All of the above-mentioned allows attributing this pathology of the
tooth-jaw system not only to the medical, but also
to an important social problem [9].

Improvement of the existing methods and mod-
dern creation of the periodontitis treatment is one of
urgent tasks, it requires extraordinary approaches to
their solve. In that sense, attract attention quercetin
(corvin), which refers to flavonols with antioxid-
ant, anti-ischemic, membrane-stabilizing and im-
munomodulatory properties [10, 11]. It has great
reducing potential and declares anti-inflammatory,
anabolic, anti-apoptotic properties [12]. The antiox-
idant activity of the drug due to its ability to sup-
press lipid peroxidation, reduce the concentration of
the free radicals and toxic peroxidation products,
stimulate catalase and superoxide dismutase activi-
ty. Anti-inflammatory and antiallergic its effects are
also related of the quercetin ability to suppress cal-
cium ATPase and the synthesis of leukotrienes.
This flavonol is able to suppress the activity of hya-
luronidase, 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 pathomor-
phological changes will allow establish mechani-
isms of damage of the periodontal complex struc-
tures 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 experi-
mental bacterial-immune periodontitis and treat-
ment 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 compli-
ance with the general rules and provisions of the
"European Convention for the Protection of Verte-
brate Animals used for Research and Other Scien-
tific 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 – ani-
imals with experimental periodontitis on the 14th
day of the study; III – animals with experimental peri-
dontitis on the 14th day of the study, which was
administered quercetin (corvin).

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 injec-
tions 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 esti-
mation of the degree structural changes in tissues of
the maxillofacial area was used morphological in-
vestigation. The experimental animals were sacri-
ficed 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 immu-
nological disorders in the mechanisms of inflamma-
tion development in the periodontal complex. Study of
experimental periodontitis in that version and indi-
ces 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 peri-
dontium with accumulation of sufficiently large
number of different cells, including neutrophils,
macrophages, fibroblasts. The inflammatory reac-
tion 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 tis-
ues 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 thick-
ening and papillomatous enlargement to the depth of
its own plate was observed in the places of pres-
served epithelial layer. The atrophic areas were
found side by side with mentioned above changes.
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 arteriolar spasm was observed. Capillaries acquired the form band of connective tissue (Figure 2).

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).
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 parakeratosis, acanthosis, and increase homogeneously 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 7th and 14th days of 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 became 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 perivascularly and was poorly expressed, that evidenced about significant reduction of vascular permeability and associated with it transudation 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 14th day of the experiment. The number of resorptive gaps was decreased significantly. In part of cases, this phenomenon was absent.
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

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 orientated; 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 inflammatory process development (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


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