2,5 рази) і інтервалу SUB-G0G1 (в 2,7 рази), що свідчить на недостатню компенсацію проліферативної активності клітин селезінки на фоні посиленого апоптозу.

Ключові слова: показники клітинного циклу, фрагментація ДНК, селезінка, щури, опік шкіри, 0,9 % розчин NaCl.

Стаття надійшла 5.11.2017 р.

(почти в 2,5 раза) и интервала SUB-G0G1 (в 2,7 раза), что свидетельствует о недостаточной компенсации пролифера-тивной активности клеток селезёнки на фоне усиленного апоптоза.

Ключевые слова: показатели клеточного цикла, фрагментация ДНК, селезёнка, крысы, ожог кожи, 0,9 % раствор NaCl.

Рецензент Єрошенко Г.А.

DOI 10.26.724 / 2079-8334-2018-1-63-120-122
УДК 616.155.3-008.13-002.43-06:616.314.18-002.4-022.7]-092.9

A. Ye. Demkovych
I. Horbachevsky Ternopil State Medical University, Ternopil

THE NECROTIC-APOPTOTIC CHANGES IN BLOOD MONONUCLEAR PHAGOCYTES IN THE EXPERIMENTAL BACTERIAL-IMMUNE PERIODONTITIS DEVELOPMENT

demkovushae@tdmu.edu.ua

Mechanisms of the inflammatory process development in the periodontal complex include a number of complicated processes leading to the generalization and chronicization, tooth loss and the occurrence of complications in the other organs. The purpose of the study was to determine the level of apoptotic changed and necrotic mononuclear blood phagocytes in the dynamics of development of experimental bacterial-immune periodontitis. The paper presents the results of studies of early and late apoptosis indices of blood monocytes on the 7th and 30th days of the development of the inflammatory process in periodontal tissues. Monocytes were isolated from blood of experimental animals by gradient centrifugation. Evaluation of necrosis and apoptosis of mononuclear phagocytes was carried out by the flow laser cytofluorimetry. The results were statistically processed using parametric and nonparametric statistical methods. Dynamics of dead cells number was revealed during the formation of the focus of inflammation in the periodontal complex. In particular, the progress of experimental periodontitis was accompanied by the increase of annexin-positive (early apoptosis) and necrotic monocytes content, which is associated with increased intensity of their formation in response to antigen stimulation. In the simulated pathology the induced cell death was achieved mainly by apoptosis.

Key words: Bacterial-immune periodontitis, inflammation, mononuclear phagocytes, necrosis, apoptosis.

The paper is a part of the RSW "Systemic and organic violations due to the actions of extraordinary factors on the body, mechanisms of their development and pathogenetic correction" (registration number 0116 U003390) and "Pathogenetic approaches to treatment the main dental diseases on the basis of studying the mechanisms of damage of the oral cavity tissues against the background of accompanying somatic pathology "(state registration number 0116 U005076).

The etiology and pathogenesis of periodontal diseases is insufficiently studied and form one of the important problems of theoretical and practical medicine [7]. The main role in this belong to infectious factors and the inability of immune defense (local cellular nonspecific and general adaptive) to form an adequate nature of the development and progress of the pathological process in the oral cavity. This fact is crucial for the effectiveness of therapeutic interventions and preventive measures [6]. Among the most common diseases connected with periodontal complex is periodontitis, particularly its generalized form, in which inflammatory-dystrophic processes implicate all its tissues. The mechanisms, leading to inflammatory-destructive lesions of periodontal tissues due to local and general factors, various in nature and specificity, are poorly understood to date [16]. Notably, the development of chronic inflammation process involves destruction of periodontal and bone tissue, the immune response to oral microorganisms, which is achieved uncommonly. In the most cases, the process develops along with a low bactericidal potential of phagocytic cells, in particular, mononuclear phagocytes, polyclonal activity of B-lymphocytes, and a high level of antibacterial antibodies and dysfunction of T-lymphocytes [10,11]. At the same time, accretion of granulations, as violation of proliferative processes, an imbalance in the production of cytokines, apoptosis activation and development of hypoergic inflammation occur [3, 5]. Cytokines derived from monocytes as well as T cells modulate apoptosis, implicating regulatory circuits in monocyte survival. The capacity to therapeutic regulate monocyte apoptosis promises to have in promoting rapid healing or reducing chronic inflammation.

The purpose of this study was to determine the level of necrotic- and apoptotic-changed mononuclear blood phagocytes in the dynamics of experimental bacterial-immune periodontitis development.

Materials and methods. White outbred clinically healthy rats, 150-200 g weight, which were kept in conditions of vivarium in accordance with the sanitary standarts and GLP were involved into study. The experiments were performed according to 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 Animal Experimentation” (Kyiv, 2001).

The animals were random and divided into groups: I – intact animals, control (n = 10); II – animals with experimental periodontitis on the 7th day of the research (n = 8); III – animals with experimental periodontitis on the 30th day of the research (n = 8). Experimental periodontitis was induced in the experimental animals by introducing complex mixtures of microorganisms diluted in egg protein into

periodontal tissue [1]. Simultaneously with the injection of the pathogen a complete Freund's adjuvant was injected in the rat's paw to enhance the immune response. When conducting studies with animals of group III, on the 14th day, repeated entry of pathogenic and injection of adjuvant was carried out. The experimental animals were exsanguinated on the 7th and 30th day under thiopental anesthesia. For further testing, blood serum was selected, with which the monocytes were isolated by gradient centrifugation [13]. FITC-labeled annexin V from the ANNEXIN V FITC reagent kit (Beckman Coulter, USA) was used to evaluate apoptosis and necrosis of monocytes using flow cytofluorimetry [14]. The results were presented in percent (the ratio of the number of annexin-positive cells to the total number of the phagocyte fraction). The results were statistically processed using parametric and nonparametric statistical methods using the Excel software ("Microsoft", USA) and "STATISTICA" 10.0 (Statsoft, USA) [4]. The reliability of the differences in the values between independent quantitative values was determined with a normal distribution according to the Mann-Whitney U criterion [8].

Results and discussion. We used the bacterial-immune model of experimental periodontitis, because the mechanisms of the inflammatory process formation in the periodontal tissues include complex of pathogenic links, which might be similar to manifestations of the human generalized periodontitis [2, 9]. The apoptosis plays an important role in the development and issue of inflammatory diseases. There is a disbalance between the proliferative, necrotic processes and apoptosis of mononuclear phagocytes in generalized periodontitis [12, 15]. The results of our research have been shown that monocytes occupy one of the main positions in maintaining local immunity and in contact with antigens may expose to apoptosis. As mentioned above, it may be assumed that apoptosis of mononuclear phagocytes is one of the mechanisms controlling the development and progress of the inflammatory process in the periodontal complex. It is important to compare the results of apoptosis and necrosis of mononuclear phagocytes in the experimental bacterial-immunode periodontitis. In particular, studies have found that changes in the total number of dead cells and their correlation in the blood for inflammation in the periodontal complex increased as compared to controls, especially at the earlier stages (Table 1).

Table 1

The levels of necrotic- and apoptotic-changed monocytes of the rat's blood in dynamics of the experimental bacterial-immune periodontitis development (M ± m)

Indices	Control, intact animals	Animals with experimental periodontitis	
Experiment duration (days)	-	7	30
Number of animals	10	8	8
Necrotic-changed cells, %	0.95±0.02	2.11±0,06 p_1<0.01	1,21±0,07 p_1<0,01; p_2<0,01
Apoptotic-changed cells, %	5.00±0.02	7.89±0.05 p_1<0.01	6.67±0.05 p_1<0.01; p_2<0.01
Cells died, %	5.95±0.03	10.00±0.11 p_1<0,01	7,86±0,09 p_1<0.01; p_2<0.01
Unchanged cells, %	94.05±0.03	90.00±0.11 p_1<0.01	92.14±0.09 p_1<0.01; p_2<0.01

Notes: 1. p_1 – reliability of differences in relation to intact animals; 2. p_2 – reliability of differences in relation to animals with experimental periodontitis on the 7th day of the research.

Our study showed that at the early stage of the inflammatory process formation in the periodontal complex (the second research group), which included the period from the 1st to the 7th day of the experiment, the total number of damaged monocytic cells significantly increased (by 1.68 times; p<0.01). At the same time, a high level of death was mainly due to mononuclear phagocytes with signs of late apoptosis / necrosis, which exceeded by 2.22 times (p<0.01) the indices of the intact group. In cells with signs of early apoptosis, their level was also significantly higher (by 1.56 times; p<0.01) as compared to control values.

The analysis of the results obtained on the 30th day of the study has shown a similar pattern of changes, that is, an increase in the total number of cells that died as compared to the control group (by 1.32 times; p<0.01). In addition, the induced death of monocytes arose due to both apoptosis and necrosis, indices of which exceeded the control values by 1.33 (p<0.01) and 1.27 times (p<0.01), respectively. The analysis of annexin-positive mononuclear phagocytes (early apoptosis) in the blood, has shown significantly lower level on the 30th day of the experiment as compared with the data on the 7th day – by 1.18 times (p<0.01). At the same time, the number of monocytes with signs of late apoptosis / necrosis decreased by 1.74 times (p<0.01), as compared with the results on the 7th day of research.

Conclusions

1. The development of the experimental bacterial-immune periodontitis is accompanied by the increase of monocytes number with the signs of cell death in the blood. The realization of induced cell death in this pathology occurs due to apoptosis mainly.
2. The progress of the experimental periodontitis is accompanied by the increase of annexin-positive (early apoptosis) and necrotic mononuclear phagocytes in the blood due to the intensity of their formation in

response to the antigen stimulation and may indicate about chronic course of the inflammatory process in the periodontal complex.

References

1. Demkovych AIe, Bondarenko YuI. Patohenetychni osnovy modeliuvannia parodontytu u tvaryn. Zdobutky klinichnoi i eksperymentalnoi medytsyny. 2015 Sich;1(22):54-57.
2. Demkovych AIe. Osoblyvosti formuvannia mikrobiotsenozu v rozvytku zapalnykh zakhvoriuvan parodonta. Infektsiini khvoroby. 2015 Liut;1(79):87-92.
3. Kozak DV, Hudyma AA. Vplyv politravmy na dynamiku piznoho apoptozu tkanynnykh limfotsytiv. Klinichna khirurhiia. 2013;9:70-72.
4. Orlov AI. Matematika sluchaya: Veroyatnost i statistika – osnovnyie faktyi: uchebnoe posobie.Moskva:MZ-Press;2004.100 s.
5. Shmarov DA, Pogorelov VM, Kozinets GI. Sovremennyie aspektyi otsenki proliferatsii i apoptoza v kliniko-laboratornoy diagnostike (obzor literaturyi). Klinicheskaya laboratornaya diagnostika. 2013 Yanv;1:36-39.
6. Arimatsu K, Yamada H, Miyazawa H, Minaqawa T, Nakajima M, Ryder MI, et al. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. Sci Rep. 2014 May 6;4:4828.
7. 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 Jul-Sep;11(3):407-410.
8. Berger RL, Casella C. Statistical Inference. 2nd ed. Florida: Duxbury Press; 2001. 374 p.
9. Demkovych A, Bondarenko Yu, Hasiuk PA. Oxidative modification of proteins in the process of experimental periodontitis development. Interventional Medicine and Applied Science. 2017 Dec;9(4):218-221.
10. Herrmann JM, Meyle J. Neutrophil activation and periodontal tissue injury. Periodontol 2000. 2015 Oct;69(1):111-127.
11. Hirschfeld J. Dynamic interactions of neutrophils and biofilms. J Oral Microbiol. 2014 Dec 17;6:26102.
12. Jia SH, Parodo J, Charbonney E, Tsanq JLY, Jia SY, Rotstein OD, et al. Activated neutrophils induce epithelial cell apoptosis through oxidant-dependent tyrosine dephosphorylation of caspase-8. Am J Pathol. 2014 Apr;184(4):1030-1040.
13. Looney MR, Matthay MA. Neutrophil sandwiches injure the microcirculation. Nat Med. 2009 Apr;15(4):364-366.
14. Maianski NA, Maianski AN, Kuijpers TW, Roos D. Apoptosis of neutrophils. Acta Haematol. 2004;111(1-2):56-66.
15. Srinivas M, Chethana KC, Padma R, Suraqimath G, Anil M, Pai BS, et al. A study to assess and compare the peripheral blood neutrophil chemotaxis in smokers and non smokers with healthy periodontium, gingivitis, and chronic periodontitis. J Indian Soc Periodontol. 2012 Jan;16(1):54-58.
16. Srivastava N, Nayak PA, Rana S. Point of Care – A Novel Approach to Periodontal Diagnosis – A Review. J Clin Diagn Res. 2017 Aug;11(8):ZE01-ZE06.

Реферати

НЕКРОТИЧНО-АПОПТИЧНІ ЗМІНИ У МОНОНУКЛЕАРНИХ ФАГОЦИТАХ КРОВІ В МЕХАНІЗМАХ РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО БАКТЕРІАЛЬНО-ІМУННОГО ПАРОДОНТИТУ

Демкович А. Є.

Механізми розвитку запального процесу в пародонтальному комплексі включають низку складних процесів, що приводять до генералізації і хронізації його, втрати зубів та появи ускладнень з боку інших органів. Мета дослідження полягала у визначенні рівня апоптично змінених та некротизованих мононуклеарних фагоцитів крові в динаміці розвитку експериментального бактеріально-імунного пародонтиту. У статті наведено результати досліджень показників раннього та пізнього апоптозу моноцитів крові на 7-му і 30-ту добу розвитку запального процесу в тканинах пародонту. У піддослідних тварин відбирали кров, з якої виділяли моноцити методом градієнтного центрифугування. Оцінку апоптозу та некрозу мононуклеарних фагоцитів проводили методом проточної лазерної цитофлуориметрії. Отримані результати статистично опрацьовували із застосуванням параметричних і непараметричних методів статистики. При цьому виявлена характерна динаміка змін кількості загиблих клітин в процесі формування запального вогнища у пародонтальному комплексі. Зокрема, перебіг експериментального пародонтиту супроводжувався підвищенням вмісту алексин-позитивних (ранній апоптоз) та некротизованих моноцитів, що пов'язано з посиленням інтенсивності їх утворення у відповідь на антигену стимуляцію. При даній модельованій патології реалізація індукованої смерті клітин відбувалася переважно шляхом апоптозу.

Ключові слова: бактеріально-імунний пародонтит, запалення, мононуклеарні фагоцити, апоптоз, некроз.

Стаття надійшла 15.11.2017 р.

НЕКРОТИЧЕСКО-АПОПТИЧЕСКИЕ ИЗМЕНЕНИЯ МОНОНУКЛЕАРНЫХ ФАГОЦИТОВ КРОВИ В МЕХАНИЗМЕ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО БАКТЕРИАЛЬНО-ИММУННОГО ПАРОДОНТИТА

Демкович А. Е.

Механизмы развития воспалительного процесса в пародонтальном комплексе включают ряд сложных процессов, приводящих к генерализации и хронизации его, потери зубов и появления осложнений со стороны других органов. Цель исследования заключалась в определении уровня апопти-ческих измененных и некротизированных мононуклеарных фагоцитов крови в динамике развития экспериментального бактериально-иммунного пародонтита. В статье приведены результаты исследований показателей раннего и позднего апоптоза моноцитов крови на 7-е и 30-е сутки развития воспалительного процесса в тканях пародонта. У подопытных животных отбирали кровь, из которой выделяли моноциты методом градиентного центрифугирования. Оценку апоптоза и некроза мононуклеарных фагоцитов проводили методом проточной лазерной цитофлуориметрии. Полученные результаты статистически обрабатывали с применением параметрических и непараметрических методов статистики. При этом выявлена характерная динамика изменений количества погибших клеток в процессе формирования воспалительного очага в пародонтальном комплексе. В частности, ход экспери-ментального пародонтита сопровождался повышением содер-жания алексин-положительных (ранний апоптоз) и некротизи-рованных моноцитов, что связано с усилением интенсивности их образования в ответ на антигенную стимуляцию. При дан-ной моделируемой патологии реализация индуцированной смерти клеток происходила преимущественно путем апоптоза.

Ключевые слова: бактериально-иммунный пародонтит, воспаление, мононуклеарные фагоциты, апоптоз, некроз.

Рецензент Старченко І.І.