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BIOMARKERS OF PRO- AND ANTIOXIDANT SYSTEMS IN GUINEA PIGS' BLOOD IN LATE PERIODS OF EXPERIMENTAL ALLERGIC ALVEOLITIS DEVELOPMENT AND THEIR CORRECTION WITH THIOTRIAZOLIN

Mariana Mukchailivna Fyrduchco – Regeda[1], Zoryana Ljubomurivna Solvar [2]
Mariana Ljubomurivna Baida[3]

Danylo Halytsky Lviv National Medical University

[1] Department of pathological physiology, PhD

[2] Department of pathological physiology, postgraduate student

[3] Department of pathological physiology, PhD, Assistant Professor

Lviv, Ukraine

Abstract

Hypersensitivity pneumonitis is a potentially fatal immunological lung disease caused by occupational or environmental exposure to specific antigens and resulting from lymphocytic and frequently granulomatous inflammation of the peripheral airways, alveoli, and surrounding interstitial tissue which develops as the result of a non-IgE-mediated allergic reaction to a variety of organic materials or low molecular weight agents that are present in the workplace. The offending agents can be classified into six broad categories that include bacteria, fungi, animal proteins, plant proteins.The cornerstone of treatment is early removal from exposure to the eliciting antigen, although the disease may show an adverse outcome even after avoidance of exposure to the causal agent. The primary insults cause the greatest production of reative oxygen species, which contributes to oxidative damage by attacking all macromolecules, including lipids, proteins and nucleic acids, leading to defects in their physiological function.

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

Introduction

Hypersensitivity pneumonitis (HP) is a potentially fatal immunological lung disease caused by occupational or environmental exposure to specific antigens. A number of etiological factors of HP have been reported, including contaminated forced-air systems and water reservoirs, but the type and source of offending environments are sometimes difficult to identify from clinical history. [1]

This lung pathology resulting from lymphocytic and frequently granulomatous inflammation of the peripheral airways, alveoli and surrounding interstitial tissue which develops as the result of a non-IgE-mediated allergic reaction to a variety of organic materials or low molecular weight agents that are present in the workplace [2]. The offending agents can be classified into six broad categories that include bacteria, fungi, animal proteins, plant proteins, low molecular weight chemicals, and metals. Because the disease is not only confined to the alveoli, but also involves the bronchioles (i.e. alveolobronchiolitis), the term 'hypersensitivity pneumonitis' is more appropriate and is currently most commonly used [3, 4].

The aetiology of this disease is still unknown, although there is evidence that cellular redox status and oxidative stress contribute to progression.

The massive production of antimicrobial and tumoricidal eactive oxygen species in an inflammatory environment plays an important role in bodily defences but when inflammation becomes chronic it induces persistent activation of macrophages and neutrophils that become a persistent source of oxidative damage of DNA and cell components [5].

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

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 male 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 complete 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 tooked 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].

Thiotriazolin was used for 10 days(from 44^{th} to 54^{th} days) intramuscularly at a dose of 100 mg / kg.

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

A significant difference was found between activity of prooxidant system and enzyme of antioxidant defence. Thus, content of CD in the animals` blood was significantly increased in experimental allergic alveolitis development compared with control group and was increasing proportionally to the duration of this experimental model of disease respectively by 80,32% ($p<0,05$) and 154,84% ($p<0,05$) on 44^{th} to 54^{th} day. Profile of MDA was similar to CD. Results of our investigation have showed that content of MDA in the blood was elevated by 70,25% ($p<0,05$) and 130,50% ($p<0,05$) respectively on 44^{th} to 54^{th} day of experiment.

Researching of enzyme of antioxidant system- supreroxide dismutase in the blood has showed slight increasing of this enzyme activity on 44^{th} day by 34,90% ($p<0,05$) in comparison with healthy animals. It shows compensatory ability of antioxidant to neutralize overprodused free radical. But on 54^{th} day SOD content was decreased by 32,0% ($p<0,05$) against control. It means about suppression of antioxidant defence activity and inability to maintain positive oxidative balance .

Percentages of antioxidant biomarker were significantly increased but other measured parameters were significantly decreased in group treated with thiotriazolin .Thus, SOD activity was elevated by 43,15% ($p<0,05$) after using of this drug in comparison with level of this marker in guinea pigs which didn`t reseave treatment (Table 2).

Reduction of CD and MDA content in blood by 47,96% ($p<0,05$) and 44,68% ($p<0,05$) respectively against the group of guinea pigs without treatment have been reported (Table 1).

Table 1

Action of thiotriazolin on CD and MDA content in blood before and after treatment in EAA (M±m, n=30)

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<0,05	34,79 ± 0,50 p<0,05
	After treatment with thiotriazolin	10	16,49 ± 0,24 p<0,05 p_1<0,05	22,28 ± 0,33 p<0,05 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.

Table 2

Action of thiotriazolin on COD activity in blood before and after treatment in EAA (M±m, n=30)

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<0,05
	After treatment with thiotriazolin	10	116,19 ± 0,37 p>0,05 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 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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