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DISTURBANCES OF FUNCTIONAL CONDITION OF PRO-OXIDANT AND ANTIOXIDANT SYSTEMS IN GUINEA PIGS' LUNGS IN EXPERIMENTAL ALLERGIC ALVEOLITIS

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Abstract

We have analyzed the results of experimental work of alterations in indices of pro-oxidant (conjugated diene and malondialdehyde) and antioxidant (superoxide dismutase, catalase) systems in guinea pigs' lungs in experimental allergic alveolitis. The results of investigation showed that a significant increase in conjugated diene level in animals' lungs was observed at all stages of experimental allergic alveolitis development under the conditions of immobilization stress as compared with control group, indicating activation of this marker.

The same changes occurred with malondialdehyde content, indicating excessive accumulation of this lipid peroxidation product in lung tissue. Oxygen-derived free radicals produced by phagocytes are believed to contribute to lung tissue damage. A lung defence mechanism against oxidative stress produces antioxidant molecules. In the early period of experiment (24^{th}, 34^{th} days) compensatory increasing superoxide dismutase and catalase in the lungs of guinea pigs has been reported. Late period of this pathology (44^{th}, 54^{th} days) was accompanied with reduction of these antioxidant enzymes. Increased oxidant levels and decreased antioxidant defences can contribute to the progression of experimental allergic alveolitis.

Keywords: experimental allergic alveolitis, peroxide lipid oxidation, antioxidant system.

INTRODUCTION

Diffuse lung diseases (DLD) are a heterogeneous group of lung disorders with different aetiologies that evolve towards pulmonary fibrosis of variable severity. They are characterized by various pathogenetic mechanisms: sarcoidosis and lung diseases involving connective tissue are systemic immunoinflammatory diseases, exogenic allergic alveolitis. The aetiology of this disease isnot complete studied, although there is evidence that cellular redox status and oxidative stress contribute to progression. Defence mechanisms against oxidants involve enzyme and non-enzyme antioxidant systems. An imbalance between generation of ROS/RNS and antioxidant defences leads to a negative condition known as oxidative/ nitrosative stress [1, 2].

Because of their anatomy, location and function, the lungs are highly susceptible to oxidative damage. Lung is exposed to higher oxygen tension than other tissues. Exogenous oxidants and pollutants further increase oxidant production and activate inflammatory cells to generate free radicals. Many of these agents, including hyperoxia, cigarette smoke, asbestos fibers, drugs, and radiation, are also known to be associated with fibrotic interstitial lung reactions. a wide variety of oxidants are produced in response to injuries leading to pulmonary fibrosis. These oxidants can activate several genes related to cell growth, cell death, and fibroblast proliferation [3].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals generated physiologically during oxidative phosphorylation.[7] They have various physiological roles and are removed rapidly from the body: their persistence can cause cell dysfunction and cell death[7-9] Defence mechanisms against oxidants involve enzyme and non-enzyme antioxidant systems. An imbalance between generation of ROS/RNS and antioxidant defences leads to a negative condition known as oxidative/ nitrosative stress in which cell antioxidants are insuffi- cient to keep ROS/RNS below a toxic threshold due to excessive production of ROS/RNS and/or loss of cell antioxidant defences.Oxidative/nitrosative stress can affect proteins, lipids, carbohydrates and nucleic acids that are the main components of cells. Several studies suggest that oxidant–antioxidant imbalances in the lower respiratory tract play a critical role in the pathogenesis of IPF. For example, pulmonary inflammatory cells of patients with IPF generate higher levels of oxidants than those in control patients [4-8].

Imbalance between oxidants and antioxidants is a pathogenetic mechanism also recognized in other DLD.Oxidative stress is thought to induce progression of pathological procces by interaction of oxygen radicals with vascular endothelium components and fibroblasts.

The lungs are protected from the negative effects of oxidant persistence in tissues by endogenous agents named antioxidants. Antioxidants may be non-enzyme or enzyme proteins. The former include glutathione, vitamins (alphatocopherol and ascorbic acid), beta carotene and uric acid; the latter, superoxide dismutases, catalases and peroxidases [9].

Extracellular-SOD (EC-SOD), copper/zinc-SOD (Cu/Zn-SOD) and manganese-SOD (Mn-SOD) are different superoxide dismutases that protect the lungs against oxidative stress by reducing superoxide anion to hydrogen peroxide, which is then converted to water by catalase and glutathione peroxidase [10].

EC-SOD normally protects the lungs against fibrosis by preventing oxidative degradation of the matrix and by binding type I and type IV collagen via its heparin/matrix binding domain. Its expression has been evaluated in lung tissue samples from patients with extrinsic allergic alveolitis (EAA), sarcoidosis, IPF, desquamative interstitial pneumonia (DIP) and chronic obstructive pulmonary disease (COPD) [11].

Catalase is a 240-kDa protein mainly found in macrophages, pneumocytes and lung fibroblasts. It exerts its antioxidant function by reducing hydrogen peroxide (produced by SODs) to water. The real involvement of this enzyme in oxidative lung damage is still unclear and controversial data is available on oxidant-mediated catalase production. Catalase and Mn-SOD were expressed in alveolar regions of DIP and usual interstitial pneumonia (UIP) and in granulomas of sarcoidosis and extrinsic allergic alveolitis, suggesting a protective role against progression of diffuse lung diseases [12, 13, 14]. The aim of the research was to study of changes of functional activity of lipid peroxidation processes and the antioxidant protection in guinea pigs' lungs in different periods of experimental allergic alveolitis (EAA) formation.

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 92 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). Experimental allergic alveolitis (EAA) was induced by the method of O.O. Orehov and Y.A. Kyrylov [14]. 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, 44th, 54 th days after EAA. The content of conjugated dienes was determined by the method of V.B. Havrylov and M.I. Myshkorudina [15], malondialdehyde (MDA) – by E.N. Korobeinikov method [16], superoxide dismutase activity – by R.Fried method [17], catalase activity – by R. Holmes [18].

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

Inflammatory process in the lungs accompanied with changes of indicators of pro-oxidant system. Thus, content of conjugated dienes had tendency to increasing on 24 th, 34 th and 44 th days by54,08% ($p<0,01$) ,106,25 % ($p<0,01$) and 117,25% ($p<0,01$) and reached up of pick on 54 th day by 195,08% ($p<0,01$) respectively, in comparison with the control (Fig.1). Determination of another indicator that evaluated the condition of the pro -oxidant system, in particular the level of MDA in the lungs, showed that degree of activity and direction of change was similar to the content of the DK, but with somewhat lower severity, but elevated. It was found that on 24 th and 34 th days of this immunocomplex disease the content of MDA increased by 38.39% ($p <0.01$) and 51.66% ($p <0.01$) relative to the group of healthy animals. Further studies carried out in other terms (the 44 th day) of the experiment showed an even greater degree of expression of the MDA content in the lungs. It increased significantly by 59.95% ($p <0.01$) and reached a peak of 127.51% ($p <0,01$) on the 54 th day of observation against the intact group of guinea pigs (Fig 1).

These results allowed us to detect changes in lipoperoxidation indices, which gradually increased depending on the duration of iflammation in the lungs.

For characteristic of antioxidant defence capacity we were used the content of superoxiddismutase (SOD) and catalase (CT) in the animals' lungs (Fig 1.). Activity of ezymes had a different way of changes. Firstly, on the early period of this model of disease(on 24th day), increasing of SOD in the lungs was observed by 21,36% ($p<0,01$), later, beginning from 34th and on 44th ,54 th days it was decreased by12,81% ($p<0,01$), 17,57% ($p<0,01$) i 43,79% ($p<0,01$) respectively, in comparison with the data of intact animals.

Analysis of enzymatic activity of catalase had showed the simple like SOD changes. Activation of catalase activity on 24 th day in lung`s tissure by 17.32% ($p<0.05$) was noticed in the dynamics of EAA. But latter this indicator of antioxidant system was redused by36,38% ($p<0,01$), 40,59% ($p<0,01$) and 56,26% ($p<0,01$) respectively, in comparison with the control.

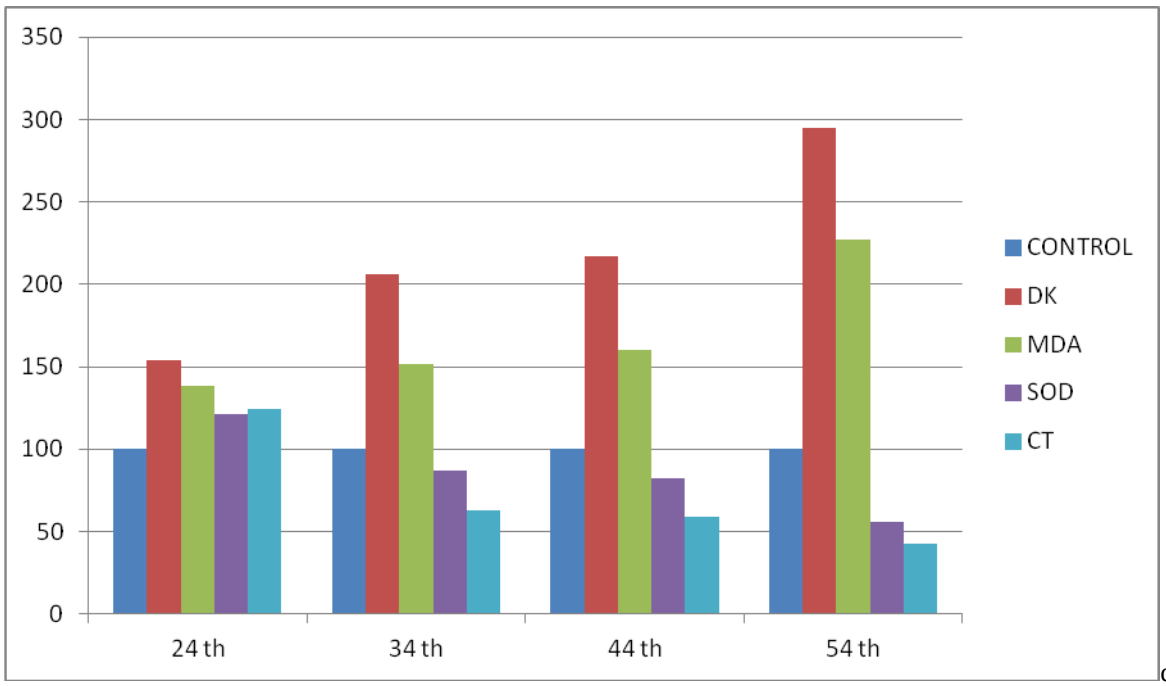

Fig.1. Condition of pro-oxidant and antioxidant systems in the animals' lungs in EAA (in % of control).

CONCLUSIONS

The obtained results indicate that a gradual accumulation of lipid oxidation products occurs in EAA , reaching its peak on the 54th day of the experiment. Free radicals are overprodused.This oxidative burs activates at the initial stages of EAA a compensatory reactions, characterized by the increase activity of investigated antioxidants(SOD , catalase)with their further exhaustion on the 34th and especially on 54th day of the experiment.

A potential role of oxidative stress in the pathogenesis of exogenic allergic alveolitis has been demonstrated.

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