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CHANGES OF LIPOPEROXIDATION AND ANTIOXIDANT SYSTEM IN PARIODONTAL TISSUES IN EXPERIMENTAL BRONCHIAL ASTHMA UNDER CONDITIONS OF CHRONIC PERIODONITIS AND CORRECTION OF THESE CHANGES WITH THIOTRIAZOLINE

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Abstract

We have analyzed the results of the research of changes in indices of lipid peroxidation (conjugated dienes and malondialdehide) and antioxidant (superoxide dismutase, catalase, ceruloplasmin) systems in guinea pigs' periodontal soft tissues in experimental bronchial asthma in the dynamics of asthma under conditions of chronic periodontitis and correction of these changes with thiotriazoline. The research was conducted on 50 male guinea pigs weighting 250-270 g, divided into 5 groups: I – intact guinea pigs (n=10), II – guinea pigs (n=10) with asthma under conditions of chronic periodontitis before correction (4^{th} day), III - guinea pigs (n=10) with asthma under conditions of chronic periodontitis before correction (18^{th} day), IV - guinea pigs (n=10) with asthma under conditions of chronic

periodontitis before correction (25th day), V - guinea pigs (n=10) with asthma under conditions of chronic periodontitis after correction (25th day). The results of experimental studies showed the significant increase of conjugated dienes and malondialdehide levels in animal's periodontal soft tissues at all observed stages of asthma development under conditions of chronic periodontitis before the correction as compared with control group. Intensive synthesis of LPO's products caused increase on 4th day with further decrease on 18th and 25th days of activity levels of superoxide dismutase, catalase and ceruloplasmin in animal's periodontal soft tissues before the correction as compared with indices of the control group. After correction with thiotriazoline the the results showed decrease of indices of LPO and increase of activity levels of antioxidant enzymes.

Key words: experimental bronchial asthma, chronic periodontitis, lipid peroxidation, antioxidant system.

Introduction. Bronchial asthma is considered as a chronic inflammatory disease, which is usually characterized by bronchial hyperreaction, which leads to bronchospasm [1]. According to the literature data, in different countries of the world, from 1 to 10% of the population suffers from asthma. It is believed that asthma affects at least 2% of the total population of the planet. As a result of this disease, about 2 million people die annually. In countries of Europe and America, asthma occurs in 2-11% of the adult population [2].

Periodontitis is a periodontal inflammation accompanied by apical migration of the connective epithelium and leads to destruction of connective tissue and loss of alveolar bone. Chronic periodontitis is the most common form of periodontal disease. Studies show a connection between periodontitis with many chronic diseases [3]. The relationship between the general health and changes in the periodontium is complicated. Affected periodontal tissues may create pathogenic effects on the organism and complicate the course of diseases.

It is known from many literature sources that an important role in the pathogenesis of development of both asthma [4-6], and periodontitis is played by changes in the prooxidant-antioxidant system [7-9].

In the available to us literature we could not find data on changes in the indices of LPO and AOS in periodontal tissues with combined pathology of bronchial asthma and chronic periodontitis.

Numerous studies point to an association between periodontitis and asthma. Thus, the prevalence of mouth breathing is 23.4% higher in patients diagnosed with bronchial asthma compared to nonasthmatics. This is due to obstruction of the respiratory tract caused by

asthma, which triggers the need for aspirating more air by oral route [10]. Mouth breathing causes to fluid evaporation which decreases oral homeostasis [11, 12]. Also, bronchial asthma and steroid therapy negatively affect the course of generalized periodontitis by suppressing the severity of inflammatory response, leading to cytological disturbances in periodontal tissues in the form of dystrophy, necrobiosis, and necrosis of epithelial cells. Systemic hormonal drugs have a more negative influence compared to inhaled steroids [13]. On the other hand, the anatomical continuity between the lungs and the oral cavity makes the last one a potential reservoir of respiratory pathogens [14]. Dentogenous and other oral and extraoral source of infection can play a role in the emergence of respiratory infections that manifest themselves as sinusitis, tonsillitis, pneumonia, bronchial asthma, abscess, etc. [15].

Also, in the available literature sources we found no information on the state of the prooxidant-antioxidant system in this combined pathology and the use of the drug "Thiotriazoline" for the correction of indices in the periodontal soft tissues. It is known that it has antioxidant, membrane stabilizing, anti-ischemic and anti-inflammatory properties [16].

The aim of our study was to determine the indeces of the of lipid peroxidation and antioxidant system in guinea pigs' periodontal soft tissues in different periods of experimental bronchial asthma under conditions of chronic periodontitis and the corrective effect of thiotriazoline.

Materials and methods

All experiments on laboratory animals were conducted according to the principles of bioethics of *European Convention for protection of vertebrate animals* used for experimental and other scientific purposes (Strasbourg, 1986), EU Directive 2010/63/EU, Law of Ukraine № 3447-IV "On protection of animals from cruel treatment", general ethic principles of experiments on animals, approved by the 1st national congress of Ukraine on bioethics (2001).

The experiment was conducted on 50 male guinea pigs weighting 250-270 g. The animals were divided into 5 groups:

I – intact guinea pigs (n=10);

II – guinea pigs (n=10) with asthma under conditions of chronic periodontitis before correction with thiotriazoline (4th day);

III - guinea pigs (n=10) with asthma under conditions of chronic periodontitis before correction with thiotriazoline (18th day);

IV - guinea pigs (n=10) with asthma under conditions of chronic periodontitis before correction with thiotriazoline (25th day);

V - guinea pigs (n=10) with asthma under conditions of chronic periodontitis after correction with thiotriazoline (25th day). The thiotriazoline was injected intramuscularly for 7 days in the period from 18th to 25th day in a dose of 100 mg/kg of a guinea pig mass.

Experimental bronchial asthma was reproduced by the method of V. I. Babych [17]. Preliminary animals were once sensitized with normal horse serum (0.1 ml intraperitoneally). Subcutaneously 0.1 ml of normal horse serum with autoclave BCG (1 mg BCG for 1.0 ml normal horse serum) were injected for the next three days. The next 14 days for 30 minutes a day in a tightly sealed chamber animals were subjected to inhalation with a spray gun of normal horse serum by 1.0 ml per each guinea pig. After that, the inhalations were conducted every seven days.

Chronic periodontitis was reproduced by the method of O. N. Voskresenskyj [18] using the model of reduced chewing function, according to which the animals were on a paste-like diet, with a norm of 63 g per day for 25 days. The model chosen for reproduction of chronic periodontitis is a classic model recommended for preclinical drugs properties study.

Animals were decapitated at the 4th, 18th, 25th days of experiment and the indices of the LPO products and enzyme activity of AOS were determined. The content of malondialdehyde (MDA) – by E. N. Korobeinikov method [19], conjugated dienes was determined by the method of V. B. Havrylov and M. I. Myshkorudna [20], superoxide dismutase activity – by R. Fried method [21], catalase activity – by R. Holmes and C. Masters method [22], and ceruloplasmin – by V.H. Kolb and V.S. Kamyshnikov method [23].

All digital resultswere statistically processed usinf arithmetical mean (V), margin of error of arithmetical mean (m), and Student's criterion "t". the calculations were performed using means of statistical and graphic analysis of Microsoft Excel electron tables (Microsoft office programs). Statistically reliable were the results with $P \leq 0,05$.

Results and their discussion

The results of experimental studies showed the significant increase of LPO products in the periodontal soft tissues. The level of conjugated dienes on 4th, 18th and 25th days of experimental bronchial asthma under conditions of chronic periodontitis before correction by thiotriazoline was 64,5% ($p \leq 0,05$), 83,3% ($p \leq 0,05$), 102,0% ($p \leq 0,05$) respectively, compared with control group; the level of malondialdehide on 4th, 18th and 25th days before correction by thiotriazoline was 56,1% ($p \leq 0,05$), 82,1% ($p \leq 0,05$), 91,7% ($p \leq 0,05$) respectively compared with control group, indicating an intensive formation of free radical compounds. After application of thiotriazoline intramuscularly for 7 days in the period from 18th to 25th day in a dose of 100 mg/kg of a guinea pig mass the levels of conjugated dienes and

malondialdehide decreased by 34,0% (p≤0,05) and 27,8% (p≤0,05) respectively on 25th day of the experiment compared to the group of animals without correction (table 1, figure 1).

Table 1

Content of conjugated dienes and malondialdehide in guinea pegs' periodontal soft tissues in experimental bronchial asthma under conditions of chronic periodontitis (M±m)

Form of the experiment	Duration of the experiment	Number of animals	Conjugated dienes in nmol/ml (g)	Malondialdehide in nmol/ml (g)
Intact guinea pigs	Control	10	4,8±0,1 p≤0,05	7,3±0,6 p≤0,05
Experimental bronchial asthma under conditions of chronic periodontitis	4th day	10	7,9±0,2 p≤0,05	11,4±0,5 p≤0,05
	18th day	10	8,8±0,2 p≤0,05	13,3±0,4 p≤0,05
	25th day without correction	10	9,7±0,3 p≤0,05	14,0±0,4 p≤0,05
	25th day with correction	10	6,4±0,1 p≤0,05	10,1±0,5 p≤0,05

Note. P – reliability of indices difference in comparison with results in control group

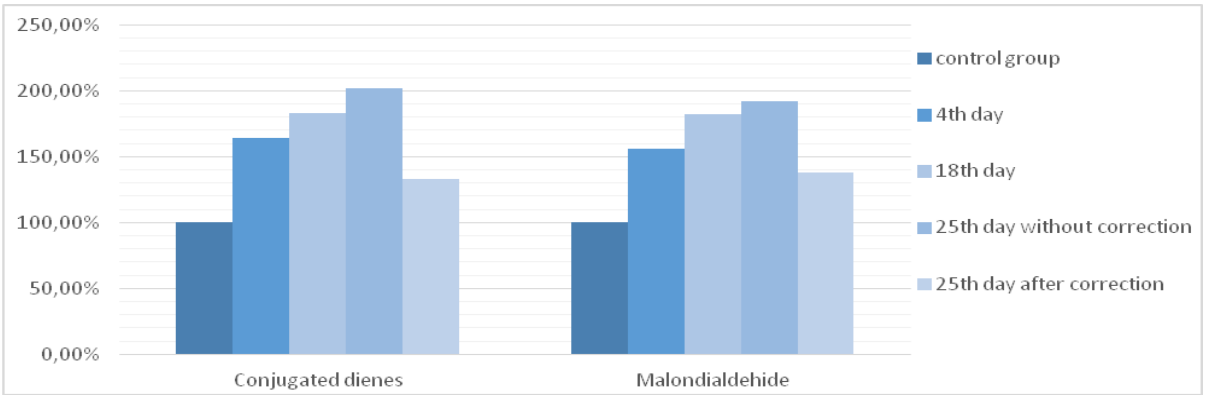

Figure 1. Content of LPO products in the dynamics of bronchial asthma development under conditions of chronic periodontitis before and after correction by thiotriazoline (in % of control)

Intensive formation of free radical compounds caused compensatory activation of antioxidant defense system enzymes. Thus, as a response to increased levels of LOP's products there was an increase in activity levels of superoxide dismutase, catalase and ceruloplasmin on 4th day of the experiment by 29,6% (p≤0,05), 35,5% (p≤0,05) and 82,5%

(p≤0,05) respectively compared with control group. Further the activity of enzymes gradually decreased: superoxide dismutase by 35,4% (p≤0,05) and 51,2% (p≤0,05); catalase by 50,2% (p≤0,05) and 55,1% (p≤0,05); ceruloplasmin by 47,5% (p≤0,05) and 55,0% (p≤0,05) on 18th and 25th days before correction by thiotriazoline respectively, compared with control group. After application of thiotriazoline intramuscularly for 7 days in the period from 18th to 25th day in a dose of 100 mg/kg of a guinea pig mass the levels of superoxide dismutase, catalase, ceruloplasmin increased by 54,6% (p≤0,05), 64,4% (p≤0,05) and 83,3% (p≤0,05) respectively on 25th day of the experiment compared to the group of animals without correction (table 2, figure 2).

Table 2

Content of superoxide dismutase, catalase and ceruloplasmin in guinea pegs' periodontal soft tissues in experimental bronchial asthma under conditions of chronic periodontitis (M±m)

Form of the experiment	Duration of the experiment	Number of animals	Superoxide dismutase in CU/ml (g)	Catalase in IU/ml (g)	Ceruloplasmin in mg/l (g)
Intact guinea pigs	Control	10	46,2±2,8 p≤0,05	18,3±1,4 p≤0,05	4,0±0,2 p≤0,05
Experimental bronchial asthma under conditions of chronic periodontitis	4th day	10	59,9±3,4 p≤0,05	24,8±1,8 p≤0,05	7,3±0,4 p≤0,05
	18th day	10	29,8±2,1 p≤0,05	9,1±0,6 p≤0,05	2,1±0,1 p≤0,05
	25th day without correction	10	22,5±1,9 p≤0,05	8,2±0,5 p≤0,05	1,8±0,1 p≤0,05
	25th day with correction	10	34,8±2,1 p≤0,05	13,4±0,8 p≤0,05	3,3±0,1 p≤0,05

Note. P – reliability of indices difference in comparison with results in control group

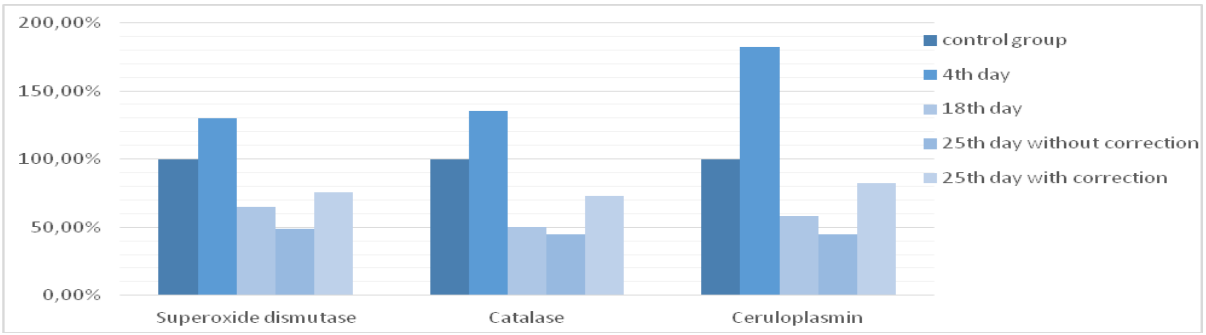

Figure 2. Activity levels of AOS enzymes in the dynamics of bronchial asthma development under conditions of chronic periodontitis before and after correction by thiotriazoline (in % of control)

Conclusions. The obtained results indicate that gradual accumulation of LOP products occurs in experimental bronchial asthma under conditions of chronic periodontitis, reaching it's peak on 25th day of the experiment. In it's turn, at the initial stage of the experiment it caused a compensatory reaction, characterized by the activity of all investigated antioxidant enzymes with the further exhaustion on 18th and 25th days of the experiment signaling about development of oxidative stress. The application of thiotriazoline lowered the levels of LPO products and increased the activity levels of AOS enzymes indicating the antioxidant properties of thiotriazoline.

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