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Comparative clinical and morphological analysis of the periodontitis of different genesis

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Summary. Comparative clinical and morphological analysis of the periodontitis of different genesis.

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The injections of the bacterial endotoxin to animals leads to the development of inflammatory and degenerative changes of the periodont. These changes are analogous to human periodontitis too. This allows to use this model for the study of parodontoprotecting activity of new drugs.

Key words: periodontal disease, rats, experiment

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Резюме. ПОРІВНЯЛЬНИЙ КЛІНІКО-МОРФОЛОГІЧНИЙ АНАЛІЗ ПАРОДОНТИТА РІЗНОГО ГЕНЕЗУ.

О.М.Бабай, Ю.В.Меркулова, Т.В.Деєва

Модель пародонтиту при введенні бактеріального ендотоксину дозволяє направлено викликати у тварин комплекс запально-дистрофічних змін пародонту, типових для пародонтопатій у людини і патогномонічних пародонтиту людини, що обґрунтовує доцільність використання даної моделі при вивченні пародонтопротекторної активності нових лікарських засобів.

Ключові слова: пародонтит, щурі, експеримент

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Резюме. СРАВНИТЕЛЬНІЙ КЛИНИКО-МОРФОЛОГИЧЕСКИЙ АНАЛИЗ ПАРОДОНТИТА РАЗЛИЧНОГО ГЕНЕЗА.

О.Н.Бабай, Ю.В.Меркулова, Т.В.Деева

Модель пародонтита при введении бактериального эндотоксина позволяет направленно вызывать у животных комплекс воспалительно-дистрофических изменений пародонта, типичных для пародонтопатий у человека и патогномоничных пародонтиту человека, обосновывает целесообразность использования данной модели при изучении пародонтопротекторной активности новых лекарственных средств.

Ключевые слова: пародонтит, крысы, эксперимент

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Introduction. Chronic generalized periodontitis (CGP) is one of the most common stomatological diseases. Morbidity of population is not reduced, despite of a number of studies of periodontal disease and methods of treatment [1, 2].

Several factors play a role in the development of periodontal disease. The violation of the periodontal trophic as a result of deterioration of the microcirculation, microbial factor, imbalance in the immune and barrier systems, biochemical changes, violations of SRO and AOP are considered the most important in the pathogenesis of periodontitis. Emotional stress, antioxidant deficiency, intoxication with pro-oxidants can also play a role. However, many fundamental aspects of CGP remain unclear and controversial [3, 4].

Objectives of the study - a comparative analysis of pathomorphological data obtained during the experimental modeling of the inflammatory and degenerative changes of the periodont of different genesis. We used a homogeneous food (pasty diet) and endotoxins of gram-negative bacterias to reproduce this pathology.

It is known, that endotoxins (lipopolysaccharides) play an important role in etiology of the periodontitis, causing rapid development of the pathology, accompanied by functional and biochemical changes characterized for human generalized periodontitis [5, 6].

Model of reduction of chewing function in which animals get a special paste-like diet, is common in experimental pharmacology and traditionally used in study of parodontoprotecting activity of new drugs [7].

Materials and methods. A group of 40 sexually mature Wistar rats, male and female weighing 183-250 g, was divided into a control group of 20 rats and an experimental group of 20 rats. In the first experimental group periodontitis was reproduced by the special paste diet during 30 days (model of reduction of chewing function) – the first group.

In the second experimental group periodontitis was reproduced by 4 multiple injections of the bacterial lipopolysaccharide (LPS) in to the gum tissue and dentogingival junction (50 MKRL LPS Escherichia coli; 1 mg / ml) in the lower region of the first molar jaw every 48 hours for two weeks – the second group.

Every day, before the manipulation, animals were examined. State of periodontal tissues were determined by the results of visual and instrumental studies [8].

Animals were killed by decapitation under tiopental narcosis according to the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [9]. Then morphological examination of the gum tissues and pieces of jaw with teeth was performed.

The material was fixed in 10% neutral formalin solution, decalcified in 5% of 1N formic acid solution. After decalcification biological samples were embedded in paraffin-celloidin.

Sections of 6-8 microns thickness were stained with hematoxylin and eosin. Morphometric measurements were made for objectification of data [10,11].

Statistical data analysis was performed on a PC using the software system «Statistica 6.0» with using of the nonparametric methods (Kruskal-Wallis test and test Mann-Whitney). The differences between the values of comparable parameters were regarded as statistically significant when you reach the level of statistical significance (p) less than 0.05 ($p < 0.05$). **The results obtained in the study data are presented as mean (M) ± standard error of average (m).**

Results. Both models successfully reproduced the experimental periodontitis in animals, but had their own peculiarities. Animals of both experimental groups exhibit clinical symptoms of the periodontitis: varying degrees of the gums hyperemia was detected in 70% of cases in the first model and in 100% of cases in the second (table 1). Significant swelling of the gums was developed in 50% of the animals with experimental periodontitis in the first model, and in 80% - in the second model. Periodontal pockets between teeth and gums were found in half of animals in both groups.

Pathological pockets were marked mainly near the first molar of the lower jaw. Pockets were sufficiently deep and opened the tooth roots, this has led to loosening of the teeth in 10% and 30% of the rats.

Table 1

State of the periodontal tissues in rats with experimental periodontitis

Model of periodontitis	Indicators of periodontal, %			
	hyperemia	swelling	periodontal pocket	loosening of teeth
Model of reduction of chewing function	70	50	50	10
Model of the bacterial endotoxin	100	80	50	30
Control	0	0	0	0

Notes:

1. Quantity animals in each model - 10;

2. Calculated as quantity of animals to a specific pathological condition in periodontal, expressed as a percentage (%) to the total amount in the model.

In animals of the first group histological changes were observed in microcirculatory bed, bone tissue and in the periodontal soft tissues. Vessels (mainly veins) have been dilated, their lumens were lined with swollen endothelium and contain blood cells.

Despite of the damage of the vessels, investigations at this stage found only a moderate and minor inflammatory processes in the soft tissues of periodontal and vacuolization of gum epithelial cells. Active lacunar resorbtion of the bone tissue was found: alveolar bones had rounded crests, corrugated lateral surfaces, apexes of the interradical septums were resorbed too.

Formed deepening and lacunas were replaced by a connective tissue. The morphometric parameters were changed respectively: compared with the control distance between cemento-enamel junction and the crest of alveolar bone increased, as well as distance between the point of bifurcation and interradical septum (Table 2).

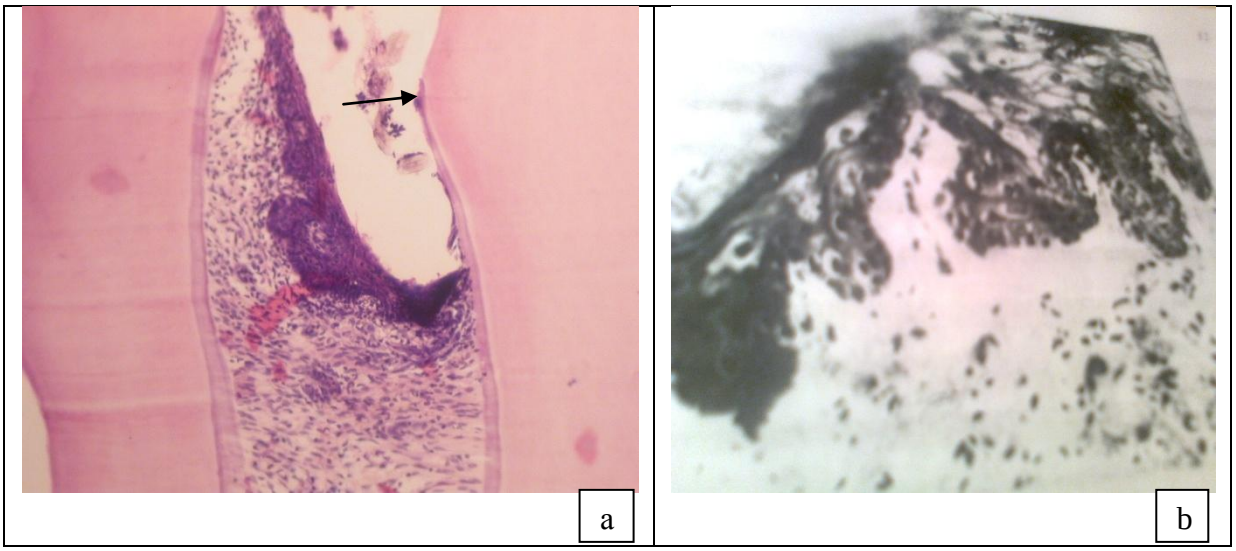
Table 2

Morphometric parameters of the periodontal tissues of the rats

Model / Indicators	height of the gingival papilla, mk	distance bitween the point of bifurcation and interradical septum, mk	distance between cemento-enamel junction and alveolar bone, mk	number of teeth exposing tooth root (%)	depth of periodontal pocket, mk
Control	238,6 ±22,8	72,0 ± 10,28	291,1 ± 30,9	0	167,0±30,3
Reduction of chewing function	343,2 ±43,0	155,0 ± 33,7	344,6 ± 38,09	23	345,0±70,1
Bacterial endotoxin introduction	272,0± 34,7	130,0 ± 33,7	650,0 ±111,7	55,5	476,0±142,8

Dystrophic changes of the soft tissues surrounding the tooth led to the formation of the periodontal pockets: the moderate hyperplasia of the epithelial layer was observed and subsequent apical migration of the junctional epithelium of the most teeth (Picture 1,a). Perinuclear swelling of the spinosum cells has been founded, a typical arrangement of the nuclei of the basal cells has been broken (Picture 1,b). The epithelial junction was located above the cemento- enamel junction level only in 15 % of the cases, in 60% - equal with it, and in 23% cases destruction of gingival sulcus, denudation of the anatomical tooth root, formation of the pathological tooth-gingival pockets were noted. The bottom of this pockets was located lower of the cemento- enamel junction at 20 -200 microns. At the same time formation of the pathological pockets in intact animals were not found.

More active vegetation of the epithelium bands along the cementum of the tooth root and a more significant depth of periodontal pockets were fixed in the animals with endotoxin-induced periodontitis (Table 2). The epithelial junction never was located above the cemento- enamel junction level, in 44.4% of cases equal with it and in 55.5% - dipped below at 40-1000 microns. The epithelium of the gum pocket was differentiated, hyperplastic and forms like bulb thickening. Sometimes the epithelium fells to the bottom third of the tooth root or to the bifurcation in multi-rooted teeth (Picture 2,a).



Picture 1. Rat with periodontitis provoked a decrease of chewing efficiency:

a - periodontal pocket, the epithelial layer is fixed below the level of cemento- enamel junction (arrow), leukocytes are collected in periodontal pocket. Hematoxylin and eosin x 150

b - perinuclear swelling of the spinosum cells, a typical arrangement of the nuclei of the basal cells are broken. Hematoxylin and eosin x 400

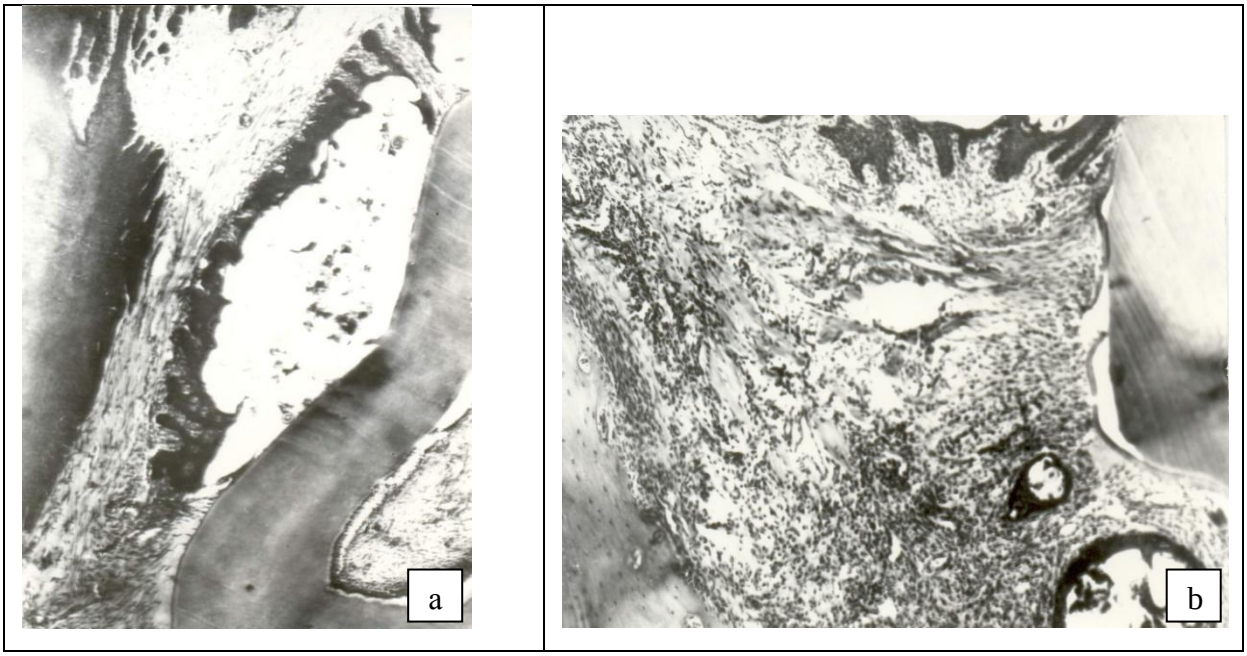
Compared with the previous group is more much expressed inflammatory processes along the minimal vascular reaction.

A migrating deeply epithelial layer was infiltrated with leukocytes, cellular infiltration sometimes defined in subepithelial connective tissue. Horizontal and vertical bone resorption occurred more much active than that was triggered by decreasing of chewing function. Alveolar processes were significantly lower, their arexes lost conical shape.

In some cases, the resorption was so strongly expressed that in place of alveolar bone a few thinned bone lamellae were only. The lamellae were located among infiltrated by leukocytes fibrous connective tissue in the form of islands (Picture 2,b).

Sometimes large cysts filled with dreggy contents were defined in the apex of the tooth root. Resorption of the alveolar processes and simultaneously teeth pushing upon the destruction of gingival sulcus led to an increase of the distance from the cemento-enamel junction to alveolar process. And distance increases

from the bifurcation point to the interradical septum, that is significant for atrophy of this part of the alveolar bone.



Picture.2. The rat with periodontal endotoxin-induced periodontitis:

a - periodontal pocket, the epithelial layer migrated into pericement and moved to the lower third of the root; Hematoxylin and eosin x 250

b - resorption of the alveolar bone, vacant proliferation of the connective tissue, cellular infiltration, epithelial cysts. Hematoxylin and eosin x 160

Discussion of research results. According to the results of histological examination it is obviously that both models leads to development of periodontitis of moderate severity with generalized character of lesion in the experimental animals.

Characterized for this disease inflammatory, dystrophic and destructive processes, exactly- formation of the periodontal pockets, due to damage of the cemento-epithelial junction and apical migration of the junctional epithelium; lacunar resorption of alveolar bones and interradical septums were observed.

However, each model has its own characteristics. Periodontitis caused by special paste diet, has a predominantly destructive nature with mild inflammation. At the same time, the endotoxin-induced periodontitis, along with the destruction of

solid tissues leads to the development of more strongly expressed inflammatory-dystrophic processis in soft tissues.

Conclusions. Thus, injections of the bacterial endotoxin cause the development of inflammatory and degenerative changes of periodontal analogous to human periodontitis. So we substantiate appropriateness of the model in the study of parodontoprotecting activity of new drugs.

Prospects studies. The study of mineral metabolism changes taking place in the alveolar crest at a selected model of experimental periodontitis. This allows to use this model for the study of parodontoprotecting activity of new drugs.

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