різноманітних ланок мікроциркуляторного русла з епітеліальними екскреторними протоками в сльозових залозах людини.

Ключові слова: сльозова залоза, екскреторні протоки, пластична реконструкція.

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о микротопографии взаимо-отношения различных звеньев микроциркуляторного русла с эпителиальными экскре-торными протоками в слезных железах человека.

Ключевые слова: слезная железа, экскреторные протоки, пластическая реконструкция.

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INDICATORS CELL CYCLE AND DNA FRAGMENTATION OF SPLEEN CELLS IN EARLY TERMS AFTER THERMAL BURNS OF SKIN AT THE BACKGROUND OF INTRODUCTION 0.9% NaCl SOLUTION

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In the experiment, during 7 days, changes in cell cycle and DNA fragmentation of spleen cells in rats after burn injuries were studied in the background of the introduction of 0.9% NaCl solution. After skin burn in the background of the introduction of 0.9% NaCl solution, after 1 day, greater values of the interval SUB-G0G1 and the G0G1 phase and, at the same time, lower values of the phases S, G2+M and the index of proliferation were determined, indicating the pathological induction of apoptosis and violations of the synthetic processes of splenocytes. After 3 days after burning the skin, large average values of the G0G1, proliferation and proliferation indexes were determined and, at the same time, the highest possible apoptosis rate compared to similar animal numbers in the 1 day after burn, which could be considered as the activation of mechanisms for compensating for pathological effects thermal damage at the given time. 7 days after skin burn, the average values of the G0G1 and the proliferation index are close to the similar figures for a group of animals without burn injuries of the skin, with the introduction of 0.9% NaCl solution, while the values of the S-phase (at almost 2.5 times) and the SUB-G0G1 interval (2.7 times), indicating insufficient compensation for the proliferative activity of the spleen cells against the background of increased apoptosis

Key words: cell cycle indexes, DNA fragmentation, spleen, rats, burn skin, 0.9% NaCl solution.

A prerequisite for the development of infectious complications in burning skin is the complex of immunity lesions that occurs due to the toxic effects of metabolism and toxins, which can lead to sepsis and death of the patient [3]. One of the most important components of the development of immune deficiency against the background of burns is the lesion of the spleen, as the main organ of humoral immunity and reticuloendothelial system [4, 13]. The study of the characteristics of the response of the spleen cells against the background of burn injury has been carried out quite long ago, but the data obtained are quite contradictory [1,10] and do not allow to form unambiguous views on the damage of this organ at the cellular level, which inhibits the development of effective methods for correction of immunosuppression with burn injury to the body.

The purpose of the study is to establish the characteristics of the cell cycle and DNA fragmentation of the cells of the spleen 1, 3 and 7 days after burn injury at the background of the introduction of a 0.9% solution of NaCl.

Material and methods. Within the framework of scientific cooperation between National Pirogov Memorial Medical University, Vinnytsya and SI "Institute of blood pathology and transfusion medicine of NAMS of Ukraine" (Lviv) and National Pirogov Memorial Medical University, Vinnytsya and the National Medical University named after O.O. Bogomolets an experimental study of the effect of the control infusion drug - 0.9% solution of NaCl on the structure of the spleen of the intact rats, as well as in the early stages (1, 3 and 7 days) after a burn injury to the skin. The research was carried out on laboratory white rats, males weighing 155-160 g, obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. During the experiment all animals were kept under vivarium of National Pirogov Memorial Medical University, Vinnytsya (indoor temperature - within 24-25 ° C, humidity - within 40-60%) on a standard water and food ration, with free access to water and food. All experiments were carried out taking into account the recommendations of the European Commission on conducting medical-biological research on the use of animals and medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for the clinical evaluation of safety of pharmacological agents (GLP)" [8, 14] and the rules of humane treatment of experimental animals (approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsya - Minutes № 1 by 14.01.2010). The 0.9% solution of NaCl were injected into the lower vena cava after its catheterization in aseptic conditions through the femoral vein at a dose of 10 ml/kg body weight of the animal. After each administration of 0.9% solution NaCl, the lumen of the catheter under the skin was filled with titrated heparin solution (0.1 ml of

heparin per 10 ml of 0.9% NaCl solution). The first introduction of colloid-hyperosmolar solutions (within 5-6 minutes) was carried out 1 hour after the start of the experiment (depilation of the lateral surfaces of the trunk and burning of the skin), and subsequent infusions - 1 time per day during the first 7 days of the experiment. Shaving of the lateral surfaces of the trunk of the rat, catheterization of the veins, staining of skin burns and decapitation of the animals were carried out under conditions of intravenous propofol anesthesia (at the rate of 60 mg/kg of animal weight). 30 rats were divided into 2 groups in the experiment: 1th groups - rats without thermal trauma who were infused with 0.9% NaCl solution in dose 10 ml per kg. In the 2th groups, 0.9% NaCl solution infusion were administered to the rats at a dose of 10 ml per kg after skin burn. Burn skin damage was caused by applying to the pre-depilated lateral surfaces of the trunk of the rats for 10 seconds by four copper plates (two plates on each side, each with a surface area of 13.86 cm^2) which were preheated for 6 minutes in constant temperature water 100 Cº [5, 12]. According to the formula M. O. Lee [9], the total area of skin surface damage in rats was 21-23%. This area at this exposure is sufficient for the formation of the second-third degree burns (according to the classification adopted at the 20th Congress of Surgeons of Ukraine, Ternopil, 2000) and the induction of a shock state of moderate severity [6]. The content of DNA in the cells of the spleen cells of rats was determined by flow cytometry. Sputum cell nucleus suspensions were prepared using a special CyStain DNA spotting solution (Partec, Germany), in accordance with the manufacturer's protocol. This solution allows you to quickly and simultaneously perform extraction of nuclei and mark the nuclear DNA with 4'-6-diamidino-2-phenylindole (DAPI), which is part of its composition. In the process of manufacturing the nucleic suspensions, special single-use CellTrics 50 μm filters (Partec, Germany) were used. The flow analysis was performed on a multi-functional flow-through cathometer "Partec PAS" from Partec, Germany, at the research center of the National Pirogov Memorial Medical University, Vinnytsya. UV radiation was used to stimulate DAPI fluorescence. From each sample of the nucleic suspension of the analysis subject to 20 thousand events. Cell cycle analysis was carried out using FloMax software (Partec, Germany) in full numeric matching according to a mathematical model, which determined: G0G1 - percentage ratio of G0G1 phase cells to all cells of the cell cycle (DNA content = 2c); S - percentage ratio of the phase of DNA synthesis to all cells of the cell cycle (DNA content> 2c and <4c); G2 + M - percentage ratio of the G2 + M phase to all cells of the cell cycle (DNA = 4c); IP - the index of proliferation, which is determined by the sum of the indices S + G2 + M; BP - block of proliferation, which is evaluated by the ratio S / (G2 + M). The statistical processing of the obtained results was carried out in the licensed package "Statistica 6.1" (license number BXXR901E246022FA) with the use of nonparametric methods for evaluating the results. Evaluated the character of the distribution of signs for each of the obtained variation series, set the average values of each characteristic, studied and the values of standard quadratic deviations. The reliability of the differences between independent quantitative values was determined using the Man-Whitney U-criterion.

Results and its discussion. The results of cell cycle and fragmentation of spleen cell DNA after burn skin damage on the background of the introduction of 0.9% NaCl solution (Table 1) suggest that there is a reserve cell group in the spleen that provides an immune response in burn-up stress. In favor of this hypothesis, the changes recorded by us *1 day after burning of the skin with the background of the introduction of 0.9% NaCl solution* indicate that the average values of the interval of the SUB-G0G1 22,5% were and set almost 2.5 times greater ($p < 0.01$) of the phase G0G1 ($p < 0.01$), as well as the lower mean values of the S-phase (39.2%, $p < 0.01$) and G2 + M phase (33.5%, $p < 0, 01$). The index of proliferation ($p < 0.01$) is reduced by almost three times, indicating a possible protective mechanism of the spleen cells in the form of inhibition of synthetic processes and protection against pathological stimulation of apoptosis. In our opinion, the findings suggest an increase in spleen cell damage by apoptosis and a protective increase in cells inactive after 1 day after burn injuries to the skin. It should be noted that the cell cycle cell count of spleen cells in the day after skin burn in our study is somewhat different from the results of other researchers [1], which did not show significant inhibition of the S phase and significant changes in the G2 + M phase. We can assume that this is due to different methods of causing burn injuries. Thus, the parameters of the cell cycle of the spleen cells after 1 day after burning of the skin showed the existence of significant damage to this subpopulation of cells in the early stages after burn, which, in our opinion, is a prerequisite for the development of immunosuppression in the subsequent stages of the pathological process. In favor of the existence of a possible protective mechanism of the spleen cells in the form of inhibition of synthetic processes and protection against pathological stimulation of apoptosis are also evidenced by the results of some studies regarding the violation of splenic cell division from 1 day after burn skin damage [2]. Also, the data we received to some extent agree with other studies [4, 15], in which a sharp decrease in the number of splenocytes in the first hours after thermal damage to the skin. This phenomenon is associated with a sharp increase in the level of adrenal hormones that develops in response to burn injury and potentially inhibits the subsequent immune response [13]. In favor of this assumption, changes are established *after 3 days with the*

burn skin damage and correction with 0.9% NaCl solution - higher average values of the parameters of the G0G1 phase (by 14.4%, p <0.05) with the highest increase in apoptosis (SUB-G0G1 is almost 4 times higher than that in animals without skin burn, p <0.01). It should be noted that when comparing cell cycle indices and DNA fragmentation in animals with burn injury in the background of the introduction of 0.9% NaCl solution in 3 and 1 day, higher mean values of the index of proliferation (62.6%, p <0, 01) and the block of proliferation (in 2 times, p <0,05). That is, the compensation of the pathological effect is already 3 days after the thermal damage and is to increase the synthetic processes. In addition, the results indicate the acute nature of changes occurring at the intracellular level 3 days after burning the skin, as well as insufficient correction of these changes when using 0.9% NaCl solution. In the scientific literature there are only isolated publications, which indicate the beginning of the restoration of lymphoid proliferation, in particular in the spleen, 48 hours after the thermal damage of the skin [10].

Table 1

Indicators of cell cycle and fragmentation of spleen cell DNA after burn skin damage on the background of administration of 0.9% solution NaCl (M±σ)

Day	Groups of animals (n=5)	Cell cycle indexes					
		G0G1	S	G2+M	IP	BP	SUB-G0G1
1	0,9 % solution NaCl	74,38±5,01	5,826±1,095	19,79±4,27	25,62±5,01	0,302±0,066	4,876±1,201
	burn + 0,9 % solution NaCl	91,08±3,01	2,284±0,753	6,638±2,308	8,922±3,007	0,356±0,077	12,03±3,27
	p	<0,01	<0,01	<0,01	<0,01	>0,05	<0,01
3	0,9 % solution NaCl	74,74±5,34	5,690±1,193	19,57±4,35	25,26±5,34	0,290±0,035	5,166±1,374
	burn + 0,9 % solution NaCl	85,49±3,21	5,866±1,606	8,646±2,305	14,51±3,20	0,718±0,294	19,38±2,24
	p	<0,05	>0,05	<0,01	<0,05	<0,01	<0,01
7	0,9 % solution NaCl	72,45±3,52	5,484±1,215	22,06±2,98	27,55±3,53	0,252±0,054	4,850±1,860
	burn + 0,9 % solution NaCl	69,74±2,96	13,17±2,17	17,09±1,45	30,26±2,96	0,774±0,122	13,18±3,34
	p	>0,05	<0,01	<0,05	>0,05	<0,01	<0,01
$p_{(NaCl)1-3}$		>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
$p_{(NaCl)1-7}$		>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
$p_{(NaCl)3-7}$		>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
$p_{(burn+NaCl)1-3}$		<0,01	<0,01	>0,05	<0,01	<0,05	<0,05
$p_{(burn+NaCl)1-7}$		<0,01	<0,01	<0,01	<0,01	<0,01	>0,05
$p_{(burn+NaCl)3-7}$		<0,01	<0,01	<0,01	<0,01	>0,05	<0,01

Notes: p - the reliability of the differences in the indicators between the corresponding groups of animals with burn and without skin burn; $p_{(...)1-3}$ - the reliability of the differences between the respective groups of animals in 1 and 3 days from the beginning of the experiment; $p_{(...)1-7}$ - the reliability of the differences between the respective groups of animals in 1 and 7 days from the beginning of the experiment; $p_{(...)3-7}$ - the reliability of the differences in the indices between the corresponding groups of animals after 3 and 7 days from the beginning of the experiment.

However, it should be noted that these studies were conducted at the cytological level without the use of DNA cytometry. *After 7 days using the application of 0.9% NaCl solution* the mean values of the G0G1 (69.74 ± 2.96) and the proliferation index (30.26 ± 2.96) were approximated to the indices of the non-burning skin group 0.9% solution of NaCl (p> 0.05 in both cases), while maintaining high mean values of the proliferation unit (more than 3 times, p <0.01) and the maximum values for the S-phase (almost 2.5 times, p <0.01). The values in the interval of SUB-G0G1 greater than 2.7 times (p <0.01) indicate insufficient compensation of spleen cells during this observation period. This is also indicated by the absence of significant differences in mean values for the indicator of proliferation (p> 0.05) between 3 and 7 days after skin burn. The data established by us confirm the opinion of the whole group of researchers [4, 15] on the formation of suppression of the spleen cells precisely in the interval from 7 to 10 days after burn, in contrast to the data on a greater decrease in the synthesis of splenocytes from 10 days [7]. Using the method of DNA cytometry, Cho K. et al. [1] 7-8 days after skin burns an avalanche-like increase in the mean values of the S-phase has been established. Also, the increase in synthesis against the background of enhanced apoptosis of splenocytes was fixed at a similar time by other scientists [11, 15], which is regarded by them as signs of the formation of an immune response to damage.

Conclusions

1. Burning of the skin with a 0.9% NaCl correction in 1 day is characterized by higher mean values of the SUB-G0G1 and G0G1 phases and, at the same time, lower average values of the phases S, G2 + M and IR, indicating the pathological induction of apoptosis and violations of the synthetic processes of splenocytes regardless of the use of this drug.

2. After 3 days of burn of the skin, with correction of 0.9% NaCl solution, set higher average values of the G0G1 (14.4%, p <0.05), proliferation and proliferation index and, at the same time, the highest possible level apoptosis compared with similar indicators of a group of animals 1 day after burn with correction of 0.9%

NaCl solution, which can be estimated as activation of the mechanism of compensation of the pathological influence of thermal damage in the given period.

3. 7 days after skin burn simulation and application of 0.9% NaCl solution, the average values of the G0G1 and the proliferation index are close to the similar indices of the group of animals without burning skin damage, with the introduction of 0.9% NaCl solution, and at most as high as all the term of the study was the value of the S-phase (almost 2.5 times, $p < 0.01$) and the SUB-G0G1 interval (2.7 times, $p < 0.01$), indicating insufficient compensation for the proliferative activity of the spleen cells on the background of increased apoptosis.

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Реферати

ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ І ФРАГМЕНТАЦІЇ ДНК КЛІТИН СЕЛЕЗІНКИ В РАННІ ТЕРМІНИ ПІСЛЯ ТЕРМІЧНОГО ОПІКУ ШКІРИ НА ФОНІ ВВЕДЕННЯ 0,9 % РОЗЧИНУ NaCl

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В експерименті на протязі 7 діб вивчені зміни показників клітинного циклу і фрагментації ДНК клітин селезінки щурів після опікового пошкодження шкіри на фоні введення 0,9 % розчину NaCl. Після опіку шкіри на фоні введення 0,9 % розчину NaCl через 1 добу встановлені більші значення інтервалу SUB-G0G1 та фази G0G1 і, одночасно, менші значення показників фаз S, G2+M та індексу проліферації, що вказує на наявність патологічної індукції апоптозу та порушень синтететичних процесів спленоцитів. Через 3 доби після опіку шкіри встановлені більші середні значення показників фази G0G1, індексу проліферації та блоку проліферації і, одночасно, максимально високий рівень апоптозу порівнянно із аналогічними показниками групи тварин через 1 добу після опіку, що може бути розцінено, як активація механізмів коменсації патологічного впливу термічного ушкодження в даний термін. Через 7 діб після опіку шкіри середні значення показників фази G0G1 та індексу проліферації наближаються до аналогічних показників групи тварин без опікового ушкодження шкіри на фоні введенням 0,9 % розчину NaCl, а максимально більшими за весь термін дослідження виявились значення показника S-фази (майже в

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА И ФРАГМЕНТАЦИИ ДНК КЛЕТОК СЕЛЕЗЁНКИ В РАННИЕ ТЕРМИНЫ ПОСЛЕ ТЕРМИЧЕСКОГО ОЖОГА КОЖИ НА ФОНЕ ВВЕДЕНИЯ 0,9 % РАСТВОРА NaCl

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В эксперименте на протяжении 7 дней изучены изме-нения показателей клеточного цикла и фрагментации ДНК клеток селезёнки крыс после ожогового повреждения кожи на фоне введения 0,9 % раствора NaCl. После ожога кожи на фоне введения 0,9 % раствора NaCl через 1 день установлены большие значения интервала SUB-G0G1 и фазы G0G1 и, одновременно, меньшие значения показателей фаз S, G2+M и индекса пролиферации, что указывает на наличие патологической индукции апоптоза и нарушение синтетических процессов спленоцитов. Через 3 дня после ожога кожи установлены большие средние значения показателей фазы G0G1, индекса пролиферации и блока пролиферации и, одновременно, максимально высокий уровень апоптоза в сравнении с аналогичными показателями группы животных через 1 день после ожога, что может быть расценено, как активация механизмов компенсации патологического влияния термического повреждения в данный период. Через 7 дней после ожога кожи средние значения показателей фазы G0G1 и индекса пролиферации приближаются к аналогичным показателям группы животных без ожогового повреждения кожи на фоне введения 0,9 % раствора NaCl, а максимально большими за весь период исследования выявились значения показателя S-фазы

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.

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(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.

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

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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