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ACTIVITY OF SUPEROXIDE DISMUTASE IN GUINEA PIGS' BRONCHI IN LATE PERIODS OF EXPERIMENTAL ALLERGIC ALVEOLITIS DEVELOPMENT AND THEIR CORRECTION WITH THIOTRIAZOLIN

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Abstract

The aim of work was to make biochemical investigations for detection of pro-oxidant and antioxidant systems markers in guinea pigs` bronchi before and after treatment of thiotriazolin during experimental allergic alveolitis development. Gradual elevation of conjugated dienes and malonic dialdegyde level in the bronchi was recorded on the 44th day and 54 th of experimental allergic alveolitis development in comparison with intact animals, indicating excessive accumulation of lipid peroxidation products. In the same time activity of superoxide dismutase was reduced. Oxidative stress occurs when the balance between antioxidants and reactive oxygen species(ROS) are disrupted because of either depletion of antioxidants or accumulation of ROS. Reduction of conjugated diene and malondialdehyde content and elevation of catalase activity have been reported in animals with this experimental model of disease after using of thiotriazolin. The study of oxidative stress can provide insights into etiopathogenesis and favour the discovery of new treatments.

Key words: **experimental allergic alveolitis, free radicals, superoxyde dismutase, thiotriazolin.**

Introduction

Hypersensitivity pneumonitis (HP) develops after inhalation of many different environmental antigens, causing variable clinical symptoms that often make diagnosis uncertain. Initially, it was associated with farming (moldy grain or hay handling) hence the term farmer's lung With time, a large variety of environmental settings and antigens have been described [1-3]. Among the other most common settings are contacts with birds (pigeons, parakeets), humidifiers, moldy wood, and a variety of other settings where moulds abound. Although most antigens are organic particles, some chemical compounds (e.g. isocyanates, zinc,) can act as haptens which link to the host albumin to create an anti.genic particle.[2] The inflammatory response of the alveolar mucosa is a hypersensitivity reaction of type 3 (immune-complex-mediated) or type 4 (T lymphocytes mediated). 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.[2, 4]

Many HP offending agents are small slowly degradable particles, which explain their retention within the lung. Antigenic substances can interact with complement, antibodies and cells to produce inflammation. This adjuvant effect causes the release of reactive oxygen species, prostaglandins, leukotrienes and proteolytic compounds by leucocytes as well as the production and secretion of IL-1, TNF, IL-12, IL-6, MCP-1 and MIP-1α by macrophages [2, 5].

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.

The prevalence of HP is higher than recognized, especially its chronic form. Mechanisms of disease are still incompletely known. Strategies to improve detection and diagnosis are needed, and treatment options, principally avoidance, are limited.

The aim of work was to make biochemical investigations for detection of markers of pro-oxidant and antioxidant systems in guinea pigs bronchi before and after treatment with thiotriazolin during experimental allergic alveolitis development [5].

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 30 female guinea pigs weighing 0.18-0.20 kg. Experimental allergic alveolitis (EAA) was induced by the method of O.O. Orehov and Y.A. Kyrylov [6]. 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 10^{th} day. Decapitation was made on 44^{th} and 54^{th} days and we took bronchi for observation. The content of conjugated dienes was determined by the method of V.B. Havrylov and M.I. Myshkorudina [7], malondialdehyde (MDA) – by E.N. Korobeinikov method [8], superoxide dismutase activity – by R.Fried method [9].

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

Data from experimental studies have shown that in the late period (44^{th}) of EAA development rising the content of DC in bronchi was observed. It was characterized by 104.55% ($p<0.01$) respectively with control group of animals . And this increasing has atchieved a highest level by 169.55% ($p <0.01$) on 54^{th} day of the experiment relatively to group of intact guinea pigs The changes with MDA had a tendency to rising.Thus, a gradual elevation of MDA level in the bronchi was recorded on the 44^{th} day of EAA development by 80,16% ($p<0,01$) and 87,04% ($p<0,01$) on 54^{th} respectively, in comparison with intact animals, indicating excessive accumulation of lipid peroxidation products.

Researching of enzyme of antioxidant system- supreroxide dismutase in the bronchi has showed a suppression of antioxidant defence activity . It decreased gradually depend on the duration of experiment. It is demonstrated in our results-reduction of enzymatic activity on 44^{th} by 18,53% ($p<0,01$) and on54^{th} by 25,17% ($p<0,01$) in comparison of healthy animals.

Antioxidant thiotriazolin has coused corrective effect on changed indicators as pro- as antioxidant systems. Thiotriazolin was used for 10 days(from 44^{th} to 54^{th} days) intramuscularly at a dose of 100 mg / kg. Reduction of CD and MDA content in bronchi by 45,37% ($p<0,01$) and 35,95% ($p<0,01$) respectively against the group of guinea pigs which didn`t reseved this drug have been reported (Table 1).Activity of superoxide dismutase had a

different direction.Iit`s level has elevated by28,96% ($p<0,01$) in comparison with animals without treatment (Table 2).

Table 1

Action of thiotriazolin on CD and MDA content in bronchi before and after treatment in EAA (M±m, n=58)

Form of investigation		Amount of animals	CD in nmol/ml (g)	MDA in nmol/ml (g)
Intact animals. Control		10	11,20 ± 0,60	18,60 ± 0,80
Guinea pigs with EAA	Before treatment	10	30,19 ± 0,36 p<0,01	34,79 ± 0,50 p<0,01
	After treatment with thiotriazolin	10	16,49 ± 0,24 p<0,05 p_1<0,01	22,28 ± 0,33 p<0,05 p_1<0,01

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

p_1 – reliability of indices difference in comparison with the results in EAA before treatment and after treatment with thiotriazolin.

Table 2

Action of thiotriazolin on COD activity in bronchi before and after treatment in EAA (M±m, n=58)

Form of investigation		Amount of animals	SOD in c.u/ml (g)
Intact animals. Control		10	120,40 ± 3,80
Guinea pigs with EAA	Before treatment	10	90,10 ± 0,34 p<0,01
	After treatment with thiotriazolin	10	116,19 ± 0,37 p>0,05 p_1<0,01

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

p_1 – reliability of indices difference in comparison with the results in EAA before treatment and after treatment with thiotriazolin.

CONCLUSIONS Late period of experimental allergic alveolitis accompanies with activation of reactive oxygen specious formation and depression of antioxidant enzyme capacity. Oxidative stress occurs when the balance between antioxidants and pro-oxidants are disrupted because of either depletion of antioxidants or accumulation of free radicals. When oxidative stress occurs, cells attempt to counteract the oxidant effects and restore the redox balance by activation or silencing of genes encoding defensive enzymes, transcription factors, and structural proteins. A potential role of oxidative stress in the pathogenesis of extrinsic allergic alveolitis has been demonstrated.Antioxidant thiotriazolin has corrective positive effect on this disbalance.

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