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Changes of phospholipids during the lipid peroxidation in workers exposed to coal-rock dust

The article presents the results of a study of lipid peroxidation when exposed to coal- rock dust on the organism of miners depending on length of service and in experimental animals. It was found that fairly significant change in the fractional composition of phospholipids in the blood of miners there after having worked for more than 15 years and is dependent on the activation of lipid peroxidation. In experimental animal studies, a decrease of phosphatidylcholine and phospholipids of the inner side of the cytoplasmic membrane — phosphatidylinositol and phosphatidylserine, phosphatidyl — ethanolamine may be indicative of increasing the permeability of the plasma membrane in the conditions of formation of fibrosis.

Key words: coal- dust pedigree, miners, experimental animals, blood, lungs, lipid peroxidation, phospholipids.

The ability of dust to cause pulmonary fibrosis or bronchial inflammation depends on its physiological and chemical properties. Pneumoconiosis and bronchitis affects working-age population, which defines the social significance of pathology.

The relevance of the study of the fundamental mechanisms of pneumoconiosis proven by, in our opinion, the following points:

– firstly, lung diseases of dust etiology dominate among miners morbidity due to insufficient information about the exact damaging mechanism of coal-rock dust (CRD) on lung tissue;

– secondly, pneumoconiosis and occupational bronchitis, characterized by the irreversible duration of disease and the loss of both professional and general capacity for work, significantly change the quality of life of patients and lead to a large amount of social loss;

– thirdly — susceptible persons to the risk of pneumoconiosis and occupational bronchitis are the ones, who are in the most active working age and employed in key industries.

Primary and compulsory link in the chain of pathological changes in the lung tissue after inhaling CRD is the activation and death of macrophages with engulfed particles of CRD due to the activation of the enzyme system of NADPH oxidase peritoneal macrophages of human blood called «respiratory burst» [1–3].

It is known that during phagocytosis of dust particles NADPH oxidase activated in that part of the membrane which is in contact with the dust [4]. However, the interaction of the different dust particles to cells occurs in different ways: contact of chrysotile asbestos particles to cells is carried out by the electrostatic attraction of different pole groups on the membrane; — quartz particles are bound by the formation of multiple hydrogen bonds; — the interaction of coal dust and a number of other low fibrogenic aerosols with cells caused by superoxide anion radicals [5]. Damaging effect of reactive oxygen species (ROS) in the lung tissue when exposed to fibrogenic dusts depends on the amount of ROS and its qualitative composition, which has a significant role in the formation and on the severity of the pathology of the lung tissue [6,7].

Unlike coal dust itself, which is well phagocytized and relatively easily removed from the lungs, CRD of Karaganda coal basin contains free silica for more than 10 % and is in phase III of metamorphism, therefore it is removed slowly and cumulated in the lungs due to rapid death of phagocytes [5].

Objective: To study the nature of the changes of phospholipids in the lipid peroxidation under prolonged exposure to coal and rock dust

Materials and methods:

Total 140 coal miners of Karaganda coal basin was surveyed. The main groups by length of exposure: 0–5 years, 24 persons — I group; 6–10 years, 24 persons — II gr.; 11–15 years, 25 persons — III gr.; 16–20 years, 23 persons –IV gr.; 21 or more years, 23 persons — V gr. The control group — 21 male subjects, 30 years old, have no contact with CRD.

The experiment was performed on male Wistar rats with an average weight of 200–230 gr. They had a single intratracheal instillation of 50 mg. CRD slurry 27K12 m. «Stakhanov». Animals were divided into 3 groups according to the duration of exposure: I group of animals — after 1 month; II gr. — 4 months; III gr.

— 6 months from the start of exposure to dust. Rats were kept in vivarium under natural light mode, on a standard diet with free access to water.

Fractional composition of plasma phospholipids and lung homogenate was determined by the method of Folch [8], the lung tissue was homogenized beforehand, then by flash chromatography on silica gel 5/40 CSSR using chloroform — methanol — water — acetic acid mixture in the ratio 60:50:1:4 with distillation produced by Cates M. [9]. The following fraction of phospholipids were determined: lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE). Activity of phospholipase A2 (PLA2) by Tuzhilin A. (1975). The quantitative content of phospholipid fractions was determined by phosphorus [10].

Evaluation of peroxide metabolism was based on the determination of the total products of LPO — diene conjugates (DC) triene conjugates (TC), double bonds (DB), malondialdehyde (MDA) [11,12].

Statistical analysis was performed on a personal computer using a well-known statistical programs. Significant differences were determined by Student's t test.

Results and discussion:

As shown in Figure 1, the process of phagocytosis in lung tissue of coal miners in groups with exposure 0–5 and 6–10 years, was accompanied by a decrease in the activity of lipid peroxidation process in comparison with the control group.

For workers with exposure period of 11–15, 16–20 and 21 or more years was observed increase in LPO activity in the form of excess generation of malondialdehyde, significantly higher than the LPO as in control subjects and coal miners in exposure groups 0–5 and 6–10 years. Changes in the level of malondialdehyde significant for assessment of lipid peroxidation, as malondialdehyde able to participate in redox reactions on the particle surface, thereby affecting the process of phagocytosis.

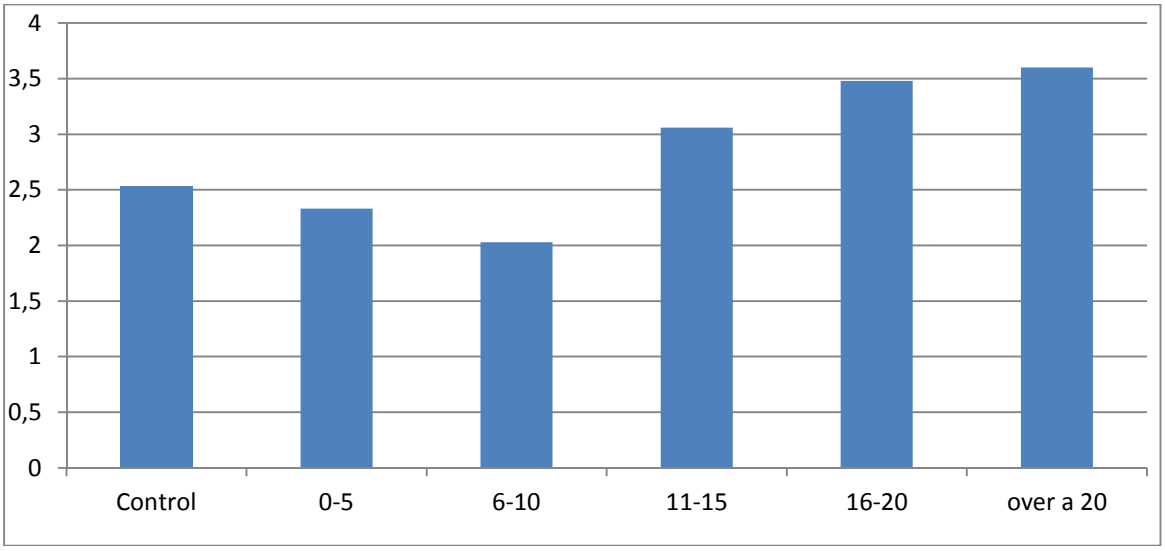

Figure 1. Activity of malondialdehyde under the influence of CRD, depending on years of exposure

Danger of LPO activation is that the process of phagocytosis of the dust particle is accompanied by a high level of cell consumption of the oxygen: so when activated by quartz particles macrophage O_2 consumption increased by 4 times, by particles of titanium dioxide — 1.5 times. Wherein the phagocyte NADPH system alters the electronic structure of the oxygen molecule, converting it into an aggressive oxygen and peroxide radicals [7], which in turn react with lipids, the lipid molecules losing (LH) hydrogen atom with the formation of free lipid radicals (L), which reacts with the dissolved molecular oxygen in the environment, resulting in a new free radical — radical lipid peroxides LO_2, which attacks one of the adjacent phospholipid molecules LH to form lipid hydroperoxide LOOH and new radical L.Alternation of the last two reactions leads to the development of a chain of lipid peroxidation [13,14].

The results of experimental studies are shown in Table, which illustrates that after 1 month of the experiment level of PE and PS, PC and SM in the lung tissue of the experimental group animals unchanged in comparison with those of the control group animals.

T a b l e

Phospholipids of the lung tissue of experimental animals after intratracheal instillation of coal dust (nM / ml) (M ± m)

Animal groups	n	LPC	SM	PC	PI	PS	PE
After 1 month	C=6	136,86 ±13,49	214,98 ±33,80	1505,92 ±33,40	508,34 ±19,40	360,08 ±41,80	311,92 ±82,50
	M=6	160,50 ±56,60	202,50 ±49,56	1340,80 60,20±	508,80 ±16,26	251,70 ±50,59	248,70 ±60,23
After 4 month	C=6	178,06 ±12,90	388,44 ±11,70	1256,70 ±19,90	565,80 ±19,50	382,60 ±9,50	656,40 ±20,00
	M=6	129,65 ±15,20	181,20 ±2810*	1159,16 ±27,60	448,00 ±11,60	208,40 ±12,80*	520,16 ±23,30*
After 6 month	C=6	107,90 ±25,10	218,38 ±28,30	373,20 ±96,20	240,98 ±52,70	151,96 ±72,40	361,90 ±56,10
	M=6	184,37 ±30,30	221,30 ±31,20	283,46 ±69,30	110,12 ±31,70	111,18 ±25,90	204,90 ±44,30

*Note**: The difference of main group with the control significant at ($p<0,05$).

This may indicate the presence of sustainable cell membranes and is regarded by us as a sign of lung tissue forming adaptation to CRD. However, increased LPC at the same experimental period may show vulnerability of plasma lipoproteins to the phospholipase A2 and its peroxidation by free radicals, and also indicates the increased phospholipase hydrolysis of phospholipids of lung tissue.

After 4 months of the experiment PE and PS in animals of the main group changes to the opposite direction — their content is reduced from 4 % to 24 % and reaches significant values compared with the values of the control group. Besides, there is a clear tendency to reduction in the level of LPC, PC and SM. Simultaneous decrease in phospholipids of the internal and external layer of the membrane to the impact of CRD after 4 months can be explained by the high sensitivity of the inner layer membrane phospholipids to CRD, and by the fact, that the PE and PS have the highest degree of saturation of fatty acid composition which makes them more susceptible to lipid peroxidation.

Consequently, the results of the experiment with CRD in 4 months can be regarded as another phase, when there is destabilization of phospholipid spectrum of lung tissue, which can be regarded as a voltage phase.

After 6 months of the experiment the impact of CRD further add to the PE and PS decrease from 18 % to 26 %, PC to 46 % at the highest level of LPC from 31 % to 70 % respectively in the plasma and lung tissue in the experimental group compared with those of the control group.

Figure 2 presents the results of the analysis of the total phospholipid content of plasma membranes of the lung tissue of experimental animals at different times. The figure shows that priming of animals with CRD has a significant destabilizing effect on the plasma membrane of the lung tissue: so the total content of PE and PS is reduced by 15–29 % (depending on the duration of the experiment), whereas the amount of PC + SM increased by 16–20 % in rats of the experimental group compared to the total phospholipid content of the lung tissue of control group rats. These results also indicate that the continued impact of CRD at the lung tissue of experimental animals can damage both internal and superficial layers of membranes.

Shifts of phospholipids of the lung tissue can be explained in two ways: compensatory and altering (damaging). First explanation — following the collapse of PC under the action of phospholipase A2 cleaves unsaturated higher fatty acids, which are included in the link «oxidation» and can serve as a generator of the power source. Second explanation — transition of PC to LPC promotes disruption of membrane integrity, enhances the intracellular Ca^{2+} [15].

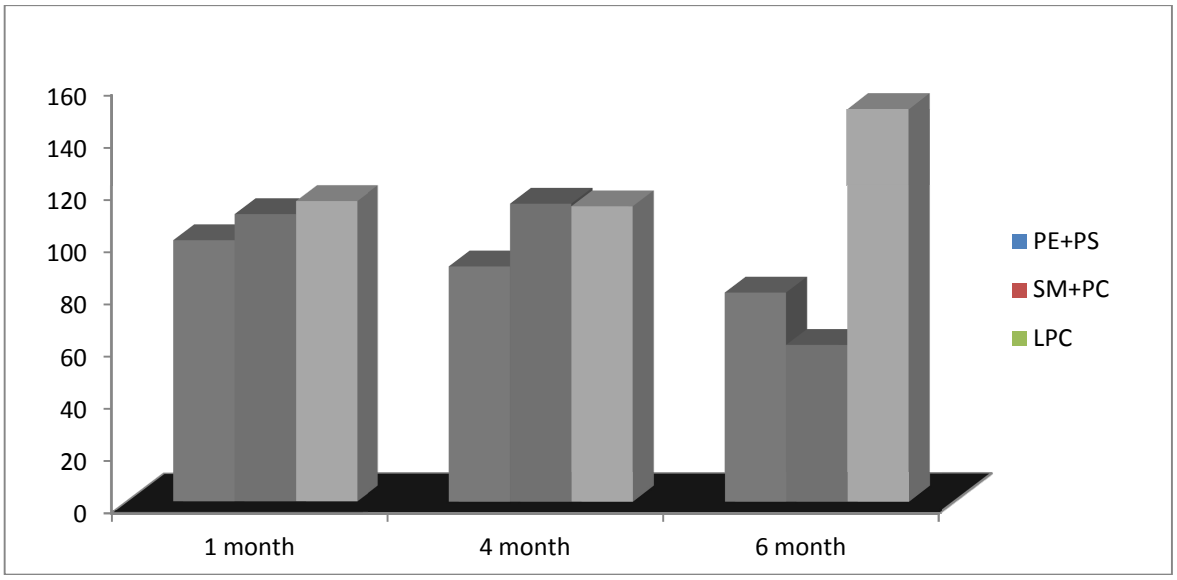

Figure 2. Ratio of total phospholipids of lung tissue of experimental rats after intratracheal dusting (%)

Because lung tissue phospholipids almost completely localized in the membranes, our results indicate directly the damage of the lipid bilayer membrane under the action of the CRD. Consequently, in the 6 months of the experiment with CRD was observed damage of the cell membranes, which can end up with sclerosis of cells, in this case pulmonary fibrosis.

The foregoing shows that the activation of the enzyme system under the influence of CRD is an irreversible process, since the object of phagocytosis as CRD, does not disappear, moreover, avalanche increase of LPO processes can cross the boundaries of phagosome and lead to oxidative damage of not only the cell structures of the lung tissue, but its membranous structures.

Based on the foregoing it can be assumed that the impact of CRD on the organism of miners leads to the following:

– firstly, mainly those fractions of phospholipids (PC, SM) are destroyed, which in its composition has a high content of easily oxidized fatty acids;
– secondly, simultaneously significantly are activated peroxidation (MDA) in lung tissue and in blood plasma;

Under the influence of CRD phagocyte produces even more antibacterial protection — oxygen radicals. But if in case of an infectious or viral pathogen they are harmful, the dust particle is not sensitive to them, on the contrary, an excess of dust load leads to a sharp increase in reactive oxygen species that become dangerous not only to the phagocytes, but also for the surrounding lung tissue. Thus, the amplification of LP — enrich membrane PC, PE, PS; braking LP — SM, PS.

That is, during phagocytosis of CRD bactericidal ability of macrophages may be useless, as the continued production of reactive oxygen species can lead to oxidative degradation of the phagocyte. Consequently, suffers not only the barrier function of the lungs, as phagocytosis is active and passive capture, absorption, long-lasting hold, enzymatic metabolism — the main ways of inactivation of xenobiotics, including solid, practically insoluble, low-toxic and small disperse dust particles, but also identifies the nature of three specific manifestations of dust pathology: frequency of lesions of the respiratory tract, the genesis of obstructive syndrome, irreversible course of anthracosilicosis [9].

Overall, based on the results of our research, we can assume that prolonged exposure to CRD increases the intensity of lipid peroxidation, i.e. CRD damaging effect on lung tissue is obliged to secondary radicals, resulting in a continuous chain reactions of lipid oxidation [16,17]. Thus, a mechanism of the pathological effect of coal-rock dust expressed in its cytotoxicity.

Conclusions:

1. Impact of CRD on the organism leads to the predominantly destruction of those fractions of phospholipids (PC), which in its composition has a high content of easily oxidized fatty acids;

2. CRD stimulates not only phagocytosis, but also activates peroxidation in lung tissue and in blood plasma with increased generation of reactive oxygen species as preferential production of secondary form of radicals. This allows us to consider the active forms of oxygen as the primary link in the mechanism of the damaging effect of the CRD on macrophages, which seems to determine the pathogenesis of anthracosilicosis.

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Ағзаға көмір-кенді шаңмен әсер еткенде липидтердің асқын тотығу процесіндегі фосфолипидтердің өзгерісі

Мақалада тау-кен жұмысшыларының жұмыс тәжірибесінен тәуелсіз ағзасы мен жануарлар көмір-кенді шанның әсеріндегі липидтердің асқын тотығуын зерттеудің нәтижелері берілген. Тау-кен жұмысшыларының қанындағы фосфолипидтердің фракциялық құрамының нақты айқын өзгерісі 15 жылдан астам уақыт жұмыс тәжірибесінде дамитындығы және липидтердің асқын тотығуына байланысты екендігі дәлелденген. Жануарларға сынақтық зерттеулер жүргізу кезінде цитоплазмалық мембрананың ішкі бетіндегі фосфолипидтер мен фосфатидилхолиннің фосфатидилинозитид, фосфатидилсерин және фосфатидил-этаноламинның төмендеуі пневмофиброз даму жағдайындағы плазмалық мембрананың жоғары өткізгіштігін көрсетті.

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Изменения фосфолипидов в процессе перекисного окисления липидов при воздействии угольно-породной пыли на организм

В статье представлены результаты исследования перекисного окисления липидов при воздействии угольно-породной пыли на организм горнорабочих в зависимости от стажа работы и в эксперименте на животных. Установлено, что достоверно значимое выраженное изменение фракционного состава фосфолипидов крови у горнорабочих наблюдается при стаже работы более 15 лет и зависит от активации процессов перекисного окисления липидов. Отмечено, что в экспериментальных исследованиях на животных снижение фосфатидилхолина и фосфолипидов внутренней стороны цитоплазматической мембраны — фосфатидилинозитида и фосфатидилсерина, фосфатидил-этаноламина — может свидетельствовать о повышении проницаемости плазматической мембраны в условиях формирования пневмофиброза.

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