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PECULIARITIES OF CYTOKINE PROFILE CHANGES UNDER THE CONDITIONS OF EXPERIMENTAL PERIODONTITIS AND IMMOBILIZATION STRESS FORMATION AND ITS PHARMACOLOGICAL CORRECTION

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The study was devoted to establishing the character of the disturbances content level of TNF- α , IL-6 and IL-10 in the serum of guinea pigs under the conditions of experimental periodontitis and immobilization stress and to evaluate the effectiveness of the thioacetam use. Experimental studies were performed on 50 guinea pigs, which were divided into three groups. The concentration of IL-6, IL-10 and TNF- α in the blood serum was determined. Experimental combined pathology was accompanied by a gradual and significant increase in the blood content of TNF- α and IL-6 with the highest degree of manifestation on day 15 of the experiment and significant depletion of anti-inflammatory cytokine, indicating a significant predominance of proinflammatory activity of the cytokine profile. The administration of thioacetam had a positive effect on the cytokine profile and contributed to a decrease in the proinflammatory cytokine link and a significant increase in the level of interleukin-10 under conditions of experimental periodontitis and immobilization stress.

Key words: experimental periodontitis, immobilization stress, tumor necrosis factor- α , interleukin-6, interleukin-10, thioacetam.

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ОСОБЛИВОСТІ ЗМІНИ ЦИТОКІНОВОГО ПРОФІЛЮ ЗА УМОВ ФОРМУВАННЯ ЕКСПЕРИМЕНТАЛЬНОГО ПАРОДОНТИТУ ТА ІММОБІЛІЗАЦІЙНОГО СТРЕСУ ТА ЙОГО ФАРМАКОЛОГІЧНА КОРЕКЦІЯ

Дослідження було присвячено вивченню характеру порушень рівня вмісту TNF- α , IL-6 та IL-10 у сироватці крові морських свинки за умов експериментального пародонтиту та іммобілізаційного стресу та оцінити ефективність застосування тіоцетаму. Експериментальні дослідження проводили на 50 морських свинках, яких розподілили на три групи. Визначали концентрацію IL-6, IL-10 та TNF- α в сироватці крові. Експериментальна поєднана патологія супроводжувалася поступовим і достовірним підвищенням у крові вмісту TNF- α та IL-6 з найбільшим ступенем прояву на 15 добу експерименту та значне виснаження протизапального цитокіну, що свідчить про значну перевагу прозапальної активності цитокінового профілю. Введення тіоцетаму позитивно впливало на цитокіновий профіль та сприяло зниженню прозапальної ланки цитокінів та значне підвищення рівня інтерлейкіну-10 за умов експериментального пародонтиту та іммобілізаційного стресу.

Ключові слова: експериментальний пародонтит, іммобілізаційний стрес, фактор некрозу пухлин- α , інтерлейкін-6, інтерлейкін-10, тіоцетам.

The study is a fragment of the research project "The role of metabolic and immune disorders in the pathogenesis of allergic and inflammatory processes, stress, adrenaline myocardial damage and their pathogenetic therapy", state registration No. 0120U105779.

Today, periodontal diseases have become a priority problem of modern dentistry due to their significant spread, tooth loss and treatment difficulties. According to WHO, periodontal diseases occur in 65 % of the adult population of developed countries and in more than 90 % of the population of developing countries [6]. The high incidence of periodontitis, the severity of the course of some forms of its pathology, the loss of teeth and, as a result, significant changes in the maxillofacial system, reduced work capacity, reduced quality of life of the population – all this makes it possible to consider this disease not only a serious medical problem, but also an important social problem [3]. Although fundamental studies of the etiology and pathogenesis of the most common periodontal disease – generalized periodontitis have been conducted, the mechanisms of the occurrence and development of this pathology have not been definitively elucidated. For many years, there has been a tendency towards an earlier onset of this disease, its aggressive course and the development of the inflammatory-dystrophic process.

The pathological process in the periodontium is a focus of infection, oral sepsis and has a significant negative impact on the body as a whole [6, 15]. Based on general ideas about their development, periodontitis should be considered as a multifactorial pathology caused by the unfavorable influence of general and local factors, as well as aggressive environmental factors. For the realization of the action of the pathogenic potential, the favorable effect of certain systemic factors is important. Among them, an

important place is occupied by the reduction of the resistance of the host's immune system [10]. This can be caused by various somatic diseases, chronic stress, etc. [15]. Strong or long-term stress suppresses the acquired, to a lesser extent, the innate immune response, changes the level of cytokines [13]. Thus, the broad planned problem of emotional stress requires further research into the role of immunological, genetic, and social factors for a deep understanding of the nature of emotional stress, its role in the etiology and pathogenesis of dental diseases, and the justification of effective therapeutic and preventive measures for their correction [2]. Therefore, the question of researching the pathogenesis of periodontitis in conditions of combined pathology in combination with stress is relevant. It has been established that excessive immobilization and stress are factors that can cause functional changes at various levels of the body [2].

Immune mechanisms of its development play an important role in the pathogenesis of generalized periodontitis. Activation of periodontopathogenic microflora of monocytes and macrophages increases the production of pro-inflammatory cytokines by these cells, which causes an imbalance between their pro-inflammatory and anti-inflammatory pools [8]. This is one of the main causes of periodontal damage, it disrupts the relationships in the local system of the periodontal complex, and as a result leads to the pathology of the regeneration of periodontal tissues, the formation of periodontal pockets and bone resorption of the alveolar process of the jaws [11].

Increased release of pro-inflammatory cytokines leads to degradation of the extracellular matrix and, as a result, to damage to periodontal tissues and resorption of alveolar bone. Progressive destruction of bone tissue in patients with periodontal disease is associated with a combination of cytokines IL-1 β , IL-6, TNF- α and prostaglandins E2 [14]. These cytokines can activate osteoclastogenesis and bone resorption by osteoclasts. Strengthened migration of macrophages under the influence of cytokines and their constant presence in tissues enhances destructive processes in periodontium [9]. At the same time, the degree of alveolar bone resorption depends on the severity of the body's immune response to bacterial invasion. Unlike pro-inflammatory cytokines, which play an important role in the initial development of the inflammatory response in periodontal tissues, anti-inflammatory cytokines inhibit osteoporosis and suppress the activity of macrophages, contributing to the relief of the inflammatory response in the periodontal complex [12].

In recent years, scientists have been actively studying the cytokine profile in periodontal tissue diseases to establish the pathogenetic significance of cytokines in the development of generalized periodontitis. Despite significant achievements in this field, many questions remain unanswered. This determines the relevance of our research.

The purpose of the study was to establish the character of the disturbances content level of pro-inflammatory tumor necrosis factor – α , interleukin-6 and anti-inflammatory cytokine interleukin-10 in the serum of guinea pigs under the conditions of experimental periodontitis and immobilization stress and to evaluate the effectiveness of the thiocetam use.

Material and methods. Experimental studies were performed on 50 guinea pigs (males), which were divided into three groups (one control group and two experimental ones): the first (10 guinea pigs) – intact animals – control; the second (experimental) group (30 guinea pigs) – animals under the conditions of experimental periodontitis and immobilization stress, which were divided into three subgroups (10 in each) depending on the day of the experiment development (3rd, 5th and 15th), and third (experimental) group (10 guinea pigs) – animals on the 15th day of the conditions of experimental periodontitis and immobilization stress after treatment with thiocetam, which was injected at a dose of 250 mg/kg intramuscularly from the 6th day of the experiment for 10 days.

Two models were chosen for the experiment: the immobilization stress model and the experimental periodontitis. These models are scientifically justified [5, 7]. Animals of the experimental groups were subjected to prolonged immobilization stress (3h) and are on a diet that includes 1 g of dry lyophilized cattle liver, 10 g of dry skimmed milk and 20 g of breadcrumbs (the diet is calculated for one day of one guinea pig) and then animals were decapitated under anesthesia using diethyl ether and removed from the experiment on the 3rd, 5th and 15th day of the experiment. We selected fixed days (3rd, 5th and 15th) for studies that corresponded to the classic stages of acute inflammation.

All experiments on laboratory animals carried out with following the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 2010/63/EU, the Law of Ukraine 3447 – IV “protection animals from the cruelty” the general ethics of animal experimentation adopted by the first national Congress on bioethics in Ukraine (2001). In addition, all experiments with experimental guinea-pigs were conducted under the control of the BioEthics Commission at Danylo Halytsky Lviv National Medical University (Protocol N2 dated by 26.02.2018). Laboratory animals were kept on a standard fodder in animal facility with adequate lighting and temperature conditions.

The research was conducted based on Danylo Halytsky Lviv National Medical University. The concentration of cytokines IL-6, TNF- α , and IL-10 in blood serum was determined using the solid-phase

immunoenzymatic method using the biotin-streptavidin system, increasing sensitivity and specificity of the immunoenzymatic method. In addition, a set of reagents for quantitative enzyme-linked immunosorbent assay (ELISA) of the corresponding cytokine produced by “Diaclone” (France) was used. The analysis procedure was carried out according to the attached manufacturer's instructions.

The values of the concentrations in the studied samples were calculated by constructing a graph based on the calibration curve of the dependence of the optical density values on the known concentration of the cytokine in the calibration samples. In those cases, when the optical density of the studied serum exceeded the similar value for the standard with the maximum concentration, a repeated analysis was performed by diluting the sample 10 times.

All numerical results were subjected to statistical processing using the arithmetic mean (M), the error of the arithmetic mean (m), and the Student's “t” test and Mann-Whitney test (U). Calculations were made using statistical and graphical analysis tools of Microsoft Excel spreadsheets of the Microsoft Office suite. In all cases of analysis, the critical level of significance was taken as 0.05. Statistical analysis of the results was carried out using a software package Statistica 10 (StatSoft, Inc., USA).

Results of the study and their discussion. When studying the cytokine system in experimental periodontitis and immobilization stress, it was found that in all studied days of the experiment there were probable changes in the concentration of both pro-inflammatory and anti-inflammatory cytokines in comparison with the group of intact animals.

The parameters of the cytokines level in the blood of guinea pigs under the conditions of the experimental periodontitis and immobilization stress formation is presented in Table 1.

Table 1

The parameters of the cytokines level in the blood of guinea pigs under the conditions of the experimental periodontitis and immobilization stress formation. (M±m, n=40)

Form of experiment	Duration of experiments in days	Number of animals	TNF- α , kg/ml	IL-6, kg/ml	IL-10, kg/ml
Intact animals. Control		10	0.38±0.03*	0.60±0.06*	8.9±0.41*
Guinea pigs with EP and IS	3	10	0.65±0.06*	0.98±0.09	4.9±0.39*
	5	10	0.70±0.07*	1.1±0.09*	4.6±0.38*
	15	10	0.72±0.07*	1.2±0.09*	4.0±0.37*
Guinea pigs with EP and IS (after treatment)	15	10	0.40±0.03*	0.76±0.06*	7.1±0.4*

Note. * – $p < 0.05$; EP – experimental periodontitis, IS – immobilization stress.

The dynamics of the development of the combined pathology (experimental periodontitis and immobilization stress) is accompanied by a pronounced progression of the pro-inflammatory group of cytokines, namely an increase in TNF- α by 89.4 % ($p < 0.05$) and IL-6 by 100.0 % ($p < 0.05$) on the 15th day against the control group. At the same time, we observe a decline in the functional activity of IL-10 at all stages of their formation (3rd, 5th and 15th days) with an advantage on the 15th day of the experiment (decrease by 55.0 % ($p < 0.05$) in comparison with intact animals.

We also compared the obtained data not only between groups of animals with EP and IS and intact guinea pigs, but also between different experimental subgroups of animals that were exposed to the antigenic factor and, in particular, the duration of its action. During the study of the pro-inflammatory TNF- α , a slight increase in its concentration was found almost equally by 7.6 % ($p < 0.05$), both on the 5th and by 10.7 % ($p < 0.05$) on the 15th day of the experiment compared to guinea pigs on the 3rd day of the experiment.

Carrying out a comparative characteristic of the described results among the indices of guinea pigs in different periods of the development of EP and IS, we also note a gradual increase in IL-6 depending on the duration of the pathological process: on the 5th and 15th days, this index increases by 12.2 %, respectively ($p_1 < 0.05$) and 22.4 % ($p_1 < 0.05$) relative to the group of animals on the 3rd day of the experiment.

Therefore, the results of studies of the cytokine profile in blood serum under the conditions of EP and IS established an increase in the level of the pool of pro-inflammatory CK depending on the duration of the pathological process, especially the most pronounced in animals on the 15th day of the experiment, which indicates a significant intensification of the inflammatory process in this comorbid pathology.

In order to fully characterize the cytokine status, we also conducted a study of the anti-inflammatory factor IL-10 in the blood serum of guinea pigs with EP and IS. In the case of a comparison of this index with different groups of animals, it was found that on the 5th day of the combined pathology, it decreases by only 6.1 % ($p < 0.05$) when compared to the 3rd day of the experiment. An even more significant decrease in IL-10 was recorded as EP and IS developed, namely on the 15th day by 18.3 % ($p < 0.05$) compared to the 3rd day of animals.

The influence of thiocetam at the level of cytokines indices in guinea pigs' blood in the experimental periodontitis and immobilization stress formation dynamics is presented in Fig. 1.

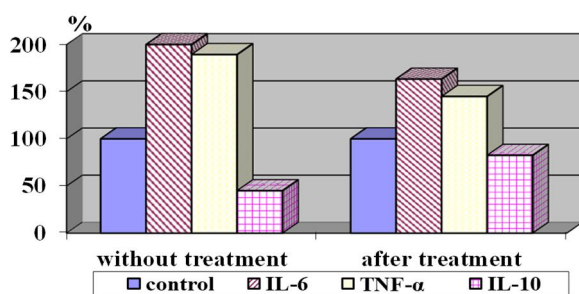


Fig. 1. The influence of thiocetam on the level of cytokines indexes in guinea pigs' blood in the experimental periodontitis and immobilization stress formation dynamics (in % comparison before and after treatment).

pharmacological effect of which is due to the mutually potentiating effect of thiotriazoline and piracetam. The first component of this medicine stimulates the activity of antioxidant defense enzymes, stabilizes cell membranes, stimulates alternative metabolic pathways in hypoxia, improves microcirculation. The second component – thiotriazoline has an antioxidant, anti-inflammatory, detoxifying and immunomodulating effect [1]. Based on the obtained results, we found an evident relationship between the level of studied inflammatory mediators and chronic inflammatory processes and stress factors [4, 13]. In particular, TNF- α plays an essential role in activating prostaglandins and other substances related to pain perception and is the central mediator of chronic pain, which is also often accompanied by periodontitis. IL-6 is produced by a variety of cells and serves as a significant signal for the release of CRP from the liver as part of the acute phase response and is also involved in the induction of cell proliferation and differentiation. Usually conceptualized as an inflammatory mediator in the context of stress and depression, IL-6 has both pro- and anti-inflammatory properties [7, 10]. Like TNF- α , IL-6 also plays an essential role in pain sensitivity. Another inflammatory mediator, IL-10, is a cytokine involved in B-cell proliferation and in the production of IgA, the main immunoglobulin in salivary immunity, which also reduces inflammation in the oral cavity [5]. The use of thiocetam in experimental periodontitis and immobilization stress significantly reduces the level of inflammatory mediators TNF- α and IL-6 and increases the level of IL-10 due to the anti-ischemic, antioxidant and membrane-stabilizing properties of the main component of this drug, namely morpholine 5-methyl-1,2,4-triazoline-5-thioacetate.

Conclusions

1. Thus, the conducted immunological studies of cytokine status showed that the experimental combined pathology is accompanied by a gradual and significant increase in the content of pro-inflammatory cytokines (TNF- α and IL-6) in the blood, with the greatest degree of manifestation on the 15th day of the experiment and a significant depletion of the anti-inflammatory cytokine, which proves the significant advantage of the pro-inflammatory activity of the cytokine profile.

2. Administration of thiocetam at the rate of 250 mg/kg intramuscularly from the 6th day of the conditions of the formation of EP and IS for 10 days had a positive effect on the cytokine profile and contributed to the reduction of the pro-inflammatory link of cytokines and a significant increase in the level of interleukin-10, the biological effects of which are aimed at suppressing the production of IL-1, IL-6, TNF- α and macrophage cytotoxic activity.

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IMMUNOCOMPETENT LIVER CELLS REACTION TO INHIBITION OF LUTEINIZING HORMONE SYNTHESIS ON THE 180TH DAY

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Hepatic macrophages play a central role in maintaining liver homeostasis and in the pathogenesis of liver injury. Liver macrophages are composed of functionally distinct cellular subpopulations. Currently, there is interest in the effect of androgens and their receptors on different liver cells and the development of liver pathology as well as the potential impact of quercetin on androgen synthesis inhibition. The blockade of luteinizing hormone synthesis by administration of triptorelin acetate on day 180 of the experiment caused morphological changes in the structure of the rat liver, in particular in the cellular, connective tissue and vascular components. Total activity of NO synthases in the liver rose up as well as nitrite concentration. Arginase activity was decreased. Administration of quercetin leads to a decrease in the total activity of NO synthases. The shift in macrophage polarization toward the predominance of M1 may be a consequence of endothelial dysfunction as a result of luteinizing hormone synthesis inhibition.

Key words: liver, macrophage, luteinizing hormone, triptorelin, quercetin, rats.

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РЕАКЦІЯ ІМУНОКОМПЕТЕНТНИХ КЛІТИН ПЕЧІНКИ НА ПРИГНІЧЕННЯ СИНТЕЗУ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ НА 180-Й ДЕНЬ

Макрофаги печінки відіграють центральну роль у підтримці внутрішньоорганного гомеостазу та в патогенезі її пошкодження. Вони складаються з функціонально відмінних клітинних субпопуляцій. В теперішній час існує інтерес до впливу андрогенів та їх рецепторів на різні клітини печінки та розвиток печінкової патології, а також потенційний вплив кверцетину на фоні пригнічення синтезу андрогенів. Блокада синтезу лютеїнізуючого гормону шляхом введення триптореліну на 180 добу експерименту спричинила морфологічні зміни в структурі печінки шурів, зокрема в клітинному, сполучнотканинному та судинному компонентах. Сумарна активність NO-синтаз у печінці зростала, як і концентрація нітритів. Активність аргінази знижувалась. Введення кверцетину призводило до зниження сумарної активності NO-синтаз. Зсув поляризації макрофагів у бік переважання M1 може бути наслідком дисфункції ендотелію внаслідок пригнічення синтезу лютеїнізуючого гормону.

Ключові слова: печінка, макрофаги, лютеїнізуючий гормон, трипторелін, кверцетин, щури.

The study is a fragment of the research project “Experimental morphological study of the effect of cryopreserved preparations of cord blood and embryofetoplacental complex, diferelein, ethanol and 1 % methacrylic acid on the morphofunctional state in a number of internal organs”, state registration No. 0119U102925.

Macrophages are myeloid immune cells that are abundant in all tissues of the body. They recognize, absorb and neutralize cellular debris, foreign material or pathogenic microorganisms and perform a central function in the organization of inflammatory processes [12]. Hepatic macrophages play a central role in maintaining liver homeostasis, as well as in the pathogenesis of acute or chronic liver damage [11]. Regarding this, they are an attractive target for the development and research of new methods of liver disease treatment. Since macrophages perform a wide range of different functions in the liver and consist