

## POSSIBLE ROLE OF IRON IN THE PATHOGENESIS OF PULMONARY EMPHYSEMA IN RABBITS

VUČEVIĆ DANIJELA, PEŠIĆ Č, ĐARMATI DANICA \* and ĐORĐEVIĆ - DENIĆ GORDANA

*Institute of Pathophysiology, Medical Faculty, Dr Subotića 9, 11 000 Belgrade, Yugoslavia; \*Health Protection Institute of Serbia, Belgrade, Yugoslavia*

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*Oxidative stress has been recognized to be responsible for pulmonary tissue damage. Having in mind the important role of iron as a catalyser of the production of some oxidants, we examined the content of this transition metal in pulmonary tissue of rabbits with experimental emphysema induced by a hypercholesterolemic diet. Pulmonary emphysema was pathohistologically confirmed. Lung iron content was quantified by atomic absorptive spectrophotometry. For this study three groups of ten rabbits each were used: C - a control group fed on the usual diet for this species, O - a control group fed on an oil - containing diet, and Ch - an experimental group fed on a hypercholesterolemic diet. After two-months treatment lung iron content was significantly ( $p < 0.05$ ) decreased in group Ch and highly significantly ( $p < 0.01$ ) decreased in group O compared to group C. In comparison with group O lung iron content was highly significantly ( $p < 0.01$ ) increased in group Ch. Our findings indicate a possible role for iron in the pathogenesis of pulmonary emphysema.*

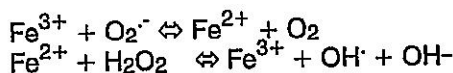
*Key words: hypercholesterolemic diet, iron, pulmonary emphysema, rabbits*

### INTRODUCTION

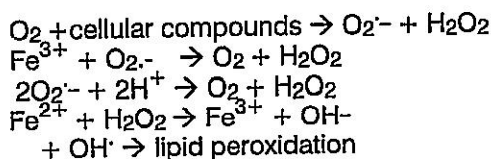
The pathogenesis of pulmonary emphysema (PE) is still unclear. In the healthy lung the oxidant burden is balanced by local antioxidant defenses. However, both an increased oxidant burden and/or decreased antioxidant defenses may reverse the physiological oxidant/antioxidant balance in favor of oxidants, leading to lung injury. The contribution of iron has become increasingly meaningful in understanding the development of PE.

Iron is an essential trace element utilized in almost every aspect of normal cell function. This transition metal carries out a wide range of biological functions as a result of its interactions with oxygen and its behavior with donor-acceptor complex formation (coordination). Although all living systems depend on transition metals to catalyze homeostatic and synthetic functions, reactive oxygen species (ROS) generated by these metals have the capacity to damage biological molecules (Ghio *et al.*, 1998). Iron is an important catalyst of oxidant-induced injury

because of its role in generating highly reactive hydroxyl radicals ( $\text{OH}^\cdot$ ) from less reactive superoxide anion ( $\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) via the Fenton reaction (Wesseliuss et al., 1994):



Elevated iron levels in lung parenchyma may lead to pulmonary injury by tipping the oxidant/antioxidant balance towards increased oxidant function. Accumulating evidence suggests that the production of ROS by the catalytic action of iron may have a role in the pathogenesis of PE by impairing antiprotease function, directly attacking lung matrix proteins or by inactivating enzymes involved in elastin synthesis and lung repair (Thompson et al., 1991). Iron may induce lipid peroxidation by catalyzing the Fenton reaction (Mohsenin and Gee, 1989; Repine et al., 1997):



Lipid peroxidation can impair membrane function, inactivate membrane bound receptors and enzymes, disturb membrane fluidity and increase permeability (Halliwell and Chirico, 1993). Lipid peroxidation products were increased in the plasma and lung lavages of patients with PE (Barnes, 1990; Petruzzelli et al., 1990; Bridges et al., 1993).

Because of its high reactivity, iron is normally bound to various iron-binding compounds, such as transferrin, ceruloplasmin and ferritin. However, this protective mechanism may be disturbed in the lungs of PE patients, since oxidants can release iron from ferritin (Moreno et al., 1992; Lapenna et al., 1995). Iron also accumulates progressively with age in men and postmenopausal women at the time when PE worsens in both sexes (Repine et al., 1977). Since iron-mediated oxidative injury may be relevant to the pathogenesis of PE, we directed our experimental goal to measuring the iron content in pulmonary tissue of Chinchilla rabbits with experimental emphysema.

#### MATERIALS AND METHODS

The experiments were performed on Chinchilla rabbits of both sexes whose initial weight was about 1600-2000g. The investigated animals (n=30) were divided into three groups (of 10 animals each):

- 1.C - control group fed on a standard diet for this species,
- 2.O - control group fed on an oil - containing diet. These animals received 6 ml of edible oil through a gastric tube five times a week for two months,
- 3.Ch - experimental group fed on a hypercholesterolemic diet. These animals received a 4% solution of crystalline cholesterol (ICN Galenika) in 6 ml of edible oil through a gastric tube five times a week for two months.

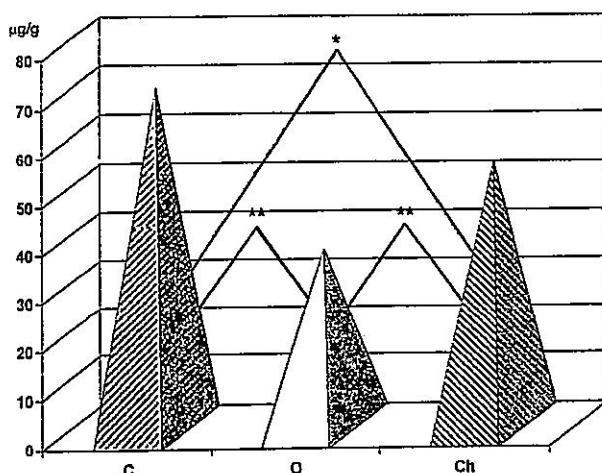
After two-months of treatment the respective groups of rabbits were sacrificed by air embolism (air injected intracardially). Pulmonary tissue sections, obtained from each group of rabbits, were placed in formalin solution to be subsequently molded and stained with haematoxylin eosin. Pulmonary tissue specimens were analysed pathohistologically by light microscopy. The iron content in lung tissue was determined by atomic absorption spectrophotometry (VARIAN AA-5). Statistical evaluation of results was performed using Students t-test.

### RESULTS

The iron content in pulmonary tissue of the rabbits is presented in Table 1 and Figure 1.

Table 1 - Iron content in pulmonary tissue of the rabbits

Fe ( $\mu\text{g/g}$ )	Group C	Group O	Group Ch
$\bar{X} \pm \text{SD}$	$70.08 \pm 13.25$	$36.71 \pm 3.94$	$54.64 \pm 11.97$



\* -  $p < 0.05$  ; \*\* -  $p < 0.01$

Figure 1. Iron content in pulmonary tissue of the rabbits

Figures 2, 3 and 4 show pulmonary tissue of the rabbits.

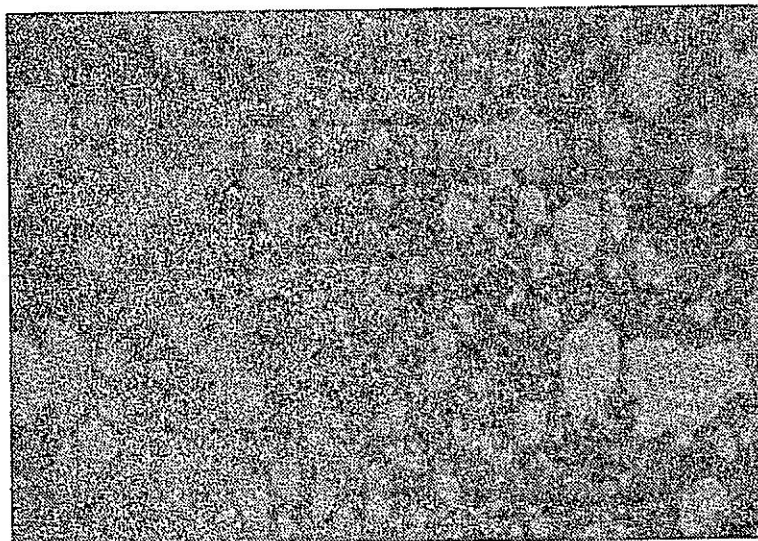


Figure 2. Pulmonary tissue of a control rabbit (C)



Figure 3. Pulmonary tissue of a rabbit fed on the oil - containing diet (O). Severe inflammation can be observed.

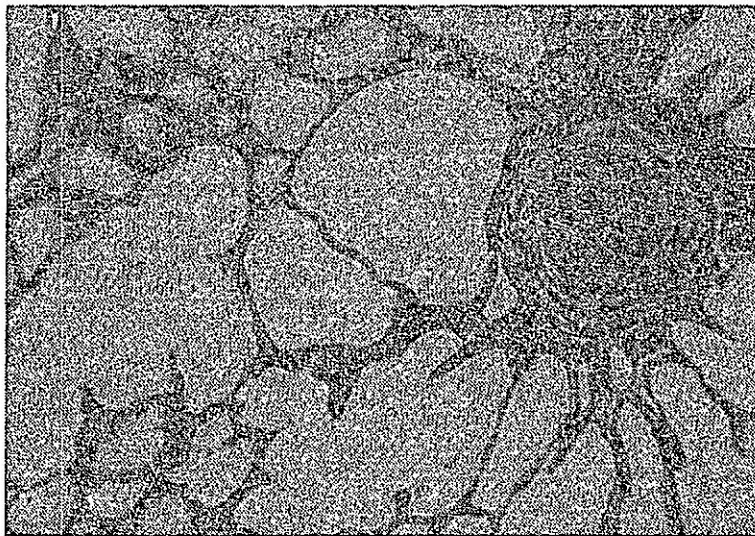


Figure 4. Pulmonary tissue of a rabbit fed on the hypercholesterolemic diet (Ch) with evident emphysema

In comparison with group C the iron content in pulmonary tissue was significantly ( $p < 0.05$ ) decreased in group Ch and highly significantly ( $p < 0.01$ ) decreased in group O. A highly significant increase ( $p < 0.01$ ) of iron content was found in pulmonary tissue of group Ch compared to group O.

#### DISCUSSION

Mechanisms involved in iron-catalyzed oxidative lung injury are still not fully understood. Direct epithelial toxicity, inactivation of enzymes required to maintain the integrity of the pulmonary tissue, alteration of the protease-antiprotease balance to favor protease activity, and augmentation of ROS formation have been postulated to account for the development of PE. Mobilization of iron from the tissue to the fluid reserve in a relatively hypoxic environment has been found by Mazzetti *et al.*, (1996), which is in agreement with the significant decrease of the iron content in pulmonary tissue of groups Ch and O ( $p < 0.05$  and  $p < 0.01$  respectively) compared to control animals. Since the production of ROS depends on iron as a catalyst, the decrease of lung iron content in groups Ch and O may be of importance in the developing pathomorphological changes in the pulmonary tissue of these animals.

The lung is a primary target for oxidant injury, because of its location, anatomy and function. Transition metal complexes may facilitate free radical processes by promoting lipid hydroperoxide breakdown to form further free radicals. Lipid peroxidation products have been shown to reduce the elastase inhibitory capacity of the alveolar lining fluid in humans (Wesselius *et al.*, 1994). Iron-mediated oxidation was involved in low density lipoprotein (LDL) modifications, that lead to uptake of these molecules by "scavenger receptors"

present on macrophages with the resultant development of foam cells (Buhl *et al.*, 1994).

In rabbits, after one-month treatment on a hypercholesterolemic diet bronchial obstruction is followed by foam cell formation (Haslam, 1994). Additionally, alterations of the cholesterol/phospholipid ratio could disturb membrane fluidity and reduce surfactant activity, so alveolar damage may be facilitated by promotion of pulmonary immune reactions (Wilsher *et al.*, 1988). Activation of complement leads to influx of inflammatory cells into lung parenchyma with subsequent release of elastases and oxidants that cause damage to elastic lung tissue (Kosmas, 1997). It has been shown that an excessive release of lysosomal enzymes from alveolar macrophages (AM) with subsequent lung tissue destruction may be induced by cholesterol. Rabbits fed a cholesterol rich diet exhibited hypercholesterolemia because of their extreme sensitivity to dietary cholesterol. In this model, the experimental protocol also caused an increase of serum cholesterol content in groups Ch and O.

Since cholesterol is an extremely immunogenic molecule, massive hypercholesterolemia induced in rabbits by special diets may increase the local lymphoproliferative response (Clarkson *et al.*, 1974; Alving and Wassef, 1999). An increase in immune and inflammatory effector cell numbers in areas adjacent to lung parenchyma injury directly confirms that parenchymal infiltration by these cells is associated with the pathogenesis of PE (Barnes, 1990). Increased numbers of polymorphonuclear cells, AM, epithelial, mast and other metabolically active lung cells that consume oxygen and release ROS could alter the oxidant/antioxidant balance in the lungs of PE patients (Wallaeart *et al.*, 1993; Halliwell, 1996; Repine *et al.*, 1997). Mitochondrial and arachidonic acid metabolism also can generate oxidants that might participate in the development of PE (Repine *et al.*, 1997). It seems that in this model the increased number and dysfunction of immune and inflammatory effector cells, as well as cholesterol immunostimulation capacity are also related to iron content and the pathohistological findings observed in pulmonary tissue specimens of groups Ch and O (Figure 2 and 3). Additionally, the oil - containing diet in some manner leads to disturbance of iron metabolism. Thus, considering the role of iron in enzyme catalyzed reactions, oil immunogenic activity may be involved with the findings presented in this study.

Iron which has been released into the cell must either be used immediately for biosynthesis or stored in a safe form. Storage as well as transport systems must function rapidly and be completely reversible under physiological conditions in order to preclude local excess (Kaim and Schwederski, 1996). Iron has been demonstrated to initiate and potentiate the inflammatory response (McGowan *et al.*, 1986). The most striking feature of this reaction is the presence of large numbers of pigment-laden macrophages in the respiratory bronchioles, alveolar ducts, and alveoli (McGowan *et al.*, 1986).

Literature data indicate that the lung parenchyma iron burden is increased in cigarette smokers and patients suffering from chronic obstructive pulmonary disease (COPD). Although cigarette smoking is the major risk factor for PE development (nearly 90% of all PE patients are smokers), the increase of iron in AM of PE patients cannot be accounted for by cigarette smoking alone. It may be speculated that chronic airway inflammation, reduction of mucociliary clearance and microhaemorrhage could partly explain the high intramacrophagic iron load observed in these patients. The greatest amount of this iron burden is sequestered



within AM in ferritin, which effectively inhibits local iron-catalyzed oxidative injury. AM in smokers and COPD patients are involved in a dynamic equilibrium with respect to iron metabolism that includes uptake of unbound or transferrin-bound iron and release of cytosolic ferritin-bound iron as AM become iron-loaded (Corhay et al., 1992; Wesselius et al., 1994). As a result of complexation of iron by transferrin and the consequent decrease in the ability of the coordinated metal to catalyze ROS generation, transferrin has been described as a major antioxidant in the alveolar lining fluid. Thus, functional abnormalities of this protein might be an additional factor contributing to the development of PE (Ghio et al., 1998). The results of the present study do not correspond to previously mentioned literature data probably because iron content was measured in the whole pulmonary tissue. Moreover, in this model experimental emphysema was induced by different mechanisms.

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## MOGUĆA ULOGA GVOŽĐA U PATOGENEZI EKSPERIMENTALNOG EMPFIZEMA PLUĆA U KUNIĆA

VUČEVIĆ DANIJELA, PEŠIĆ Č, ĐARMATI DANICA I ĐORĐEVIĆ-DENIĆ GORDANA

### SADRŽAJ

Gvožđe ima ulogu kao katalizator u reakcijama nastanka slobodnih radikala važnih u patogenezi plućnog emfizema i zbog toga je cilj našeg istraživanja bio određivanje sadržaja gvožđa u plućnom tkivu kunića sa eksperimentalnim emfizemom izazvanim hiperholesterolskom dijetom (4% rastvor kristalnog holesterola u jestivom ulju). Ispitivane životinje su bile podeljene u tri grupe: C - kontrolna grupa na ishrani uobičajenoj za ovu životinjsku vrstu (n=10), O - kontrolna grupa na dvomesečnoj uljanoj dijeti (n=10) i Ch - eksperimentalna grupa na dvomesečnoj hiperholesterolskoj dijeti (n=10). Potvrda emfizema pluća je vršena patohistološki. Sadržaj gvožđa u plućnom tkivu određivan je metodom atomske apsorpcione spektrofotometrije. Analiza dobijenih rezultata je ukazala na statistički visoko značajno smanjenje ( $p < 0.01$ ) sadržaja gvožđa u plućnom tkivu O grupe, dok je smanjenje sadržaja gvožđa u tkivu pluća Ch grupe na nivou statističke značajnosti ( $p < 0.05$ ). Sadržaj gvožđa u plućnom tkivu Ch grupe pokazuje statistički visoko značajno povećanje ( $p < 0.01$ ) u odnosu na uljanu kontrolnu grupu. Naši rezultati ukazuju na moguću ulogu gvožđa u nastanku plućnog emfizema.