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CONDITION OF PRO-OXIDANT SYSTEM IN GUINEA PIGS' BRONCHI IN EXPERIMENTAL ALLERGIC ALVEOLITIS DEVELOPMENT AND THEIR CORRECTION WITH THIOTRIAZOLIN

Mariana Ljubomurivna Baida[1], Mariana Muchailivna Fyrduchko-Regeda[2], Natalia Grugorivna Sementsiv[3]

Danylo Halytsky Lviv National Medical University

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

[2]Department of pathological physiology, PhD

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

Lviv, Ukraine

Abstract

Exogenic allergic alveolitis has been ascribed to multiple inhaled antigens found in a large variety of environmental settings. The immune mechanisms leading to this deases development are still incompletely understood. Initially, believed to be a classes III and IV immune response, we now have a clearer understanding of the complex inflammatory events involved. These include the release of pro inflammatory cytokines and a decrease in the immune control mechanisms via surfactant, dendritic and T-regulatory cells. Free radicals and reactive oxygen species could play a major role in the pathophysiology of allergic alveolitis . Despite the improved understanding, the treatment and outcome of this pathology have not known enough. This work determinate on accumulation of lipid peroxidaton products in the animals` bronchi during experimental allergic alveolitis and corrective effect of antioxidant-thiotriazolin on these indicators.

Key words: **experimental allergic alveolitis,malonic dialdegyde, conjugated diens, thiotriazolin.**

Introduction

Hypersensitivity pneumonitis (HP) is the term now most commonly used to describe a disease that was previously referred to as extrinsic allergic alveolitis. It is a disease that occurs upon exposure to organic dust[1]. The essence of this disease is an interaction between the host's immune system and external antigen, influenced by both genetic and environmental factors. A large number of occupational agents/antigens have been described as potential causative agents of HP in a wide variety of occupations. These offending agents can be classified into six broad categories that include bacteria, fungi, animal (glyco)proteins, plant (glyco)proteins, low molecular weight chemicals, and metals.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. [2-5]

In susceptible subjects, it leads to a combined type III allergic reaction of Gell and Coombs (with formation of precipitines) and a type IV lymphocytic reaction (with a granulomatous inflammation in the distal bronchioles and alveoli) [3]. Because the disease is not only confined to the alveoli, but also involves the bronchioles (i.e. alveolobronchiolitis), the term ‘hypersensitivity pneumonitis’ (HP) is more appropriate and is currently most commonly used [3, 4, 6].

The role of cells in the pathophysiology of HP has been widely studied. Neutrophils, macrophages, $CD4^+$ and $CD8^+$ lymphocytes, mast cells, natural killer (NK) cells, major histocompatibility class (MHC)-restricted and non-MHC-restricted cells have been found in the lung and BAL of HP patients [9, 10]. In most subjects, the normal immune defence mechanisms maintain a homeostasis between antigen inhalation and the host’s response. During an inflammatory process such as HP, production of the inducible nitric oxide synthase by alveolar macrophages is increased. This enzyme allows inflammatory cells to produce nitric oxide which has direct toxic effects on cells [7, 11]. Hence, free radicals and reactive oxygen species could play a major role in the pathophysiology of HP.

The aim of the research was to study lipid peroxidation processes in guinea pigs’ bronchi in experimental allergic alveolitis (EAA) formation and action on these enzymes of thiotriazolin.

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 [12]. 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 took bronchi for observation. The content of conjugated dienes(CD) was determined by the method of V.B. Havrylov and M.I. Myshkorudina [13], malondialdehyde (MDA) – by E.N. Korobeinikov method [14].

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

Our findings indicate that on the early periods which included 24^{th} and 34^{th} days day from the start of injecting antigen increasing conjugated dienes content in the bronchi was observed by 54,55% ($p<0,01$) and 79,38% ($p<0,01$) respectively, in comparison with the control, indicating an intensive formation of free radical compounds in experimental animals. Latter, gradual accumulation of this indicator of prooxidant system by 104,55% ($p<0,01$) took place on 44^{th} day. Tendency to aggravation coused that level of conjugated dienes reached pick on 54^{th} day of this model of disease by 169,55% ($p<0,01$) in comparison with healthy animals.

The changes similar to conjugated dienes occurred with MDA. Thus, a gradual elevation of MDA level in the bronchi was recorded on the 24^{th} and 34^{th} days of EAA by 44,03% ($p<0,01$) and 73,55% ($p<0,01$) respectively, in comparison with the control indices. Late period of this imunne complex pathology was characterized with more significant level of this enzyme by 80,16% ($p<0,01$) and 87,04% ($p<0,01$) respectively against control. These

results demonstrate that activation of lipid peroxidation is activated in early and late periods of allergic alveolitis.

For correction of these disturbances thiotriazolin was used. This drug is antioxidant, membranes` protector and immunomodulator.

The using of antioxidant thiotriazolin for 10 days (from 44^{th} to 54^{th} days) intramuscularly at a dose of 100 mg/kg led to reduction of CD and MDA content in bronci by 169,55% ($p<0,01$) and 87,04% ($p<0,01$) against the group of guinea pigs which didn`t reseved this drug, which indicates the positive corrective effect of this medication.

CONCLUSIONS. Our findings indicate significant accumulation of lipid peroxidation products in the animals` bronchi during experimental allergic alveolitis and corrective effect of antioxidant-thiotriazolin on these markers.

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