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CHANGES IN ACTIVITY OF SOME INDICATORS OF PRO-OXIDANT AND ANTIOXIDANT SYSTEMS IN GUINEA PIGS' LUNGS IN EXPERIMENTAL ALLERGIC ALVEOLITIS AND THEIR CORRECTION WITH THIOTRIAZOLIN

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Abstract

These changes correlate with the development of oxidative stress in animals over the entire duration of the experiment, which manifested by an increase in the content of the lipoperoxidation products (LPO) - conjugated diene level and malonic dialdehide) in the lungs in comparison with control group. Decreasing of the antioxidant activity (a significant reduction of peroxidase and ceruloplazmin level) in the lung`s tissure of guinea pigs has been reported. Correction of these shifts with the antioxidant drug thiotriazolin inhibits accumulation of LPO products and increases antioxidant defense. The experimental data most probably indicate a pronounced effect of thiotriazolin on the realization of the antiinflammatory and antioxidant effects and allows a suggestion about the antiradical mechanism of this drugs.

Key words: **experimental allergic alveolitis, peroxide lipid oxidation, antioxidant system,thiotriazolin.**

Introduction

Hypersensitivity pneumonitis (HP), also called extrinsic allergic alveolitis, is a respiratory syndrome involving the lung parenchyma and specifically the alveoli, terminal bronchioli, and alveolar interstitium, due to a delayed allergic reaction [1].

The most frequent antigens that cause HP worldwide are bird proteins (pigeon breeders' disease) and bacteria (*Saccharopolyspora rectivirgula*). However, fungi are also implicated in many cases, including occupational and nonoccupational outbreaks. The clinical course of the disease is highly variable and its diagnosis clinically challenging since no specific test or biomarker allows a consistent diagnosis. Therefore, a combination of symptoms, bronchoalveolar lavage findings, chest imaging, lab tests, and often biopsies are needed for an accurate diagnosis. [2, 3].

Regardless of the cause or the responsible environment, the histopathology is similar and usually consists of a granulomatous interstitial bronchiolocentric pneumonitis characterized by the presence of poorly formed granulomas and a prominent interstitial infiltrate composed of lymphocytes, plasma cells, and macrophages[3-6]/

This response is mediated by immune complexes in the acute form and by Th1 and likely Th17 T cells in subacute/chronic cases. Pathologically, HP is characterized by a bronchiolocentric granulomatous lymphocytic alveolitis, which evolves to fibrosis in chronic advanced case.According to a two-hit model, antigen exposure associated with genetic or environmental promoting factors provokes an immuno-pathological response. Granulomas were commonly observed in the bronchiolar wall and alveolar ducts in subacute hypersensitivity pneumonitis; they are less than 150 μm in diameter, smaller than those observed in sarcoidosis [7, 8, 9]. Precipitins against causative antigen and immunoglobulin and complement were demonstrated in vessel walls [10]. T-lymphocytes mediated hypersensitivity response is the most important type 4 immune reaction in the pathogenesis of HP. Th1-cytokine network plays a key role in the development of HP [11], and later in the chronic form develops a Th2-like immune response. In fact, the features associated with chronic HP include a gradual increase in CD4+ T cells and in the CD4+/CD8+ ratio, a modification toward TH2 T cell differentiation and cytokine profile as well as a decline of CD8+ T cells. In acute HP, pulmonary parenchyma inflammation appears to be mainly mediated by a type 3 response, as suggested by the presence of high titers of antigen-specific precipitating IgG in the serum, and an increase in lung neutrophils. Subacute and chronic forms of HP are characterized by a T cell-mediated immune response with increased T-cell migration and developing of a characteristic T-lymphocytic alveolitis[12-14].

The description of oxidant/antioxidant balance in the lungs and evidence of its involvement in the pathogenesis of this pathology is hasn`t studied enough.

The aim of the work was to study lipid peroxidation processes and the condition of antioxidant defence in guinea pigs' lungs in different periods of experimental allergic alveolitis and action on this indicators of thiatriazolin.

MATERIALS AND METHODS OF INVESTIGATION

All experiments on laboratory animals were conducted following the principles of bioethics according to the regulations of European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), European Union 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 first national congress of Ukraine on bioethics (2001).

The experiment was conducted on 110 female guinea pigs weighing 0.18-0.20 kg. The animals were divided into 5 groups:I – intact guinea pigs (n = 20);

II – guinea pigs (n = 18) with EAA (24 th day from the start of injecting antigen);

III – guinea pigs (n = 18) with EAA (34 th day from the start of injecting antigen);

IV – guinea pigs (n = 18) with EAA (44th day from the start of injecting antigen).

V – guinea pigs (n = 18) with EAA (54th day from the start of injecting antigen) before treatment.

VI – guinea pigs (n = 18) with EAA (54th day from the start of injecting antigen) after treatment with tiotriazolin in dose 100 мg/кg (from 44th to 54th day),which was injected during 10 days in allergic alveolitis development.

Experimental allergic alveolitis (EAA) was induced by the method of O.O. Orehov and Y.A. Kyrylov [15]. Prior, the animals had been immunized with Freund's c*omplete* adjuvant (0.2 ml intramuscularly into a hind leg). In 2 weeks, 0.2 ml of 1% BCG solution was introduced intravenously every 10th day. Later, the animals were decapitated; the level of LOPs and activity of antioxidant system enzymes were detected in lung homogenate on the 24 th, 34 th, 44^{th,} 54 th days after EAA. The content of conjugated dienes was determined by the method of V.B. Havrylov and M.I. Myshkorudina [16], malondialdehyde (MDA) – by E.N. Korobeinikov method [17], ceruloplasmin activity – by method [18], peroxidase activity – by O.G. Arhipova [19].

All digital results were statistically processed using arithmetical mean (M), margin of error of arithmetical mean (m), and Student's criterion "t". The calculations were performed

using means of statistical and graphic analysis of electron tables Microsoft Excel (Microsoft office programs). Statistically reliable were the results with $p \leq 0.05$.

RESULTS OF INVESTIGATION AND THEIR DISCUSSION

Results of this experimental work have shown that activation of primary*(*conjugated dienes) product of lipid oxidation by 54,08% ($p<0,01$) i 106,25 % ($p<0,01$) and by117,25% ($p<0,01$) and 195,08% ($p<0,01$) during early(24th, 34th days) and late (44th , 54th days) respectively, in comparison with the control. We were observing the evaluation of secondary product of free radicals intensive formation - MDA in the lungs. Activity and direction of change was similar to the content of the conjugated dienes , but not so significant. Thus, it was found that on the 24th and 34th days of this immunocomplex disease the content of MDA increased by 38.39% ($p<0.01$) and 51.66% ($p<0.01$) relatively to the group of healthy animals. During the latter period, this indicator had tendency to increase and was 59,95% ($p<0,01$) and 127,51% ($p<0,01$) on the 44th, 54th days in comparison with healthy animals.

Such changes of pro- oxidant system components content poin on excessive formation of lipid peroxidation products.

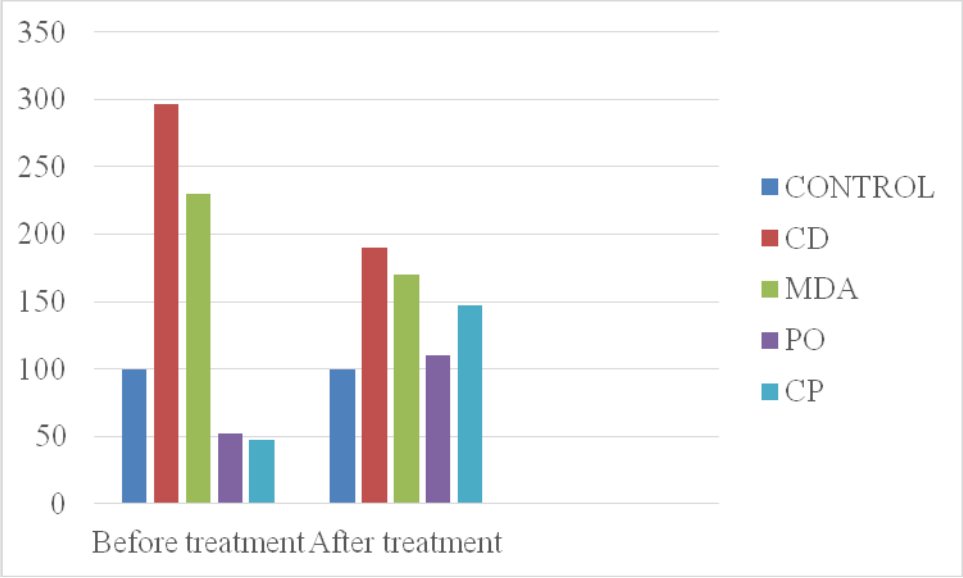

Fig 1. Influence of thiotriazolin on indicators of pro-oxidant and antioxidant systems in the animals' lungs in EAA (in % before and after treatment on 54 th day of experiment)

For characteristic of antioxidant system condition we analized activity of peroxidaze (PO) and ceruloplazmin(CP). It was found increasing of activity of PO in early period (24th day) of allergic alveolitis by 46,65% ($p<0,01$) compared with control. Further, on 34th day, this indicator was on the level of intact animals. Significant reduction of peroxidase activity

on 44th and 54th days of the experiment, respectively, by 36,91% ($p<0,05$) and 47,35% ($p<0,05$) compared with the control, have been established. It allows us to make decision about depletion of antioxidant defense. Similar changes from ceruloplasmin activity was observed. We have found that on the 24th day of the experiment it was an increase in the lungs by 42,65% ($p<0.01$) against the control. Subsequently, at the 34th day of AA this index did not differ from the group of intact animals and was subjected to opposite changes in the late period of the formation of this experimental disease model - the activity of the CP decreased by 40,69% ($p<0.01$) and 55.35% ($p<0.01$), which testified to the suppression of the antioxidant system.

The using of antioxidant tiotriazoline for 10 days intramuscularly at a dose of 100 mg / kg led to a decrease in the content of DC in 51,11% ($p <0,05$) and 48,09% ($p <0,05$) in the lungs against the group of guini pigs that have not been exposed to the drug, which indicates its positive effect on these medicine.

CONCLUSIONS

These changes correlate with the development of oxidative stress in animals over the entire duration of the experiment, which manifested by an increasing in the content of the primary and end LPO products and decreasing of the antioxidant activity in the lungs og guinea pigs . Correction of these shifts with the antioxidant drug inhibits accumulation of LPO products and increases antioxidant defense.

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