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PHAGOCYTIC ACTIVITY OF PERIPHERAL BLOOD NEUTROPHILS IN MECHANISMS OF EXPERIMENTAL BACTERIAL-IMMUNE PERIODONTITIS DEVELOPMENT

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One of the important links that leads to injury in all structures of the periodontium and formation of inflammation of various severity is phagocytic activity of polymorphonuclear leukocytes. The purpose of the study was to evaluate pathogenic role of neutrophils activity in the experimental bacterial-immune inflammation in periodontium. The article shows the results of studies on the spontaneous and activated test with nitroblue tetrazolium on the 7th, 14th and 30th days of the bacterial-immune inflammation in periodontal tissues. In this paper, the results of neutrophils activity changes in the process of formation and chronification of the inflammation in the tissues of periodontal complex are presented. It was established that the nature of the experimental inflammation course in periodontal tissues depending on the peculiarities of phagocytic activity changes in blood neutrophils. In this condition the spontaneous test with nitro blue tetrazolium of blood neutrophils accompanied the growth and indices of the oxygen-dependent metabolism reserve decreased in the induced nitro blue tetrazolium test, the coefficient of blood neutrophils metabolic activation in the rats with inflammatory process in periodontium and testified about metabolic reserves depletion of these cells and disorder of phagocytic activity.

Key words: phagocytosis, bacterial-immune periodontitis, neutrophils, immune system.

The work is a fragment of the research project "Systemic and organic violations due to the actions of extraordinary factors on the body, mechanisms of their development and pathogenetic correction", state registration No. 0116 U003390.

Inflammatory diseases of periodontal tissues idespread in the all world, and Ukraine is one of the countries with a high incidence of chronic generalized periodontal disease. According to various authors, there is a further increase of periodontal disease in our country in last years, which has both general medical and social significance [3].

The development and progression of inflammatory diseases of the periodontium is now considered not so much as a local inflammation as well as reaction of the whole organism to the bacterial invasion in the periodontal tissues. It is known that the development of periodontitis is a result of an imbalance between the microflora of the oral cavity, that is, microbiote, and immune protection of the body [12]. It should also take into account the activity of inflammatory cells and adequacy of mechanisms of resistance [8, 11]. The bacterial factor is not enough for development of inflammation one, should be a combined of pathogenetic factors. There is means fixing of them not only in the tissues of periodontium, but also in all organism, from that depends on effectiveness of standard therapy [6].

Now, much evidence has been accumulated that the risk of systemic diseases may increase as a result of periodontal disease. This concerns, for example, coronary heart disease, myocardial infarction, stroke, arteriosclerosis. There is a necessity in deep and comprehensive study of the disorder features of immunological processes in the mechanisms of this pathology development as one of important parts of pathogenesis [9]. It is generally known that one of the key links in the phagocytosis immunity is, which is considered not only as an antiinfectious immunity tool, but also as a universal effector that responds to numerous signals about destabilization of the body's internal environment and is one of key pathogenetic links in the development of immune and inflammatory reactions [10].

The purpose of the study was to determinate the pathogenetic role of neutrophils activity for the experimental bacterial-immune inflammation in periodontium.

Materials and methods. The studies were performed with the use of non-linear, clinically healthy white rat males weighing 150-200 g in the conditions of vivarium. Experiments were carried out in compliance with the general rules and regulations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and the "General Ethical Principles of Animals Experimentation" (Kyiv, 2001). Animals were kept throughout the observation period on a standard diet, balanced by elements of food with free access to water. The rats were randomly divided into four groups: I group (n = 10) – intact animals; II group (n = 8) – animals with bacterial-immune inflammation in the periodontium on the 7th day of the experiment; III group (n = 8) – animals with bacterial-immune inflammation in the periodontium on the 14th day of the experiment, IV group (n = 8) – animals with bacterial-immune inflammation in the periodontium on the 30th day of the experiment.

The experimentally modelled bacterial-immune periodontitis was produced in the rats by injection into periodontal tissues complex mixture of microorganisms diluted in protein (egg white). A complete

Freund's adjuvant was injected in the animal's paw simultaneously with the introduction of the pathogen to enhance the immune response. To IV group of animals was injected pathogen with adjuvant repeatedly on the 14th day. Thus, an increase in the reproduction efficiency of bacterial-immune periodontitis was achieved [2]. On the 30th day of the study, rats were killed by bleeding under thiopental anesthesia. Blood was taken for further research in which the bactericidal action of neutrophils was determined using the nitro-blue tetrazolium reduction test (NBT-test) [4].

The spontaneous NBT-test was performed with phagocytes which were cultivated in the presence of nitro blue tetrazolium without prior activation of cells, while carrying out an induced NBT-test, an activator of phagocytic reaction was added to the culture medium. The reaction was evaluated by a result of counting 100 neutrophils with the presence of granules and grains of diformasan in the cytoplasm. In the cytoplasm of the cells, which reacted positively with nitro blue tetrazolium, precipitation of granular diformasan blue-violet color was recorded. In the negative reaction with NBT, granulates of diformasan in the cytoplasm of the cells were absent. Reserve possibility of oxygen-dependent metabolism was determined for index of reserve and metabolic activation coefficient.

The results were statistically processed using the software STATISTICA Version 10.0 ("Statsoft", USA) [5]. The reliability of the difference in values between independent quantitative values was determined with the normal distribution by criterion U-criterion Mann-Whitney [7].

Results of the study and their discussion. In a result of this research was found for early stage of inflammation in the periodontium, which included period from the 1st to the 7th day of the experiment, excessive increase of the number formazan-positive neutrophils (by 1.83 times; $p < 0.01$) in the spontaneous nitro blue tetrazolium test (NBT-test) as compared to the intact animal group (table 1, fig. 1).

Table 1

Indices of spontaneous NBT-test of rat's blood neutrophils for different periods of modelled inflammation development in the periodontium ($M \pm m$)

Groups of animals	Duration of the experiment (days)	Number of the rats	Spontaneous NBT-positive test, %
Control, intact rats	-	10	14.39 \pm 0.84
Animals with periodontal inflammation	7	8	26.34 \pm 1.07 $p_1 < 0.01$
	14	8	22.54 \pm 0.90 $p_1 < 0.01$; $p_2 < 0.05$
	30	8	19.80 \pm 0.72 $p_1 < 0.01$; $p_2 < 0.01$; $p_3 > 0.05$

Note 1: p_1 – index of differences relative to intact animals. 2: p_2 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 7th day of the experiment. 3: p_3 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 14th day of the experiment.

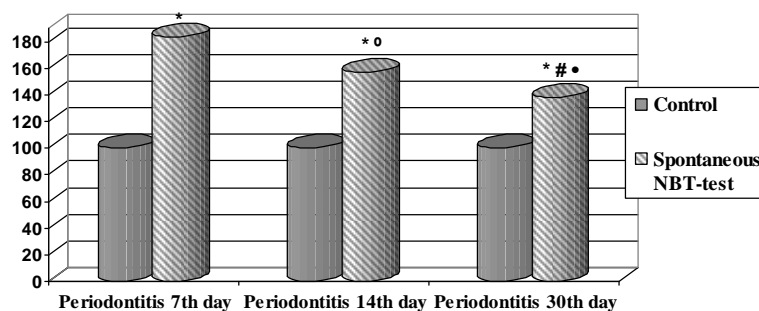


Fig. 1. – Changes in the indices of spontaneous NBT-test in the rats blood neutrophils in conditions of modelled bacterial-immune periodontal inflammation (% of control).

Note 1: * – differences between the control rats ($p < 0.01$). 2: # – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.01$). 3: ° – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.05$). 4: • – differences between rats with periodontal inflammation on the 14th day of the research ($p > 0.05$).

Studying the presence of diformasan-positive cells in activated NBT-test in the animals with experimental modelled bacterial-immune inflammation on the 14th day of the pathological process in the periodontium, number of neutrophils with these properties was found increased (by 1.19 times; $p < 0.05$) compared to animals, which were studied on the 7th day, and decreased compared to the results of the intact group (by 1.47 times ($p < 0.01$)).

The activated NBT-test characteristics functional activity of phagocytes and affects their readiness for complete phagocytosis in the presence of antigenic stimuli [1]. In a process of perform an activated NBT-test opposite-directed results were obtained (table 2, fig. 2). The number of diformasan-positive neutrophils was found decreased in the rats, which was enquire on the early stage (7th day) of the experimental periodontitis (by 1.75 times; $p < 0.01$), compared to the control rats.

Table 2

Indices of activated NBT-test of rats blood neutrophils for different periods of modelled inflammation development in the periodontium (M±m)

Groups of animals	Duration of the experiment (days)	Number of the rats	Activated NBT-positive test, %
Control, intact rats	-	10	31.07±1.01
Animals with periodontal inflammation	7	8	17.81±0.69 $p_1 < 0.01$
	14	8	21.20±0.74 $p_1 < 0.01$; $p_2 < 0.05$
	30	8	24.93±0.52 $p_1 < 0.01$; $p_2 < 0.01$; $p_3 < 0.01$

Note 1: p_1 – index of differences relative to intact animals. 2: p_2 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 7th day of the experiment. 3: p_3 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 14th day of the experiment.

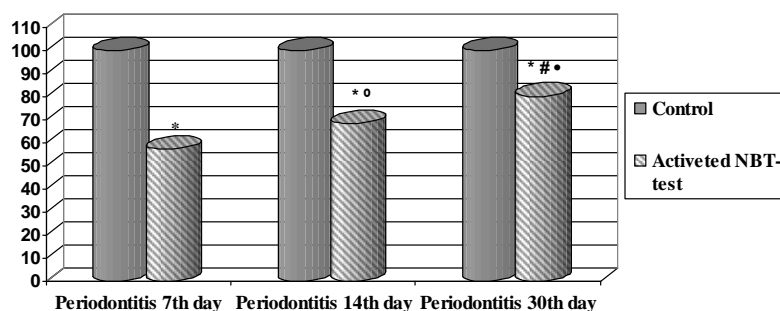


Fig. 2. – Changes in the indices of activated NBT-test in the rats blood neutrophils in conditions of modelled bacterial-immune periodontal inflammation (% of control).

Note 1: * – differences between the control rats ($p < 0.01$). 2: # – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.01$). 3: ° – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.05$). 4: • – differences between rats with periodontal inflammation on the 14th day of the research ($p < 0.01$).

In comparison of diformasan-positive cells amount in spontaneous NBT-test on the 30th day of experimental periodontitis development, were found reduced their in relation with the 14th day, but the data statistically were doubtful ($p > 0.05$), at the same time meant were significantly lower (by 1.33 times; $p < 0.01$), in comparison with the 7th day of the study. However, this indicators was also significantly higher in comparison to the intact group of animals (by 1.38 times; $p < 0.01$).

It should be noted that number of diformasan-positive neutrophils in activated NBT-test of the experimental animals with periodontitis on the 30th day of the experiment was also lower (by 1.25 times; $p < 0.01$) in relation to indicator of the intact animals. However, the indicated data were higher according to those obtained in rats on the 7th (by 1.40 times; $p < 0.01$) and 14th (by 1.18 times; $p < 0.01$) days of experiment.

The coefficient of metabolic activation of phagocytes also changed. Thus, on the 7th day of bacterial-immune periodontal inflammation, it was decreased, if compared to the intact rats (by 1.79 times; $p < 0.01$) (table 3).

As it can be seen from table 3, it means that metabolic activation of phagocytic neutrophils was found decreased (by 1.46 times; $p < 0.01$) on the 14th day of the study as compared to the control group of rats. It should be noted that this index increased on the 14th day of the study in relation to the 7th day (by 1.23 times; $p < 0.05$) (fig. 3).

Table 3

Indices of oxygen-dependent metabolic reserve and coefficient of metabolic activity of animals blood phagocytes for different periods of modelled inflammation development in the periodontium (M±m)

Experiment conditions and indices	Control, intact rats	Animals with periodontal inflammation		
Duration of the experiment (days)	-	7	14	30
Number of the rats	10	8	8	8
Reserve of the oxygen-dependent metabolism	2.24±0.17	0.69±0.05 $p_1 < 0.01$	0.98±0.07 $p_1 < 0.01$; $p_2 < 0.05$	1.27±0.04 $p_1 < 0.01$; $p_2 < 0.01$; $p_3 < 0.05$
Coefficient of metabolic activation	29.23±1.91	16.31±0.78 $p_1 < 0.01$	20.07±0.81 $p_1 < 0.01$; $p_2 < 0.05$	24.13±0.52 $p_1 < 0.01$; $p_2 < 0.01$; $p_3 < 0.01$

Note 1: p_1 – index of differences relative to intact animals. 2: p_2 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 7th day of the experiment. 3: p_3 – index of differences relative to animals with bacterial-immune periodontal inflammation on the 14th day of the experiment.

The performed studies showed that coefficient characterizing the activity of blood neutrophils on the 30th day of the study was higher, as compared to the groups of rats with experimental inflammations on the 7th and 14th day of the study, respectively (by 1.48 times; $p < 0.01$ and by 1.20 times; $p < 0.01$). But, it increased (by 1.21 times; $p < 0.01$) than in the group of animal without periodontitis (fig. 3).

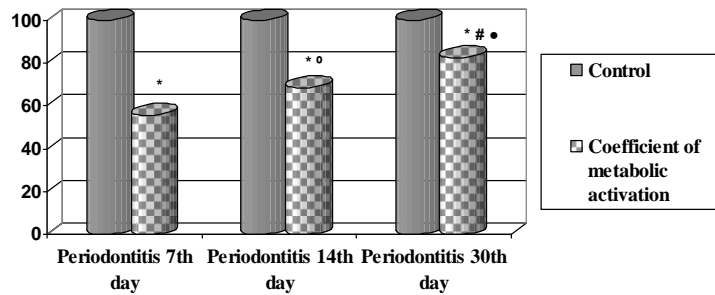


Fig. 3. – Changes in the indices of metabolic coefficient activation in the rats blood neutrophils in conditions of modelled bacterial-immune periodontal inflammation (% of control).

Note 1: * – differences between the control rats ($p < 0.01$). 2: # – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.01$). 3: ° – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.05$). 4: • – differences between rats with periodontal inflammation on the 14th day of the research ($p < 0.01$).

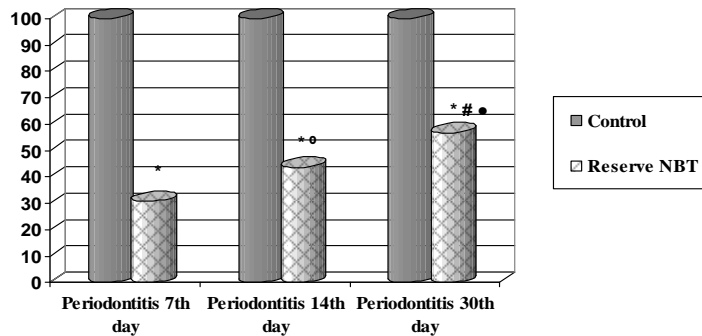


Fig. 4. – Changes in the indices of oxygen-dependent metabolic reserve in the rats blood neutrophils in conditions of modelled bacterial-immune periodontal inflammation (% of control).

Note 1: * – differences between the control rats ($p < 0.01$). 2: # – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.01$). 3: ° – differences between rats with periodontal inflammation on the 7th day of the research ($p < 0.05$). 4: • – differences between rats with periodontal inflammation on the 14th day of the research ($p < 0.05$).

A similar tendency to decrease was found in determination of the oxygen-dependent metabolic reserve index (table 3). In particular, it was decreased (by 3.25 times; $p < 0.01$) in the experimental animals with bacterial-immune inflammation on the 7th day, in relation to the intact group.

Subsequently, there was gradual increase of metabolic reserve in NBT-test (by 1.42 times; $p < 0.05$) on the next period (14th day) for the pathological process, compared with the group of rats that were studied on the 7th day of the research (fig. 4).

That is, these indices were reduced compared to the intact animal group (2.29 times; $p < 0.01$).

In the further study, on the 30th day of the inflammation development in the periodontal tissues, the index of reserve capacity of the oxygen-dependent metabolism of blood phagocytes has shown significant increase in comparison with the indices on the 7th day (by 1.84 times; $p < 0.01$), and also on the 14th day (by 1.30 times; $p < 0.05$). However, this index was significantly lower (by 1.76 times; $p < 0.01$) compared to the control group.

Conclusions

1. Increased indices of spontaneous test with nitro blue tetrazolium of blood neutrophils in experimental animals with bacterial-immune modelled periodontitis indicate the predominance of their oxygen-dependent bactericidal activity and presence in the blood of activators that can stimulate the functional activity of these phagocytes.
2. Reduced indices of induced NBT-test of oxygen-dependent metabolism, metabolic reserve rate of blood neutrophils in the rats with inflammatory process in the periodontium is a result of metabolic reserves depletion of these cells and of phagocytic processes disorder.

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Реферати

ФАГОЦИТАРНА АКТИВНІСТЬ НЕЙТРОФІЛІВ КРОВІ В МЕХАНІЗМАХ РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО БАКТЕРІАЛЬНО-ІМУННОГО ПАРОДОНТИТУДемкович А.Є., Бондаренко Ю.І., Гасюк П.А.,
Сухоалець І.О.

Однією із важливих ланок в механізмах розвитку, що призводять до ушкодження структурних компонентів пародонтального комплексу та розвитку запального процесу з різним ступенем тяжкості, є фагоцитарна активність лейкоцитів. Мета дослідження полягала в оцінці активності нейтрофілів крові при експериментальному змодельованому бактеріально-імунному пародонтиті. У статті наведені результати досліджень показників спонтанного та активованого тесту з нітросинім тетразолієм на 7-му, 14-ту і 30-ту добу експериментального бактеріально-імунного запалення в пародонті. При цьому наводяться дані щодо характеру змін фагоцитарної активності нейтрофілів у процесі формування і хронізації запального вогнища в пародонтальних тканинах. Також встановлено, що характер перебігу даного змодельованого запального процесу запалення в пародонтальних тканинах залежав від особливостей змін фагоцитарної активності нейтрофілів крові. При цьому запальний процес супроводжувався підвищенням спонтанного тесту з нітросинім тетразолієм нейтрофілів крові та зниженням показників індукованого НСТ-тесту, резерву оксиген-залежного метаболізму, коефіцієнта метаболічної активації нейтрофілів крові шурів із запальним процесом у пародонті та свідчив про виснаження метаболічних резервів даних клітин і порушення процесів фагоцитозу.

Ключові слова: Фагоцитоз, нейтрофіли, імунна система, бактеріально-імунний пародонтит.

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ФАГОЦИТАРНАЯ АКТИВНОСТЬ НЕЙТРОФИЛОВ КРОВИ В МЕХАНИЗМАХ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО БАКТЕРИАЛЬНО-ИМУННОГО ПАРОДОНТИТАДемкович А.Е., Бондаренко Ю.И., Гасюк П.А.,
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Одной из важных звеньев в механизмах повреждения структур пародонтального комплекса и формирования воспалительного процесса с различной тяжестью, является фагоцитарная активность лейкоцитов. Цель исследования заключалась в оценке активности нейтрофилов крови при экспериментальном моделированном бактеріально-імунном воспалении в пародонте. В статье показаны результаты исследований полученных показателей спонтанного и активированного теста с нитросиним тетразолием на 7-ые, 14-ые и 30-ые сутки экспериментального пародонтита. При этом приводятся данные о характере изменений активности нейтрофилов в процессе формирования и хронизации воспалительного очага в тканях пародонта. Установлено, что характер течения экспериментального воспаления в тканях пародонтального комплекса зависит от особенностей изменений фагоцитарной активности нейтрофилов. При этом воспалительный процесс сопровождался повышением спонтанного теста с нитросиним тетразолием нейтрофилов крови и снижением показателей индуцированного НСТ-теста, резерва кислород-зависимого метаболизма, коэффициента метаболической активности нейтрофилов крови крыс с воспалительным процессом в пародонте и свидетельствовал об истощении метаболических резервов данных клеток и нарушении процессов фагоцитоза.

Ключевые слова: Фагоцитоз, нейтрофилы, иммунная система, бактеріально-імунный.

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MORPHOLOGICAL CHANGES IN RAT HEART MUSCLE IN EXPERIMENTAL PERITONITIS AGAINST THE BACKGROUND OF DIABETES MELLITUS

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The purpose of the work was to study the morphological changes in the heart muscle of rats with modeled acute peritonitis against the background of diabetes mellitus compared to animals with experimental widespread acute peritonitis. Structural changes of cardiomyocytes (focal intracellular myocytolysis and pycnotically altered nuclei), pronounced perivascular edema, focal dystrophic-necrotic changes, polymorphocellular infiltrates, which were located in the stroma, perivascularly and around the necrotically altered cardiomyocytes, were revealed. Structural changes of cardiomyocytes and disturbances in all links of the microhemocirculatory bed, which were manifested in dystonia and paresis of vessels, changes in rheological properties of blood were more pronounced in the conditions of comorbid pathology.

Key words: acute peritonitis, diabetes mellitus, morphological changes of the heart muscle.

The work is a fragment of the research project "Pathogenetic features of the allergic and inflammatory processes course and their pharmacocorrection", state registration No. 0116U004503.

The urgency of timely diagnosis and treatment of widespread acute peritonitis (WAP) against the background of diabetes mellitus (DM) is due to long-term disability, complicated course and high mortality [7, 10]. One of the main provisions in all modern concepts of various diseases' pathogenesis, including WAP, is the cell membrane structure damage. As a consequence, complex morphofunctional changes of the internal organs lead to the development of complications, which are the main causes of lethality in