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THE EFFECT OF TOCOPHEROL ON SERUM LIPID PROFILE IN PULMONARY EMPHYSEMA INDUCED BY HYPERCHOLESTEROLEMIC DIET

VUČEVIĆ DANIJELA, MILOVANOVIĆ I, MLADENOVIĆ D, ŽUNIĆ-BOŽINOVSKI SNEŽANA, RADOSAVLJEVIĆ TATJANA, STOJANOVIĆ JASNA, PEŠIĆ BČ and ĐORĐEVIĆ-DENIĆ GORDANA

Institute of Pathophysiology, School of Medicine, Belgrade

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This investigation deals with the effect of tocopherol on serum lipid profile in emphysema experimentally induced by hypercholesterolemic diet. For this study six groups of twenty Chinchilla rabbits each were used: C-control group on standard diet for this species, O - control group on oil diet, Ch - experimental group on hypercholesterolemic diet, T - experimental group received tocopherol intramuscularly, OT - experimental group on oil diet receiving tocopherol intramuscularly and ChT - experimental group on hypercholesterolemic diet receiving tocopherol intramuscularly. After a two-months treatment serum content of total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL) and high density lipoproteins (HDL) were examined by the enzymatic colorimetric method. Experimental emphysema was histologically confirmed. TC and LDL contents were significantly increased ($p<0.01$) in the sera of all groups compared to group C. In comparison with group C, TG content was significantly decreased ($p<0.01$) in all tested groups. In comparison with group O, TC content was significantly decreased ($p<0.01$) in sera samples of ChT and OT groups. TC content was significantly increased ($p<0.01$) in the sera of group ChT compared to group OT. Our findings indicate the influence of tocopherol on the serum lipid profile in experimental emphysema, as well as its potentially protective role in the development of this disease.

Key words: hypercholesterolemic diet, pulmonary emphysema, rabbits, serum lipid profile, tocopherol

INTRODUCTION

Pulmonary emphysema (PE) is a widespread disease of the lungs (Heunks and Dekhuijzen, 2000). It is a condition in which there is overinflation of structures in the lungs known as alveoli or air sacs. This overinflation results from a breakdown of the walls of the alveoli, which causes a decrease in respiratory function and often, breathlessness (Chow *et al*., 2003). PE doesn't develop suddenly, it comes on very gradually (Fletcher and Pride 1984).

The pathogenesis of PE is still unclear. The clinical manifestations and progression of this chronic disease are influenced by a number of risk factors, including α_1-antitrypsin deficiency, low birth weight, air pollution, socio-economic status, recurrent respiratory infections and tobacco smoke, which plays a leading role (Del Donno and Verduri, 2000). Years of exposure to the irritation of cigarette smoke usually precede the development of PE (Fletcher and Pride, 1984).

PE begins with the destruction of air sacs (alveoli) in the lungs where oxygen from the air is exchanged for carbon dioxide in the blood. The walls of the air sacs are thin and fragile. Damage to the air sacs is irreversible and results in permanent "holes" in the tissue of the lower lungs (Fletcher and Pride, 1984; Finlay *et al*., 1996). As air sacs are destroyed, the lungs are able to transfer less and less oxygen to the bloodstream, causing shortness of breath. The lungs also lose their elasticity, which is important in keeping the airways open (Finlay *et al*., 1996).

Oxidative stress can be defined as an increased exposure to oxidants and/or a reduced defensive ability of the antioxidants (Fletcher and Pride, 1984; Repine *et al*., 1997; Rahman, 2002; Chow *et al*., 2003; Urso and Clarkson, 2003). Many data attribute a pathogenic role to oxidative stress in PE. Reactive oxygen species (ROS) can trigger a lipid peroxidation chain reaction. The resulting lipid radical reacts with oxygen to make a peroxyl radical, which then transforms polyunsaturated fatty acids into lipid hydroperoxides, capable of producing other radical compounds. Unsaturated fatty acids of polymorphonuclear leukocytes (PMN), monocytes and macrophages are particularly susceptible to attack by oxidants (Acworth and Bailey, 1995; Del Donno and Verduri, 2000; Polidori *et al*., 2001; Bowler and Crapo, 2002; Dröge, 2002; Minko *et al*., 2002; Rocksen, 2003). An increased sequestration of neutrophils has been observed in pulmonary microcirculation, from which superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydrohyl radical ($OH^{\cdot}$) are formed by means of the NADPH oxidase system. Moreover, proteases such as elastase and collagenase, which perform a proteolytic action, are released from neutrophils, since the antiproteases are inactivated by oxidants (Bast *et al*., 1991; Repine *et al*., 1997).

The alveoli are lined with a lipoprotein substance called surfactant (King, 1982). 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; Wilsher *et al*., 1990).

An increase of triglycerides and free fatty acids pulmonary tissue content has been reported during experimental atherosclerosis induced by a hypercholesterolemic diet. Lipid accumulation in alveolar macrophages (AM) of rabbits fed on a hypercholesterolemic diet could play a role in the pathogenesis of PE in this experimental model. Cholesterol is an extremely immunogenic molecule. It is capable of initiating immune responses that could be of importance both in the development of atherosclerosis and experimental emphysema (Djordjevic, 1978; Radak *et al.*, 1987).

Cholesterol exists either free or combined with fatty acids in the form of cholesterol esters. Three-fourths of the total cholesterol in normal human plasma is contained within low density lipoproteins (LDL). Under oxidative stress native

LDL particles become trapped in the subendothelial space, where they can undergo progressive oxidation and be internalized by macrophages via the scavenger receptors on the surfaces of these cells. The internalization leads to the production of lipid peroxides and facilitates the accumulation of cholesteryl esters, resulting in the formation of foam cells. Oxidized LDL (ox-LDL) particles also cause endothelial dysfunction and injury, as well as foam cell necrosis, resulting in the release of lysosomal enzymes and necrotic debris (Ross, 1993; Witztum, 1994; Libby, 1999; Ross, 1999; Libby, 2002).

Lipid accumulation in AM results in the formation of foam cells. In rabbits, after a one-month treatment on a hypercholesterolemic diet bronchial obstruction is followed by foam cell formation. One can observe abundant cytoplasmic cholesteryl ester droplets and lysosomal free cholesterol in these lipid-laden cells. Insufficiency of emulsion and lipid metabolism is a characteristic of foam cells (Spencer, 1985).

The potential of oxidants to damage pulmonary tissue dependends on the local antioxidant defense mechanisms. Tocopherol, structurally incorporated in lipoproteins, acts as an intrinsic antioxidant that blocks electron transfers involved in the initiation and propagation of lipid peroxidation. It has been shown that vitamin E deficiency results in enhanced tissue susceptibility towards reactive oxygen species (ROS) and increased lipid peroxidation *in vivo*. It has also been demonstrated that dietary intake of vitamin E suppresses elevated plasma concentrations of lipid peroxides both in patients with hyperlipoproteinemia and rabbits fed on a cholesterol rich diet (Szczeklik *et al*., 1985). Vitamin E, by the action of its phytyl tail lateral chain, stabilizes the lysosomal membranes. Thus, deficiency of this antioxidant increases the activity of lysosomal enzymes, which may alter the protease-antiprotease balance to favor protease function (Bonyan, 1967).

MATERIAL AND METHODS

The experimental procedure was obtained from previous long-standing investigations of the effects of experimental arteriosclerosis on lung tissue. Namely, Djordjevic *et al*., 1978. and Radak *et al*., 1987. have observed several times that this process, besides other changes, also brings the occurrence of PE.

The experiments were performed on Chinchilla rabbits of both sexes whose initial weight was about 1600-2000g. The investigated animals (n=120) were divided into six groups (twenty 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,
4. T – experimental group received 100 mg of tocopherol intramuscularly (i.m.) per week, divided into three equal doses, for two months,

5. OT – experimental group received oil (6 mL of edible oil, orally given five times a week for two months) and tocopherol (100 mg per week, i.m. given in three equal doses, for two months) and
6. ChT – experimental group fed on a hypercholesterolemic diet (4% solution of crystalline cholesterol /ICN Galenika/ in 6 mL of edible oil, orally given five times a week for two months) treated with tocopherol (100 mg per week, i.m. given in three equal doses, for two months).

After two months of treatment the respective groups of rabbits were bled and serum was separated from the blood samples and stored at -20°C. 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 analyzed histologically by light microscopy (magnification 100 x).

Lipid concentrations in serum (triglycerides /TG/, total cholesterol /TC/, low density lipoproteins /LDL/ and high density lipoproteins /HDL/) were measured by enzymatic colorimetric method.

An enzymatic colorimetric test from "Lighthing" (Yu Medica) was employed to measure serum TG concentration according to the method described by Trinder (Trinder, 1983). Primarily, TG fractions were converted to glycerol and fatty acids by the enzyme lipase. Secondary, specific enzymes (glycerol kinase /GK/, glycerolphosphate oxidase /GPO/ and hydrogen peroxidase /HPO/) were used. Simultaneously, a chromogen system of colour measurement was utilized, too.

$$TG \xrightarrow{lipase} \text{glycerol} + 3 \text{ fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} \xrightarrow{GPO} \text{dihydroxyacetonephosphate (DHAP)} + H_2O_2$$

H_2O_2 combines with chromogen and gives quinonimine coloured reaction, due to the catalytic action of HPO. The extinction was measured by the colorimetric method (wavelength /λ/ 540 nm) on a multiple analyzer ("Monarch plus"). In serum samples extinction is directly proportional to the concentration of TG.

Serum TC concentration was determined with enzymatic colorimetric test from "Lighthing" (Yu Medica), that includes the PAP method described by Trinder (Allain *et al*., 1974; Meipttini, 1978). According to Trinder's method, cholesteryl esters were hydrolysed under the action of cholesterol esterase (CE). Thus, free cholesterol and fatty acids were formed. Thereof, free cholesterol was oxidized due to the catalytic action of cholesterol oxidase (CO) and cholestrol-3-OH and H_2O_2 were formed. In addition, H_2O_2 reacts with chromogen and produces by the enzyme HPO a quinonimine purple coloured reaction. The intensity of the produced colour is proportional to TC concentration. Extinction at $\lambda = 500$ nm was determined by a multiple "Monarch plus" analyzer.

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholesterol-3-OH} + H_2O_2$$

$$H_2O_2 + \text{4 aminoantipirine (AAP)} + \text{p-hydroxybenzoate} \xrightarrow{HPO} \text{quinonimine colour} + H_2O.$$

Serum HDL concentration was determined according to the method by "Lighthing" (Yu Medica). In the presence of double positively charged anions the HDL fraction was measured after selective LDL and very low density lipoproteins (VLDL) precipitation by polyanion sulphates. Besides, polyanion sulphates bind cations, so indissoluble complex production ensued.

Manganese-heparin, phosphowolframic acid, magnesium chloride, magnesium sulphate and dextran sulphate use is in accordance with USA Center of Disease Control - Lipid reference section. In our experimental protocol dextran sulphate and magnesium sulphate were used. After centrifugation, HDL fraction was suspended in the supernatant. Then, supernatant was separated and the HDL fraction was determined directly from the supernatant by the PAP method. Cholesterol concentrations 1.3 mmol/L and 2.6 mmol/L were utilized for calibration.

Serum LDL concentration was calculated using the formula by Friedewald *et al.* (1972).

The values of parameter for individual animals were averaged and standard deviation (SD) was calculated. Statistical evaluation of results was performed using Student's t-test. With regard to the feature of our parameters, the significance of the differences between groups was calculated using two-tailed Student's t test. A p value of less than 0.05 was considered significant. Statistical analysis of data was carried out using a computer with the assistance of a statistical software package-SPSS 9.0 Professional Edition.

RESULTS

Lipid concentrations in serum of the rabbits is presented in Table 1.

Table 1. Lipid concentrations in the serum of investigated rabbits

Lipid fraction → Group ↓	TG [mmol/L] $\bar{X} \pm SD$	TC [mmol/L] $\bar{X} \pm SD$	LDL [mmol/L] $\bar{X} \pm SD$	HDL [mmol/L] $\bar{X} \pm SD$
C	0.80±0.46	2.18±0.14	1.07±0.12	1.55±0.03
O	0.50±0.94	3.81±1.41	2.07±1.15	1.31±0.56
Ch	0.70±0.29	3.65±1.50	4.53±1.56	1.01±0.38
T	0.38±0.21	2.35±0.33	1.24±0.17	1.22±0.11
OT	0.62±0.32	2.46±0.68	1.59±0.43	1.18±0.26
ChT	0.49±0.40	2.95±0.46	1.43±0.15	1.11±0.30

Statistical significance of the differences in serum lipid fractions between investigated animals is shown in Table 2. As it can be seen, TC and LDL concentrations were significantly increased ($p<0.01$) in the sera of all other groups compared to group C. In comparison with group C, TG content was

significantly decreased ($p<0.01$) in the sera of all other groups. In comparison with group O, TC content was significantly decreased ($p<0.01$) in the sera of ChT and OT groups. TC content was significantly increased ($p<0.01$) in the serum of ChT group compared to group OT. HDL content was significantly decreased ($p<0.05$) in the sera of O, Ch and ChT groups compared to group C as well as in the sera of Ch, ChT and OT groups compared to group O. In comparison with Ch group HDL concentration was significantly increased ($p<0.05$) in the serum of group ChT. HDL content significantly increased ($p<0.05$) in the serum of group OT compared to group ChT.

Table 2. Statistical significance of the differences in serum lipid fraction concentrations between investigated groups

Lipid fraction → Groups ↓	TC	TG	LDL	HDL
C/O	**	**	**	*
C/Ch	**	**	**	*
C/ChT	**	**	**	*
C/OT	**	**	**	ns
C/T	*	**	*	ns
O/Ch	**	ns	ns	*
O/ChT	**	ns	ns	*
O/OT	**	ns	ns	*
O/T	**	ns	ns	ns
Ch/ChT	ns	ns	ns	*
Ch/OT	ns	ns	ns	ns
ChT/OT	**	ns	ns	*

** – $p<0.01$; * – $p<0.05$; ns – non significant ($p>0.05$)

Figure 1-6 pulmonary tissue of the experimental rabbits.

Pulmonary tissue of a control rabbit (C) is presented in Figure 1. As expected, pulmonary tissue of the control group is without pathologic changes.

Pulmonary tissue of a rabbit on the oil – containing diet (O) is presented in Figure 2. Severe inflammation can be observed. Namely, inflammatory effector cell infiltration and the oil – containing diet in some manner lead to the disturbance of homeostatic mechanisms in the pulmonary tissue (Djordjevic *et al*., 1978; Radak *et al*., 1987; Zunic 1997; Vucevic 1999). Thus, oil immunogenic activity may be involved with the findings presented in this study (Figure 2).

Pulmonary tissue of a rabbit fed on the hypercholesterolemic diet (Ch) with evident emphysema is presented in Figure 3. It seems that in this model

cholesterol immunostimulation capacity and severe inflammatory processes are related to the pathohistological findings observed in the pulmonary tissue sample of group Ch (Figure 3).

Pulmonary tissue of a rabbit treated with tocopherol is presented in Figure 4. An inflammatory reaction can be noticed. One can also observe signs of inflammatory response in the pulmonary tissue of a rabbit which received oil and tocopherol (Figure 5), as well as in the pulmonary tissue of a rabbit receiving cholesterol and tocopherol (Figure 6).

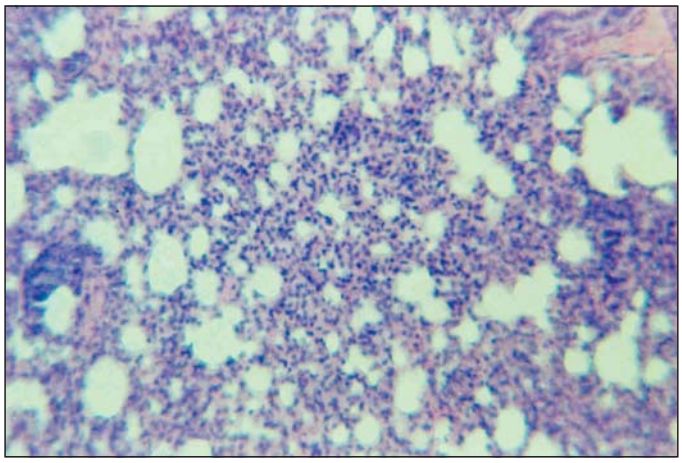

Figure 1. *Pulmonary tissue of a control rabbit (C)*

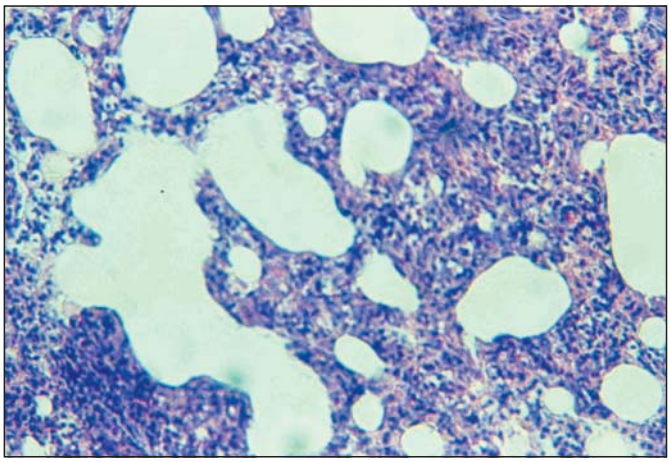

Figure 2. Pulmonary tissue of a rabbit fed the oil-containing diet (O). Severe inflammation can be observed

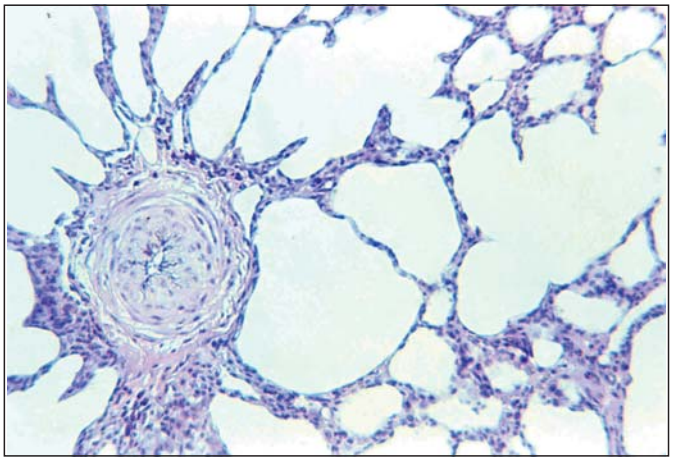

Figure 3. Pulmonary tissue of a rabbit fed the hypercholesterolemic diet (Ch) with evident emphysema

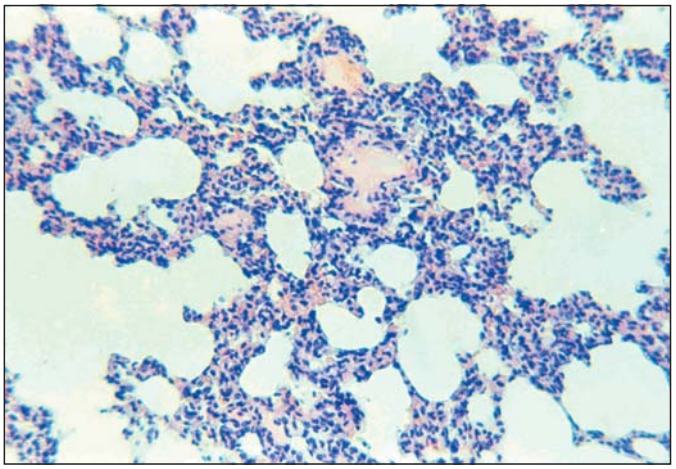

Figure 4. Pulmonary tissue of a rabbit treated with tocopherol (T). Inflammatory reaction can be noticed

Inspite signs of inflammation, the pulmonary tissue of rabbits from all experimental groups receiving tocopherol (T, OT and ChT group) is without emphysematous changes (Figure 4-6). It seems that in this study dietary supplementation with tocopherol (T, OT and ChT group) appears to have the potential to prevent experimental emphysema (Figure 4-6).

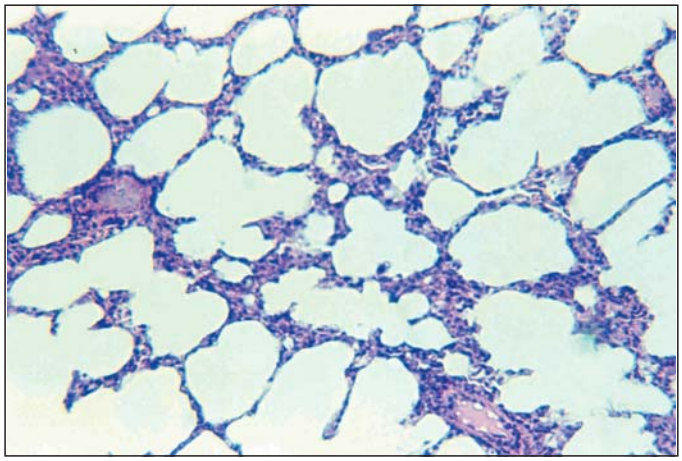

Figure 5. Pulmonary tissue of a rabbit receiving oil and tocopherol (OT), with signs of inflammatory response

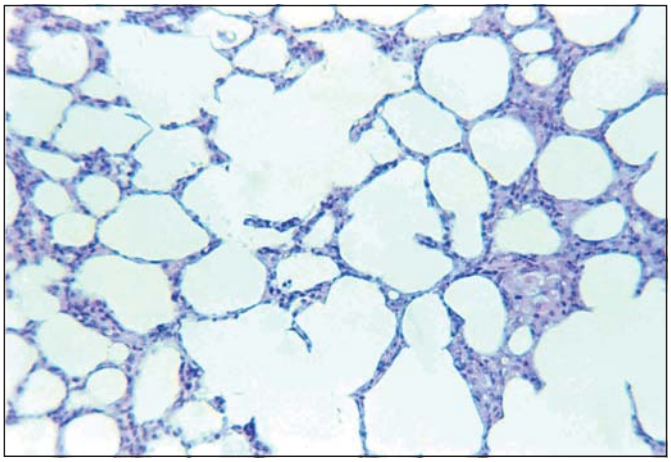

Figure 6. Pulmonary tissue of a rabbit receiving cholesterol and tocopherol (ChT), without emphysematous changes. Severe inflammation can be observed

DISCUSSION

In our investigation TC and LDL concentrations were significantly increased ($p<0.01$) in the sera of all groups compared to group C. In comparison with group C, TG content

was significantly decreased ($p<0.01$). These results may be related with the augmentation of ROS production, since the lungs are a primary target for oxidant injury, because of their morphology and function (Del Donno and Verduri, 2000).

An increased number of neutrophils, eosinophils, AM, lymphocytes, epithelial, mast and other metabolically active lung cells undoubtedly impact the inflammatory process and may alter the oxidant-antioxidant balance in patients with PE (Fletcher and Pride, 1984; Zunic, 1997; Vucevic, 1999). Circulating adhesion molecule-1 (ICAM-1) was increased in PE patients and probably altered PMN retention in the lungs (Riise *et al*., 1994; Baluk *et al*., 1996; Popper *et al*., 2002; Theriault *et al*., 2002). Recruitment and activation of PMN and other inflammatory cells into the lungs may also involve production of interleukin-8 (IL-8) by AM, epithelial, or other lung cells. IL-8 is a potent chemotaxin for PMN *in vitro* (Fletcher and Pride, 1984; Retamales *et al*., 2001). Another AM and lung cell-derived molecule that may contribute to PMN recruitment is LTB_4 (Repine *et al*., 1997; Retamales *et al*., 2001).

Increased numbers of PMN appear to be making increased amounts of oxidants in the lungs of PE patients. ROS trigger lipid peroxidation, that can impair membrane function, inactivate membrane-bound receptors and enzymes, disturb membrane fluidity and increase permeability. Phospholipid fatty acids and fatty acids esterified with glycerol and cholesterol are particularly susceptible to lipid peroxidation. In this process 40% of lecithin is converted into lysolecithin, known to be a potent chemotaxin for monocytes and lymphocytes (Acworth and Bailey 1995; Del Donno and Verduri, 2000; Polidori *et al*., 2001; Bowler and Crapo, 2002; Dröge, 2002; Minko *et al*., 2002; Rocksen, 2003). Oxidants and proteases can act as synergists and damage airspaces by degrading elastin, extracellular membrane proteins, proteoglycans and glycoproteins (Repine *et al*., 1997; Teramoto *et al*., 1999; Krettek *et al*., 2003). Oxidants also stimulate thromboxane formation, reduce surfactant activity and impair cilia function in patients with PE (Repine *et al*., 1997; Heunks and Dekhuijzen, 2000; Ginzberg, 2001; Bowler and Crapo, 2002).

An increased number of immune and inflammatory effector cells in bronchoalveolar lavage (BAL) of rabbits fed on the oil – containing diet compared to the control and emphysematous rabbits could be explained by the immunogenic influence of edible oil (Zunic, 1997). Inflammatory effector cell infiltration and the oil – containing diet in some manner lead to the disturbance of homeostatic mechanisms in pulmonary tissue (Djordjevic *et al*., 1978; Radak *et al*., 1987; Zunic, 1997; Vucevic, 1999) and may be related with the findings presented in this study (Figure 2).

The LDL receptor mediates the transport of cholesterol as part of circulating LDL, and thus is the key player in the maintenance of systemic cholesterol homeostasis. LDL that binds to this receptor is taken up by receptor-mediated endocytosis and digested by lysosomes within the cells. Cholesteryl esters of LDL are hydrolysed by a lysosomal CE, and the liberated cholesterol is used for steroid hormone synthesis, and as a regulatory molecule that suppresses the synthesis of new LDL receptors. The LDL receptor accounts for approximately two-thirds of the LDL normally removed from plasma in humans. The remaining

LDL is removed by scavenger cell pathways. Control metabolic studies have provided ample evidence that endogenous and exogenous cholesterol are directly related. It seems that there are significant individual and population genetic variations involved in the ability of exogenous cholesterol to exert an influence on the edogenous one (Haslam, 1994). Additionally, increase of TC concentration in serum of all other groups compared to group C is an expected finding, that could be explained with the induction of experimental emphysema. Our findings correspond to literature data, too. Because of the extreme sensitivity of rabbits to dietary cholesterol, this experimental protocol causes massive increases of cholesterol above normal levels. Absorption of a considerable amount of dietary cholesterol, without a compensatory increase of its degradation and excretion, is responsible for massive hypercholesterolemia in the serum of rabbits fed on a hypercholesterolemic diet (Ohnishi *et al*., 1998).

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). Increased numbers of immune and inflammatory effector cells in areas adjacent to lung injury could indicate that the accumulation of these cells correlates with the pathogenesis of PE (McLaughlin and Tueller, 1971). 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 the pathohistological findings observed in pulmonary tissue specimens of group Ch (Figure 3). Pathohistological analysis of pulmonary tissue specimens obtained from rabbits fed on a hypercholesterolemic diet indicates changes that refer to evident emphysema (damage to air sacs, i.e. breakdown of the walls of the alveoli that results in permanent „holes" in the lung tissue called *bullae*) (Figure 3).

LDL particles are the major cholesterol transporting vehicle in the organism. Hypercholesterolemia leads to chronic presence of LDL particles in the arterial wall. Insudation of LDL is promoted by elevated plasma LDL concentrations and endothelial damage. Lipids and proteins derived from circulating LDL accumulate both within cells and in the extracellular space. It is now widely held that injury from high cholesterol levels is actually a result of cytotoxic forms of LDL (Falk *et al*., 1995).

Each cholesterol-rich diet infallibly induces endogenous LDL production. Moreover, local changes of mucopolysaccharide components (namely glycosaminoglycans) lead to lipid accumulation. It is supposed that these molecules may interact with intimal proteins and alter intimal permeability, so lipids accumulate to a great extent (Haslam, 1994).

In experimental atherosclerosis induced by hypercholesterolemic diet monocytes, recruited by activated endothelial cells, become activated themselves and differentiate in the subendothelial space into macrophages. Then, macrophages ingest lipid components to form cholesterol-laden foam cells (Ross, 1993). Macrophage recruitment and activation occur in response to a variety of locally produced chemotactic and proinflammatory molecules (Hansson, 1997). Besides, the inflammatory response itself can have a profound effect on lipoprotein movement within the artery. Mediators of inflammation, such

as tumor necrosis factor α (TNFα), interleukin-1 (IL-1) and macrophage colony stimulating factor (M-CSF) increase binding of LDL to endothelium and smooth muscle cells and increase the transcription of the LDL-receptor gene. After binding to scavenger receptors *in vitro*, modified LDL initiates a series of intracellular events that include the induction of urokinase and inflammatory cytokines (IL-1, etc.). Thus, a vicious circle of inflammation, modification of lipoproteins and further inflammation can be maintained in the artery by the presence of these lipids (Ross, 1999). Additionally, a cholesterol-rich diet results in rapid endothelial expression of vascular cellular adhesive molecule-1 (VCAM-1 (Libby, 2002). Lipoprotein(a) /Lp(a)/ has been shown to upregulate the endothelial expression of some cell adhesion molecules, and it has the potentially harmful effect of interfering with plasminogen activation (Takami *et al*., 1998). Similar findings have also been documented in human subjects. Namely, after binding to LDL-receptor, LDL particles increase the expression of VCAM-1 and E-selectin at the surface of human vascular endothelial cells (Allen *et al*., 1998). However, it is not completely understood if the major atherogenic property can be attributed to cholesterol concentration, lipid configuration, relative values of lipoprotein fractions, or else.

Modifications of LDL particles that can favor foam cell formation include oxidation, aggregation, enzymatic modification, complexing with immunoglobulins, and possibly others (Ross, 1999). All lipid classes (sterols, phospholipid fatty acids, cholesteryl esters and TG) and protein particles are susceptible to oxidative modification. Ox-LDL particles are chemotactic for monocytes. Ox-LDL particles have also been associated with an increase in monocyte binding to endothelial cells in humans. Moreover, they inhibit macrophage motility from the arterial wall into the circulation and induce cholesterol accumulation in smooth muscle vascular cells. Oxidative cholesterol products derived from LDL particles appear capable of activating hydroxymethylglutaryl-coenzymeA reductase (HMG-CoA reductase). They can suppress the synthesis of the LDL-receptor gene and contribute to the maintainance of the increased LDL value in the circulation (Steinberg and Lewis, 1997).

Our investigation demonstrated that LDL concentration was significantly increased ($p<0.01$) in the serum of all other groups compared to group C. Similar results have been documented by Clarkson *et al.,* 1974. They described a multiple increase of LDL fraction in the serum in comparison with other lipid fractions (Clarkson *et al.,* 1974).

Continued inflammation results in a increased number of macrophages and lymphocytes, which both migrate from the blood and multiply within the lesion. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines and growth factors, which can induce further pulmonary tissue damage. Namely, the number of lymphocytes, mastocytes and neutrophils was in correlation with the immunostimulation of lipids in BAL patients with extrinsic allergic alveolitis (EAA). Such finding confirmed the assumption that the lipids obtained from the epithelial lining fluid could play a role in the modulation of inflammatory responses in the alveolar space (Hughes and Haslam, 1990).

Other findings have shown the association between atherogenesis and pulmonary diseases. In patients with pneumoconiosis inhibition of surfactant synthesis, followed by development of blood and tissue phospholipid deficiency, may contribute to the development of atherosclerosis. On the other hand, increase of lung phospholipid production, followed by increase of phospholipid blood concentration, is an important factor that inhibits atherogenesis in these patients (Shapiro, 1994).

In comparison with group O, TC content was significantly decreased ($p<0.01$) in the sera of groups ChT and OT as a result of a possible protective function of tocopherol. It is well known that tocopherol efficacy depends on high oxygen pressure, and consequently tocopherol concentration increases in lipid regions with the highest oxygen partial pressure, such as erythrocytes and lung membranes. One tocopherol molecule can protect about 10^3-10^8 polyunsaturated fatty acid molecules at a low peroxide levels (Kamal-Eldin and Appelqvist, 1996; Diaz *et al*., 1997). It has been shown that small α-tocopherol/ polyunsaturated fatty acids ratio in biomembranes (e.g., 1:500 α-tocopherol/ arachidonic acid molecules in erythrocyte membrane) is enough to interrupt the free radical chain reactions (Kamal-Eldin and Appelqvist, 1996). Alternatively, the presence of catalase (CAT), glutathione peroxidase (GPO) and superoxide dismutase (SOD) in biological systems may diminish the flux of radicals sufficiently, so that the small amount of available vitamin E operates only as a second line of defense (Kamal-Eldin and Appelqvist, 1996; Padayatty *et al*., 2003).

Vitamin E derivatives inhibit $O_2^{\cdot -}$ production in neutrophils (Kanno *et al*., 1996). In recent years, the effects of á-tocopherol on monocytic cell adhesion have been studied extensively. Aside from their ability to protect LDL from oxidation, á-tocopherol has been shown to inhibit monocyte adhesion by inhibiting the surface expression of endothelial adhesion molecules (Devaraj *et al*., 1996).

The lipophilicity of the molecule (as determined by the number of methyl substituents in the chroman ring and the structure and stereochemistry of the phytyl tail) is an important feature for the biological activity of the tocopherols since it determines the kinetics of their transport and retention within the membranes. The chroman group is, thus, not only responsible for the antioxidant potential of the tocopherols but also for their lipophilic properties. The phythyl tail, on the other hand, has no effect on the chemical reactivity of vitamin E, but is responsible for the high lipophilic properties of these compounds thus being important for proper positioning in the biomembranes (Kamal-Eldin and Appelqvist, 1996). The data from the study by Giasuddin and Diplock (1981) strongly suggest that the same mechanism is involved in the inactivation of membrane phospholipases and oxygenases, which is relevant in membrane phospholipid protection (Giasuddin and Diplock, 1981). All these observations could be of importance, especially if the protective tocopherol effects in PE are observed from the immunologic point of view.

Increase of TC content in the sera of group OT ($p<0.01$) compared to group ChT could be explained by the prooxidant activity of tocopherol. Namely, under certain conditions, where ROS levels are raised beyond the capacity of the

protective mechanisms, or when these mechanisms are faulty, tocopherol can act as a prooxidant. Studies performed in the recent years have shown that α-tocopherol may act either as an antioxidant or as a prooxidant depending on experimental conditions. Prooxidant tocopherol activity may be explained by synergistic effects of this vitamin with prooxidants (transiton metal ions, lipid peroxides, etc.) (Kamal-Eldin and Appelqvist, 1996). Each antioxidant/substrate combination has critical concentration ratios for their maximum stability. Below these critical concentration ratios, inhibition is below optimum and the antioxidants may invert their effects and exert their so-called coprooxidant activity (Pokorny, 1987; Kamal-Eldin and Appelqvist, 1996). Furthermore, it is found that tocopherol activity depends on temperature (Marinova and Yanishlieva, 1992). It is also documented that the higher the temperature, the less the prooxidant effect of á-tocopherol, even at high concentrations. An explanation to this may be related to the fact that at high temperatures, oxygen has lower solubility in oils so that autoxidative peroxide formation proceeds at lower rates and becomes gradually substituted by polymerization reactions. On the other hand, the higher the temperature, the higher the rate of H_2O_2 decomposition and the higher the reactivity of the transition metal ions (Kamal-Eldin and Appelqvist, 1996).

HDL content was significantly decreased ($p<0.05$) in the serum of the experimental group fed on a hypercholesterolemic diet compared to both control groups. This finding is compatible with the documented data which reports a significant decrease of this lipoprotein particle in the serum of animals fed a hypercholesterolemic diet. Decrease of HDL concentration followed by an increase of LDL was the main lipoprotein change observed in the plasma of these animals (Ikeda *et al*., 1987).

Our results showed that HDL content significantly decreased ($p<0.05$) in the sera of groups O and ChT compared to group C, as well as in the sera of groups ChT and OT compared to group O. These results suggest that some kind of abnormal relation between serum lipid fractions and tocopherol could be established. It is known that α-tocopherol is the most abundant and active dietary antioxidant present in plasma LDL and HDL particles. Phospholipid transport protein (FLTP) has been reported to catalyze the α-tocopherol transport between lipid sections. As a consequence, α-tocopherol molecules easily move from HDL to VLDL and LDL particles. This process is followed by the return of small α-tocopherol amount to the HDL fraction (Cogny *et al*., 1994). However, the mechanism of vitamin E transition from lipoproteins to tissues is still unclear. According to the data from the study by Cogny *et al*. (1994), this transition could occur during degradation of triglyceride-laden lipoproteins by the enzyme lipoprotein lipase, upon the action of LDL receptors or due to other mechanism.

Increase of HDL concentration in the sera of group ChT ($p<0.05$) compared to group Ch could be explained by an efficient antioxidant activity of tocopherol.

HDL content was significantly increased ($p<0.05$) in the sera of group OT compared to group ChT. This result could indicate a disorder of HDL concentration maintenance due to cholesterol as a relevant ethiological factor.

Efficacy of our experimental procedure was histologically confirmed. In rabbits fed on the hypercholesterolemic diet one can observe the development of pulmonary emphysema (Figure 3).

Inflammatory cell infiltration and oil diet lead to the disturbance of homeostatic mechanisms in rabbit pulmonary tissue (Figure 2).

In spite of inflammation, the pulmonary tissue of rabbits from all experimental groups receiving tocopherol (groups T, OT and ChT) is without emphysematous changes (Figure 4-6). These findings may be explained by the protective action of tocopherol (Figure 4-6).

Vitamin E in the form of α-tocopferol is quantitatively the most important lipophilic redox-active, low-molecular-weight component in the human circulation, and has thus received a lot of attention as a possible modulator of PE. Our findings indicate the influence of tocopherol on the serum lipid profile in experimental emphysema as well as its possible protective role in development of this disease. Furthermore, the questions that this manuscript raises about the potential mechanisms of tocopherol activity will provide an exciting basis for future research into the mechanisms of oxidant-induced lung injury and a possible role for tocopherol and other antioxidant drugs in the prevention or care of this disease.

Address for correspondence:
Danijela Vučević MD, Msc
School of Medicine, University of Belgrade
Dr Subotića 9, 11 000 Belgrade
Serbia
e-mail: danibovuc@med.bg.ac.yu

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EFEKAT TOKOFEROLA NA LIPIDNI STATUS U PLUĆNOM EMFIZEMU IZAZVANOM HIPERHOLESTEROLSKOM DIJETOM

VUČEVIĆ DANIJELA, MILOVANOVIĆ I, MLADENOVIĆ D, ŽUNIĆ-BOŽINOVSKI SNEŽANA, RADOSAVLJEVIĆ TATJANA, STOJANOVIĆ JASNA, PEŠIĆ BČ i ĐORĐEVIĆ-DENIĆ GORDANA

SADRŽAJ

Za ispitivanje efekta tokoferola na lipidni status u eksperimentalnom plućnom emfizemu (PE) izazvanim hiperholesterolskom dijetom korišćeno je šest grupa kunića Činčila rase: K – kontrolna grupa na ishrani uobičajenoj za ovu životinjsku vrstu (n=20), U – kontrolna grupa na uljanoj dijeti (n=20), H – eksperimentalna grupa na hiperholesterolskoj dijeti (n=20), T–eksperimentalna grupa intramuskularno tretirana tokoferolom (n=20), UT – eksperimentalna grupa na uljanoj dijeti intramuskularno tretirana tokoferolom (n=20) i HT – eksperimentalna grupa na hiperholesterolskoj dijeti intramuskularno tretirana tokoferolom (n=20). Nakon dvomesečnog tretmana, životinjama je određivana koncentracija ukupnog holesterola (UH), triglicerida (TG), lipoproteina male gustine (LDL) i lipoproteina velike gustine (HDL) u serumu. Postojanje eksperimentalnog plućnog emfizema je potvrđeno histološkim metodama. U odnosu na K grupu nađeno je visoko statistički značajno povećanje ($p<0,01$) koncentracije UH i LDL u ostalim grupama. U odnosu na U grupu visoko statistički značajno sniženje ($p<0,01$) nivoa UH registrovano je u HT i UT grupi. Sniženje serumskih vrednosti TG svih ispitivanih grupa u odnosu na K grupu je bilo na nivou visoke statističke značajnosti ($p<0,01$). Sadržaj UH u serumu životinja HT grupe u poređenju sa UT grupom je visoko statistički značajno povećan ($p<0,01$). Naši rezultati ukazuju na uticaj tokoferola na lipidni status u eksperimentalnom PE, kao i na njegovu moguću zaštitnu ulogu u razvoju ove bolesti.