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CHANGES OF CERULOPLASMIN`S ACTIVITY IN ANIMALS` BRONCHI IN LATE PERIODS OF EXPERIMENTAL ALLERGIC ALVEOLITIS DEVELOPMENT AND THEIR CORRECTION WITH THIOTRIASOLIN

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Abstract

The present study has shown that thiotriazolin possess to moderate antiinflammatory and antioxidant activities.An imbalance between generation of free radicals and antioxidant defences leads to a negative condition known as oxidative stress. Pulmonary damage caused by oxygen toxicity occurs due to the generation of reactive oxygen species and subsequent formation of more potent oxidants. Additionally, treatment with thiotriasolin has modulated enzymatic level of ceruloplasmin. The study of oxidative stress in the pathogenesis of exogenic allergic alveolitis could open new therapeutic horizons.

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

Introduction

Extrinsic allergic alveolitis also known as hypersensitive pnevmonitis(HP), is a complex syndrome that results from repeated inhalation and sensitization to a wide variety of aerosolized antigens [1]. It is caused by the inhalation of an antigen to which the individual is sensitized and hyperresponsive;

The immunopathogenesis of HP is poorly understood, although T-cell hyperreactivity and immune complex formation and deposition appear to play a prominent role. Patients with acute HP, if correctly and timely diagnosed and treated, generally have an excellent prognosis [2, 5]. Factors associated with worse prognosis include duration of exposure (eg, individuals exposed for a shorter period have a more favorable outcome than those with a longer exposure) [3]; digital clubbing ; older age [4] and greater intensity of exposure [5];

The chronic stage of HP is characterized by fibrotic changes, although evidence of active disease (eg, superimposed centrilobular fluffy nodules) may still be present. HP has been conventionally classified as acute, subacute, and chronic. Identification and removal of the offending agents remains the cornerstone of treatment and a major determinant of prognosis [6].The diagnosis of HP is often difficult and effective treatments in progressive forms are lacking.

Oxidative stress is a central feature of many diseases. The lungs are particularly susceptible to lesions by free radicals and pulmonary antioxidant defenses are extensively distributed and include both enzymatic and non-enzymatic systems [8]. The aim of our scientific work was to determinate role of oxidative stress in development of exogenous allergic alveolitis.

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 40 female guinea pigs weighing 0.18-0.20 kg. The animals were divided into 4 groups:

I – intact guinea pigs (n=10);

II – guinea pigs (n=10) with EAA (44^{th} day from the start of injecting antigen).

III – guinea pigs (n=10) with EAA (54^{th} day from the start of injecting antigen).

IV– guinea pigs (n=10) with EAA(54^{th} day from the start of injecting antigen) after treatment with thiotriazolin.

Experimental allergic alveolitis (EAA) was induced by the method of O.O. Orehov and Y.A. Kyrylov [9]. Prior, the animals had been immunized with Freund's complete 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 lipid oxidated peroxides and activity of catalase were detected in lung homogenate on the 44th 54th days after EAA. The content of conjugated dienes was determined by the method of V.B. Havrylov and M.I. Myshkorudina [10], malondialdehyde (MDA) – by E.N. Korobeinikov method [11], and ceruloplasmin – by V.H. Kolb and V.S. Kamyshnikov method [12].

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 detected that in the late period (44th *)* of EAA development elevation of DC content in bronchi was observed. It was characterized by 104.55% ($p<0.01$) respectively with control group of animals . Maximal activity of this enzyme atchieved, on 54th day of the experiment, by 169.55% ($p <0.01$) relatively to group of intact guinea pigs. The same character of MDA activity was recorded. Thus,a gradual elevation of MDA level in the bronchi was revealed on the 44th 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 reactive oxygen species. Ceruloplasmin (CP) activity in the bronchial tissue significantly decreased in the late periods of experimental allergic alveolitis and was on the minimum activities on 54th day by 35,15% ($p<0,01$) against the healthy animals.

After the started treatment with thiotriazolin improvement in ratio between pro-oxidants and antioxidants balance was noticed. Thiotriazolin was used for 10 days (from 44th to 54th 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). It was found that activity of ceruloplasmin elevates by 31,19% ($p<0,01$) in comparison with animals without treatment (Table 2). Corective positive effect of thiotriazolin on markers of pro-oxidant and antioxidant systems have been established

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 CP activity in bronchi before and after treatment in EAA (M±m, n=58)

Form of investigation		Amount of animals	CP in mg/l	p
Intact animals. Control		10	12,30 ± 0,90	
Guinea pigs with EAA	Before treatment	10	7,11 ± 0,20	p<0,01
	After treatment with thiotriazolin	10	10,80 ± 0,26	p<0,01 p_1<0,05

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

Prolonged exposure to hyperoxia results in acute lung injury. Pulmonary damage caused by oxygen toxicity occurs due to the generation of reactive oxygen species and subsequent formation of more potent oxidants. The results of the investigations showed a high

effectiveness of the thiotriazolin on disturbed balance between pro- oxidant and antioxidant systems. However, oxidant/antioxidant imbalance has been thoroughly investigated for some diffuse lung diseases while very little data is available for others.

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