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IRON CONTENT IN SERUM AND PULMONARY TISSUE OF RABBITS WITH EXPERIMENTALLY INDUCED PULMONARY EMPHYSEMA

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Summary. *The pathogenesis of pulmonary emphysema is uncompletelly understood. As a catalyzer of some oxidants production iron is involved in oxidant/antioxidant balance disturbances, associated with oxidative stress injury of pulmonary tissue. That's why the iron content was studied in serum and pulmonary tissue of rabbits with pulmonary emphysema induced by hipercholesterolemic diet. Experimental animals were divided into three groups (of 10 animals each): hypercholesterolemic (H)-on hypercholesterolemic diet (oil solution of crystalline cholesterol), oil (O)-on oil diet, and control (C)-on standard diet for that animal species. Pulmonary emphysema was pathohistologically confirmed. Iron content in serum and pulmonary tissue was determined using atomic absorptive spectrophotometric method. There is a significant increase in serum iron content in H in comparison with C group of rabbits. Pulmonary tissue iron content is found to be significantly decreased in H and O compared to C group of investigated animals.*
The changes of iron content in serum and pulmonary tissue, in relation with pathohistological findings of pulmonary tissue, could be of certain importance in developing mechanisms of pulmonary emphysema induced by hypercholesterolemic diet.

Key words: *Pulmonary emphysema, iron, hypercholesterolemic diet*

Introduction

The tissue component of emphysema is defined anatomically as the permanent destructive enlargement of airspaces distal to the terminal bronchioles with a concomitant loss of alveolar attachments. Elastic degradation is a key feature in the pathogenesis of this disease. Elastin fragmentation in emphysema is accompanied by significant collagen remodeling (1).

The pathogenesis of pulmonary emphysema is uncompletelly understood. It has long been hypothesized that the pathogenesis of emphysema may be explained by an imbalance between proteinases, enzymes released from inflammatory cells and protective proteins (antiproteinases) found in the interstitial and extracellular spaces of the lung. This is the "proteinase/antiproteinase" theory of the pathogenesis of emphysema. Emphysema develops when the balance between proteinases and antiproteinases tips towards the destruction of lung tissue by an excess proteinases.

Recently, accumulating evidence suggests that oxidative stress plays a very important role in the pathogenesis of many acute and chronic inflammatory lung diseases of the airways, including chronic obstructive pulmonary diseases (COPD), particularly emphysema (2-5).

Oxidative stress can be defined as an increased exposure to oxidants and/or decreased antioxidant capacity. In the healthy lung, the oxidant burden is balanced by the local antioxidant defenses. However, both an increased oxidant burden and/or decreased antioxidant defenses may reverse the physiologic oxidant-antioxidant balance in favor of oxidants, leading to lung injury.

The most important lung oxidants are reactive oxygen species (ROS), superoxide anion, hydrogen peroxide, and hydroxyl radical.

Superoxide anion ($O_2^{\cdot-}$) formation from oxygen is the first step in production of oxygen radicals. $O_2^{\cdot-}$ is generated primarily by mitochondrial metabolism, in reactions using molybdenum-containing enzymes (xanthine dehidrogenase, sulfite, and aldehyde oxidases), by arachidonic acid metabolism, and NADPH oxidase-dependent processes in phagocytic cells. Reaction of $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) in the presence of transient metal, usually ferrous iron (Fe^{++}), produces the highly reactive and damaging hydroxyl radical ($^{\cdot}OH$), and possibly, singlet oxygen ($^1O_2$). Either of these latter substances is able to initiate lipid peroxidation, which can then continue as a chain reaction. When catalyzed by neutrophil myeloperoxidase (MPO), H_2O_2 and a chloride form hypochlorous acid (HOCl). $^{\cdot}OH$ and HOCl are emphasized because both are extremely potent oxidants (5).

The contribution of iron, as the metal involved in many oxido-reductive processes in organism, has become increasingly meaningful in understanding the development of COPD.

There are few literature data about the iron metabolism in experimental emphysema. Cytochemical examination of iron content in alveolar macrophages (AM), stained by Perls' reaction, shows statistically significant increase in guinea-pigs with experimental emphysema compared to control animals. This increased quantity of ferruginous granules could be the consequence of destruction and cytolysis present in pulmonary emphysema development (6).

Some more results are available concerning iron disturbances in clinical studies on COPD (including pulmonary emphysema). Level of iron in pulmonary macrophages is significantly increased in patients with COPD comparing to control subjects (7). Since it's well known that smoking is the major risk factor for pulmonary emphysema development there are many studies of iron content in pulmonary tissue of smokers. The lungs of cigarette smokers are known to contain increased concentrations of extracellular ferritin-bound iron (8). Reductants present in cigarette smoke may mobilize alveolar ferritin-bound iron, which could promote oxidative injury to lung cells. Iron-mediated oxidative injury may be relevant to the pathogenesis of emphysema (8). Level of iron in pulmonary macrophages is significantly increased in asymptomatic smokers then in nonsmokers (7). Iron concentration is increased in AM of cigarette smokers, as well as in lung lining fluids obtained from cigarette smokers, than in specimens from nonsmokers (9).

The aim of this study was to determine the iron content in serum and pulmonary tissue of rabbits with experimental emphysema induced by hypercholesterolemic diet, in order to contribute to further lightening of possible role of the iron in pathogenetic mechanisms involved in this model of pulmonary emphysema.

Material and Methods

The study was done on 30 Chinchilla rabbits, both sexes, initial body weight 1600-2000g. The animals were kept under standard housing conditions and given food and water ad libitum. Investigated animals were divided into three groups:

C – control group (n = 10) – the animals on standard diet for that animal species;

H – hypercholesterolemic group (n = 10) – the animals on hypercholesterolemic diet (4% solution of crystalline cholesterol in eatable oil, orally given, 6 ml daily, for two months);

O – oil group (n = 10) – the animals on oily diet (6ml of eatable oil, orally given, daily, for two months).

Pulmonary emphysema induced by hypercholesterolemic diet was pathohistologically confirmed on pulmonary tissue samples stained by hematoxylin eosin (HE), using light microscopy (magnification 80 times).

The iron content in serum and pulmonary tissue was determined using atomic absorptive spectrophotometric method. The specimens were prepared for measurement by the combination of dry-wet digestion method. Weighed sample was dried on warmed, and then on hot heater. After that sample was heated in stove at 420^{o}C over the night. After cooling the sample was treated by concentrated nitric acid and then it was subjected to the same procedure again. When white remainder was appeared (that is the sign of complete destruction), it was treated with concentrated hydrochloric acid, dried on warmed heater, and then adjusted to determined volume with deionized water and two drops of concentrated hydrochloric acid. In the sample prepared this way the measurement was done.

MS Office 97 pro was used for data analysis and results presentation.

Results

Pathohistological examinations of pulmonary tissue samples of investigated animals gave the results presented on Figs. 1, 2 and 3. There are evident signs of pulmonary emphysema in H group (Fig. 3), as well as the beginning of the loss of alveolar attachments in O group (Fig. 2) compared to control animals (Fig. 1).

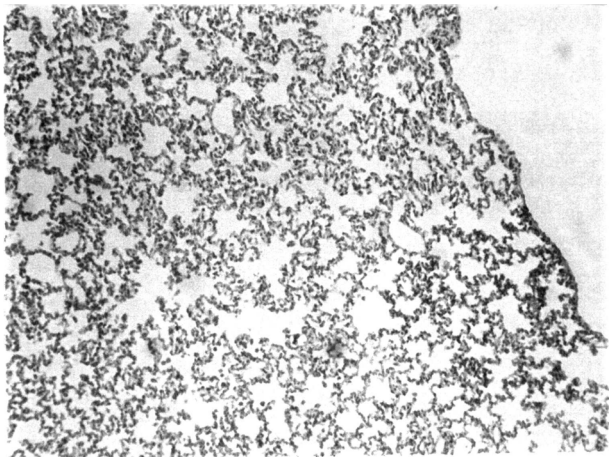

Fig. 1. Pulmonary tissue of control animal (HE ×80)
HE ×80 – pulmonary tissue sample was stained by hematoxylin eosin, magnification ×80

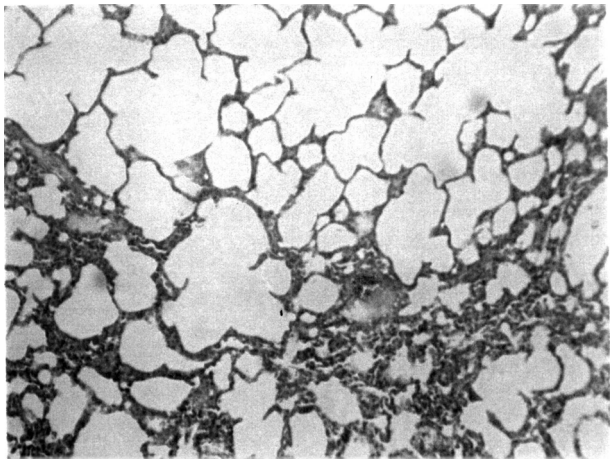

Fig. 2. Pulmonary tissue of animal on oil diet (HE ×80)
HE ×80 – pulmonary tissue sample was stained by hematoxylin eosin, magnification ×80

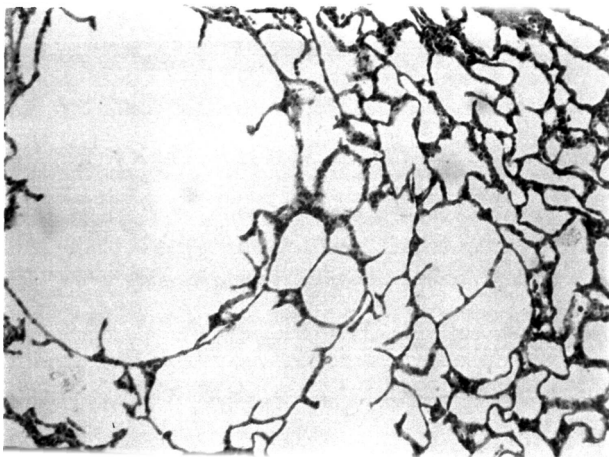

Fig. 3. Pulmonary tissue of animal on hypercholesterolemic diet (HE ×80)
HE ×80 – pulmonary tissue sample was stained by hematoxylin eosin, magnification ×80

In the group of animals on hypercholesterolemic diet there is a significant increase in serum cholesterol concentration compared to both, control animals and animals on oily diet (Table 1).

Table 1. Serum cholesterol concentrations and iron content in serum and pulmonary tissue of investigated animals

	Cholesterol (mmol/l)	Fe	
		Serum (μg/ml)	Pulmonary tissue (μg/g)
C (n=10)	2.63±0.43 100%	4.96±1.33 100%	76.64±19.42 100%
O (n=10)	2.91±0.84 111%	5.13±1.68 103%	38.27±4.78 ^{b***} 50%
H (n=10)	4.62±1.20 ^{a***c***} 176%	6.23±1.50 ^{a*} 126%	56.16±14.00 ^{a**c**} 73%

C – control animals, O – oil animals, H – hypercholesterolemic animals; n is the number of animals in each group; each value is the mean ± SD of the cholesterol or iron content; % value of each parameter is with respect to the control group of animals; statistically significant differences: a – C vs. H, b – C vs. O, c – O vs. H; * - p<0.05, ** - p<0.01, *** - p<0,001

The results of iron content determination in serum and pulmonary tissue of investigated animals are presented on Table 1 and Fig. 4.

There is a statistical significant increase (p<0.05) in serum iron concentration in H in comparison with C group of rabbits. In contrast to the increase in serum iron concentration in H group of animals there are different degrees of decreases of pulmonary tissue iron contents in both H and O group comparing to the control rabbits. According to our results pulmonary tissue iron content is significantly decreased in H and O compared to C group (p<0.01 and p<0.001 respectively), as well as in O in comparison with H group (p<0.01) of investigated animals (Table 1).

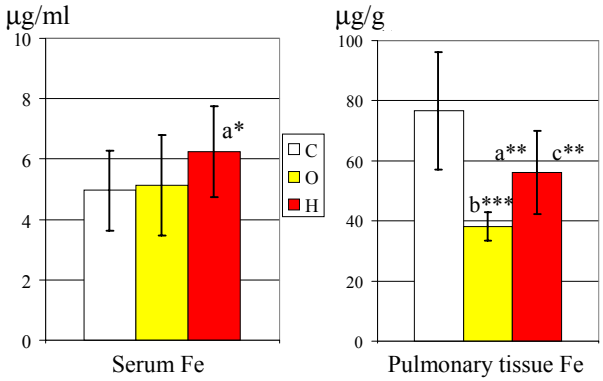

Fig. 4. Iron content in serum and pulmonary tissue of investigated animals
each value is the mean ±SD of the iron content in serum and pulmonary tissue of particular group of animals; C – group of control animals, O – group of oil animals, H – group of hypercholesterolemic animals; statistically significant differences: a – C vs. H, b – C vs. O, c – O vs. H; * - p<0.05, ** - p<0.01, *** - p<0.001

Discussion

For the determination of iron status several indicators can be used, such as serum iron, transferrin, transferrin iron binding capacity and ferritin. Thus, serum iron level itself may be one of the signs of disturbed iron metabolism and indicate possible changes in oxidant/antioxidant balance.

In our study a significant increase (p<0.05) of serum iron content is found in hypercholesterolemic compared to control group of investigated animals. The increase of iron level, available as a catalyzer for chemical reactions of ROS production, could have a part in oxidant/antioxidant imbalancing in different tissues including pulmonary tissue, and promote direct oxidative injury to lung cells. These processes might be of importance in development of typical emphysematous changes of pulmonary tissue as it was found in our experimental model (Figure 3).

Literature data from clinical studies reveal lots of evidences of damaged relation between oxidant and antioxidant in COPD including emphysema, and smoking as the major risk factor for emphysema development. Smoking, acute exacerbation of COPD and asthma are connected with marked oxidant/antioxidant imbalance in the blood, associated with signs of increased oxidative stress (10).

In this study the iron content in pulmonary tissue of animals on hypercholesterolemic diet (as well as in animals on oil diet) is significantly decreased (p<0.01 and p<0.001, respectively) in comparison with control animals. This finding is in the agreement with the data published by Mazzetti et al, 1996 (11), who found that there is a mobilization of iron from the tissue to the fluid reserve in the relative hypoxic environment. The same reason may be the origin of the disturbances in pulmonary tissue iron content in H and O group of

animals, registrated in this experimental model, and could be in relation with pathohistological findings observed in pulmonary tissue samples (Figures 2 and 3). There is also the possibility that decreased pulmonary tissue iron content may be the result, but not the cause of the disease, because of the fact that hypoxia in the emphysematous tissue intensify iron release from ferritin (11).

Clinical studies of iron metabolism in different parts of lungs of smokers and patients with COPD show various results, but predominantly the increase of iron content, particularly in lower airways and AM. Iron concentration is increased in AM of cigarette smokers, and lung lining fluids obtained from cigarette smokers contain substantially more iron than specimens from non-smokers (9). Tobacco smoke and cigarette paper contain free iron, which is deposited in the lower airways. In addition, cigarette smoke induces iron release from ferritin. Free iron plays an essential role in oxidative processes, so its de-localization from iron-binding proteins such as ferritin is regarded as a crucial step in the onset of oxidative tissue damage. Indeed, macrophages of smokers contain increased iron levels and a further increase is observed in smokers with COPD (7).

Results of the present study concerning iron content in pulmonary tissue are related to the special experimental model of pulmonary emphysema induced by hypercholesterolemic diet. The iron content was measured in the whole pulmonary tissue. That's probably why our results of pulmonary iron content are different from some literature data from clinical studies. This study can be considered as a pilot study in the field of the investigations of the possible association of iron and pathogenetic mechanisms of experimental pulmonary emphysema. Analysis of iron-binding proteins might be worthy for more detailed evaluation of the importance of iron in developing mechanisms of experimental emphysema, part of which is pointed by the present study.

The changes of iron content in serum and pulmonary tissue, in some relation with pathohistological findings of pulmonary tissue in our experimental animals, point to the possible de-localization of iron in this model. It may designate certain importance of iron in developing mechanisms of experimental pulmonary emphysema induced by hypercholesterolemic diet.

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SADRŽAJ GVOŽĐA U SERUMU I TKIVU PLUĆA KUNIĆA SA EKSPERIMENTALNO INDUKOVANIM EMFIZEMOM PLUĆA

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Kratak sadržaj: *Patogeneza emfizema pluća je nepotpuno izučena. Kao katalizator procesa produkcije nekih oksidanasa gvožđe je uključeno u poremećaje ravnoteže oksidanasa/antioksidanasa, koji su povezani sa oksidativnim oštećenjem tkiva pluća. Stoga je određivan sadržaj gvožđa u serumu i tkivu pluća kunića sa emfizemom pluća indukovanim hiperholesterolskom dijetom. Eksperimentalne životinje bile su podeljene u tri grupe (od po 10 životinja*

svaka): hiperholesterolsku (H)-na hiperholesterolskoj dijeti (uljani rastvor kristalnog holesterola), uljanu (U)-na dijeti uljem i kontrolnu (K)- na standardnoj dijeti za ovu životinjsku vrstu. Emfizem pluća je patohistološki potvrđen. Sadržaj gvožđa u serumu i tkivu pluća određivan je korišćenjem metode atomske apsorpcione spektrofotometrije. Registrovan je značajan porast sadržaja gvožđa u serumu H u poređenju sa K grupom kunića. Sadržaj gvožđa u tkivu pluća značajno je snižen u H i U u poređenju sa K grupom ispitivanih životinja.

Izmene u sadržaju gvožđa u serumu i tkivu pluća, povezane sa patohistološkim nalazima tkiva pluća, mogu biti od izvesnog značaja u mehanizmima razvoja plućnog emfizema uzrokovanog hiperholesterolskom dijetom.

Ključne reči: *Plućni emfizem, gvožđe, hiperholesterolska dijeta*