

Effect of a medicinal agent, possessing antioxidant and antihypoxant properties, on the state of oxygen homeostasis and lipid peroxidation under experimental pneumoconiosis in albino rats

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ABSTRACT

Introduction: Pneumoconioses (Pc) are interstitial pulmonary diseases of professional genesis, caused by long-term inhalation of high dust concentrations. Pcafflict 26.6-53% of the workers, exposed to dust contamination. The major feature of Pc course is irrevocability and incurability.

Purpose: The research of the effect of ethylmethyl hydroxypyridine succinate, possessing antioxidant and antihypoxant characteristics, on the oxygen metabolism, acid-alkaline state, and lipid peroxidation at the background of experimental pneumoconiosis in albino rats.

Material and methods: Pneumoconiosis was simulated on 72 albino rats through endotracheal injection of black coal suspension, on our own modification of O. Yu. Nykolenko methodology.

Results: Manifestations of experimental pneumoconiosis depended on the period of dust inhalation. Development of experimental pneumoconiosis was found to result in the decreased partial oxygen

pressure in the arterial blood, compensated hyperventilation-induced respiratory alkalosis, and marked increase in lipid peroxidation activity that could be seen in the blood content of malondialdehyde, as well as of conjugated dienes and trienes.

Conclusions: Application of ethylmethylhydroxypyridine succinate in experimental pneumoconiosis has been found to improve oxygen metabolism, acid-alkaline state, and lipid peroxidation that is revealed in normalization of the partial oxygen pressure in the arterial blood, oxygen saturation of haemoglobin, index of bases' deficiency BE (Base-Excess), and in 1.6-2.0 decrease in the content of malondialdehyde, as well as of conjugated dienes and trienes.

Keywords: experimental pneumoconiosis, methodology of pneumoconiosis stimulation in albino rats, lipid peroxidation, oxygen metabolism, ethylmethylhydroxypyridine succinate

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INTRODUCTION

Pneumoconioses (Pc) are interstitial pulmonary diseases of professional genesis, caused by long-term inhalation of high concentrations of fibrogenic dust. They can also be defined as non-specific pulmonary reaction on the inhalation of mineral or organic dust that is followed by the damage to the pulmonary tissue. Pc is characterized by chronic diffuse aseptic inflammatory process in the pulmonary tissue, accompanied by the development of pneumofibrosis [1]. The course of the disease depends on the conditions, concomitant diseases, and individual systemic sensitivity. Pc afflicts 26.6-53% of the workers, exposed to dust contamination [2]. According to different authors, Pc is diagnosed on the average of 5-6% of the total number of first revealed professional diseases [3]. The number of reported Pc cases has been stable lately that can be explained by 2 factors. On one hand, working conditions at the production facilities with detrimental dust effect improve, thus resulting in the morbidity reduction. On the other hand, with the increasing level of Pc diagnostics the total number of diagnosed cases increases.

According to ICD-10, Pc relate to "Pulmonary diseases, caused by external agents" (J60-J70). Pc cover: silicosis, silicatosis, metaloconioses, carboconioses, (antracosis, etc.), Pc from mixed dust (antracosilicosis, electric welders' Pc), Pc from organic dust (early manifestations of bisinosis, hypersensitive pulmonites, including "farmer's lung"). The most widespread are the Pc in the miners, welders, metallurgists, machine builders, builders, workers of potteries, stone quarries, and farmers.

Awareness of the general mechanisms of Pc pathogenesis is essential for effective treatment. Pc pathogenesis is intertwined with the issues of general pathology, biochemistry, physiology, and other basic branches of medicine. In particular, they cover problems of lipid peroxidation, free radical processes, mechanisms of respiratory failure onset and its management.

Pc-induced injuries to the respiratory system are various. All the Pc are characterized by irrevocability, resulting in disability and decreasing life expectancy. So far, there is no specific therapy for Pc. In view of epidemiology and difficulty in treatment, the problem of Pc prevention and treatment is particularly important. Of particular relevance are the works, dealing with the new methods of Pc treatment, particularly possibility of using new medicines, which could be applied for the treatment, at least symptomatic.

Increased lipid peroxidation (LP) is known to be a universal pathologic toxicity mechanism for the bulk of hypoxia-associated pathologic states [4]. LP activation is a major factor of pulmonary tissue

damage in Pc [5]. Research works on the efficacy of antioxidants as the drugs, decreasing LP activity in Pc, are lacking. Antihypoxants, pharmacologically capable of decreasing systemic oxygen demand, have been applied for symptomatic treatment lately [6]. Drugs, combining properties of both antioxidants and antihypoxants, are available. Elara-manufactured ethylmethylhydroxypyridine succinate (Es) is an option. In theory, the drug is expected to reduce manifestations of hypoxia in Pc and to alleviate the course of the disease. The objective of this work was to research Es efficacy in the treatment of experimental Pc in albino rats. Oxygen metabolism and LP being intrinsically linked to systemic acid-alkaline state, the indices which characterize these three homeostasis links have been taken as research objects.

MATERIALS AND METHODS

72 albino male Wistar rats (200-250 g) were used. Dust accumulation in the pulmonary tissue as a precondition of any Pc, mechanisms and quantitative regularities of dust particles sedimentation, as well as their elimination from the lungs and protracted stay there, are of the same type [8]. Therefore, simulation of a certain Pc type suggests that pathologic changes obtained are characteristic for all Pc types. Pc simulation was performed on our own modification of Nykolenko methodology [7] in the following way: the animals, fixed on the back, had 1 ml of dust suspension, containing 50 mg of coal dust, injected intratracheally into the glottis through the auricular otolaryngological funnel, with blunted 10 cm needle under superficial ether narcosis. After injection, a rat was promptly positioned vertically, the entire procedure lasting 2-4 minutes.

In order to make coal suspension, highly biotoxic black coal that had been mined at the pits of Lviv region was used. The terms of animals' priming with coal dust were determined, based on the following considerations. The average length of a lab rat life cycle is about 36 months or 3 years that is roughly correlated to 72-year human age as 1:24. Based on this extrapolation, 1 week of rat life is comparable on the average to 6 months of human life, and 20-year human age prior to working at the mine roughly matches 8-month rat age. In our research, the term of animals' priming with coal dust was 1, 3, 6, and 12 weeks, that is appropriate to correlate with 0.5, 1.5, 3.0, and 6-year man's work at the mine, respectively. These terms match early Pc manifestations. Strict adherence to the regulations of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) [9] was provided. The rats were kept in Fengshi plastic cages, 4 in each, on the litter of

wooden chips. Light conditions: 12 hours – light, 12 hours – dark. Temperature regime – 19-25° C. Relative humidity – 50-70%. Temperature and humidity indices were taken daily. Ventilation was controlled with the anemometer and by measuring carbon dioxide and ammonia air content. Ventilation regime, providing 15 room volumes per hour, carbon dioxide concentration up to 0.15 volume percent, and ammonia– up to 0.001 mg/l, was set. The rats were fed twice a day, water available ad libitum.

The animals were blindly randomized into 9 groups (n=8):

- intact rats;
- 1-week priming with coal dust;
- 3-week priming;
- 6-week priming;
- 12-week priming.

Group 6 comprised the animals that were being treated with Es (3 mg/kg intraperitoneally) in the last 3 days, at the background of 1-week dust priming. In Group 7, treatment with Es (in the same dose) was carried out in the last 3 days, following 3-week priming. In Group 8, animals were treated at the background of 6-week priming, and in Group 9 – at the background of 12-week priming. “Elara”-manufactured Es was used. The study was undertaken 1 day after last priming and last Es administration. Blood pH, PaO₂, PaCO₂ in the arterial blood, SaO₂, and BE (Base-Excess) in the arterial blood were studied with EasyBloodGas. The blood content of LP markers: malondialdehyde (MDA), as well as of conjugated dienes (DC) and trienes (TC) was determined [10].

To collect blood samples, the animals were anaesthetized with intraperitoneal propofol injection (4 mg/kg), and then laparotomized. The blood was taken through diaphragm puncture from the abdominal cavity and left ventricle. Then the wound in the abdomen was sutured up and the rats were put down with intraperitoneal propofol injection (10 mg/kg). Strict adherence to the regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the protection of animals was provided [11]. Clearance from the bioethics commission of Ternopil State Medical University was got.

Mean arithmetic value (M) and standard deviation (m) were calculated. Source data having normal distribution, the Student’s t-test was used to determine statistical significance of various mean values. Levels of statistical significance were calculated. Changes were regarded as reliable at p<0.001. MicrosoftExcel XP (USA) and Statsoft STATISTICA program were used for calculations.

RESULTS AND DISCUSSION

Most indices of oxygen and CO₂ metabolism, as well as of acid-alkaline balance in experimental rats were found to depend on the exposure to coal dust priming (Table 1).

The tendency for the decrease of partial blood pressure in the arterial blood in all groups was observed, the value depending on the period of exposure to the dust. One-week exposure resulted in 11.77 mm Hg decrease of the partial pressure, whereas for 3, 6, and 12 weeks reduction values were 14.11 mm Hg, 14.75 mm Hg, and 22.85 mm Hg, respectively. The group with 12-week exposure to the dust revealed the most evident signs of hypoxia, the values of saturation and partial pressure being equal to 91% and 71 mm Hg, that is typical for pronounced hypoxia. Partial blood pressure decrease in the arterial blood was paralleled by decreased value of oxygen haemoglobin saturation. One-week exposure decreased the index of arterial blood saturation by 4.1%, whereas for 3, 6, and 12-week exposure the values were 4.9, 5.0, and 6.4%, respectively. 60 mm Hg is regarded as a critical value, roughly corresponding to the saturation index of 90%. Decrease of the partial oxygen pressure and saturation below these values is characteristic of hypoxia, requiring artificial lung ventilation.

Partial pressure of CO₂ in the arterial blood also tended to reduction, depending on the length of exposure to the dust. One-week exposure resulted in 6.62 mmHg decrease of the partial CO₂ pressure, whereas for 3, 6, and 12 weeks reduction values were 11.05 mm Hg, 11.77 mm Hg, and 12.9 mm Hg, respectively. Normally, PaCO₂ is within 36-44 mmHg, increase or decline indicating respiratory disorders.

Alveolar hyperventilation is accompanied by PaCO₂ reduction and respiratory alkalosis, whereas alveolar hypoventilation – by PaCO₂ increase (arterial hypercarbia) and respiratory acidosis. PaCO₂ changes can be attributed to hyperventilation owing to hypoxia, caused by Pc development.

Exposure to the coal dust caused dose-dependent tendency for blood pH increase. Animals with one- and three-week exposure showed normal blood pH values, whereas after six- and twelve-week exposure they conformed to the values of compensated respiratory alkalosis. One-week exposure resulted in pH index increase by 0.03 units, three-week – by 0.07, six-week – by 0.1, and twelve-week - by 0.14 units. While being in the normal range after one- and three-week exposure to the dust, after six- and twelve-week exposure pH values were similar to those in compensated alkalosis. Generally, respiratory alkalosis is caused by hyperventilation. Evidently, it was observed in

the animals with simulated Pc. Increase of base deficiency BE (Base-Excess) index is a compensatory systemic reaction on respiratory alkalosis. Normally, it equals to "0", acceptable range of fluctuations being ± 2.3 mmol/l. With buffer bases increasing BE (Base-Excess) value becomes positive (base excess), when decreasing – negative (base deficiency). BE (Base-Excess) value is the most informative index of acid-base metabolic disorders. Base deficiency beyond acceptable range of fluctuations is indicative of metabolic acidosis, whereas base excess indicates metabolic alkalosis [12]. All the groups with simulated Pc revealed reliable ($P < 0.001$) decrease in the index of buffer base deficiency BE as compared with intact animals, in proportion to the period of exposure to the coal dust. Animals with one-week exposure to the dust revealed 1.78 times decrease in BE index, whereas for 3-6- and 12-week exposure the values were 1.91, 2.02, and 2.27 times, respectively. BE values in all groups were beyond normal and typical of metabolic acidosis.

Development of experimental Pc is accompanied by marked and reliable increase in the LP activity. After one-week exposure to the dust, DC blood content increased 3 times, whereas for 3-6- and 12-week exposure the values were 3.41, 4.42, and 5.33 times, respectively. Meanwhile, MDA content after 1-3-6-12-week exposure increased 1.69, 2.47, 2.52, and 3.50 times, respectively.

Application of Es normalized the changed indices of O_2 and CO_2 exchange as well as those of LP activity. Antihypoxant properties of the drug were revealed in the increase of PaO_2 in the arterial blood, the efficacy of Es growing with increasing dust exposure period. Application of Es after one-week exposure to the dust increased PaO_2 value by 4.25 mm Hg, whereas treatment of 12-week exposure increased the value by 11.25 mmHg. Similar changes were observed when assessing the changes in arterial blood saturation. Treatment of the animals after one-week exposure to the dust provided 1.12% increase in oxygen haemoglobin saturation, whereas for 3-6- and 12-week exposure the values were 1.5, 0.94, and 2.25 %, respectively.

Application of Es contributed to $PaCO_2$ normalization, the indices remaining within normal physiologic range. Blood pH value at the background of Es use was within normal range. In the Es-treated groups, pH index after 6- and 12-week exposure to the dust decreased by 0.06 and 0.09 units, respectively, as compared to the untreated animals. Since pH is a logarithm from the concentration of hydrogen ions, pH change by one unit implies tenfold change in the concentration of hydrogen ions. Changes of pH more than by 0.4 ($pH < 7.0$ and > 7.8) are considered to be incompatible with life [13]. Therefore, changes of

pH by 0.09 towards acidity normalization should be regarded as essential enough.

Similar changes occurred with the value of base deficiency BE. In the treated groups the value was within physiological standard. After one-week exposure to the dust, application of Es resulted in 1.95 times increase of BE index, whereas after three-week exposure the index increased by 1.44 times.

Application of Es lead to reliable decrease in LP activity. DC content at the background of treatment after 1-3-6-12-week exposure to the dust decreased 2.0, 1.78, 1.71, and 1.83 times. Decrease of MDA content was by 42.0, 36.52, 35.54, and 42.18 % in the groups 5, 7, 9, and 11, respectively, changes in the TC content being similar.

To understand better the mechanisms of Es therapeutic effect, Pc pathogenesis should be recalled. Some dust particles remain in the tissue of interalveolar septa or are carried through lymphatic ways into perivascular and peribronchial tissue, under pleura, into intrapulmonary lymph ducts, and extra pulmonary lymph nodes. First, dust particles are phagocytosed by macrophages, which then die. The rate of macrophages death is directly proportional to fibrogenic dust aggressiveness [14]. Then repeated phagocytosis of dust particles by the other macrophages occur, followed by their death. Immune restructuring develops with immunologic reactions of cellular and types (B- and T- lymphocytic) prevailing [15]. LP activation, as a universal mechanism of cellular structure damage, plays a major role in the processes of pulmonary tissue and lymph system damage, mentioned above. Damage to the cell membrane, resulting from LP intensification, is followed by the injury of both alveolar structures and lung connective tissue framework.

Presumably, development of experimental Pc in the animals resulted in the pulmonary tissue damage that lead to hypoxia of different degree: from mild in the animals with one-week exposure to dust – to severe in twelve-week exposure. Hyperventilation is the first compensatory systemic response to hypoxia. At the early stages of Pc, hyperventilation causes decline of blood $PaCO_2$ and pH respiratory alkalosis occurs. The body tries to compensate for it by the changes in the buffer system activity, which leads to compensatory metabolic acidosis. Ultimately, respiratory alkalosis occurs, partly compensated by metabolic acidosis. If the terms of experimental Pc are extrapolated to the humans, it can be said that research findings confirm formation of the disease in 3-5 years of work, sometimes in 1-2 years.

Our findings indicate that Pc manifestations depend on the period of exposure to dust. Processes of pulmonary tissue damage correlated with intensification of LP processes.

Blood LP activity and intensity of the disease manifestations were directly related to the length of exposure to dust.

Table 1. Effect of ethylmethylhydroxypyridine succinate on the indices of acid-alkaline state, oxygen balance, and lipid peroxidation in albino rats at the background of simulated pneumoconiosis

Period of exposure to coal dust suspension	Group	pH, unit	Pa CO ₂ , mm Hg	BE, mmol/l	SaO ₂ , %	paO ₂ , mm Hg	MDA mmol/l	DC, mcmol/l	TC, mcmol/l
	Intact rats	7.35 ± 0.05	40.2 ± 4.01	1.6 ± 0.18	98.02 ± 0.95	89.0 ± 2.4	1.44 ± 0.10	0.12 ± 0.01	0.11 ± 0.01
1 week	Pneumoconiosis	7.38 ± 0.03	33.58 ± 2.36	-2.84 ± 0.15*	94.00 ± 1.00	77.23 ± 2.49	2.43 ± 0.05*	0.36 ± 0.02*	0.27 ± 0.03*
	Pneumoconiosis + Es	7.36 ± 0.01	35.89 ± 1.90	-1.45 ± 0.26***	95.12 ± 2.08	81.48 ± 3.02	1.41 ± 0.10**	0.18 ± 0.02***	0.16 ± 0.02***
3 weeks	Pneumoconiosis	7.42 ± 0.05	29.15 ± 1.17	-3.06 ± 0.31*	93.25 ± 1.19	74.89 ± 2.33	3.56 ± 0.26*	0.41 ± 0.04*	0.35 ± 0.01*
	Pneumoconiosis + Es	7.38 ± 0.02	33.35 ± 2.35	-2.12 ± 0.19***	94.75 ± 0.96	78.08 ± 1.36	2.26 ± 0.19***	0.23 ± 0.05***	0.18 ± 0.01***
6 weeks	Pneumoconiosis	7.45 ± 0.03	28.43 ± 0.60	-3.23 ± 0.17*	93.12 ± 0.92	74.25 ± 0.71	3.63 ± 0.25*	0.53 ± 0.11*	0.38 ± 0.02*
	Pneumoconiosis + Es	7.39 ± 0.02	32.33 ± 1.33	-2.53 ± 0.06***	94.06 ± 0.76	77.12 ± 2.32	2.34 ± 0.12***	0.31 ± 0.02***	0.21 ± 0.02***
12 weeks	Pneumoconiosis	7.49 ± 0.03	27.3 ± 0.65	-3.63 ± 0.12*	91.0 ± 1.00	66.15 ± 1.06	5.05 ± 0.30*	0.64 ± 0.09*	0.62 ± 0.10*
	Pneumoconiosis + Es	7.40 ± 0.04	31.2 ± 0.09	-2.85 ± 0.13***	94.00 ± 0.86	77.40 ± 2.72	2.92 ± 0.10***	0.35 ± 0.03***	0.32 ± 0.03***

Footnotes: *-reliable (P < 0.001) changes regarding control animals; **- reliable (P < 0.001) changes regarding untreated rats in the corresponding group.

Application of Es reliably decreased the content of all studied LP markers in the blood serum. As an antioxidant, Es declines LP activity, and as an antihypoxant– contributes to decreased systemic oxygen consumption. Oxygen demand decreases, whereas oxygen saturation of haemoglobin and partial oxygen pressure in the blood increase. Positive changes in the oxygen metabolism cause normalization of acid-alkaline state, that manifests in decreasing pH and increasing BE.

The body possesses the complex of specific cell, tissue, and systemic mechanisms which resist to hypoxia, the latter resulting in significant Lpactivation [16]. Pc patients’ systemic adaptation to hypoxia is largely due to the complex of intracellular antioxidant enzyme systems, resisting to the oxidative stress and neutralizing active oxygen forms. Antioxidants break the chains of molecules in free radical oxidation reactions and destroy peroxide molecules, thus restricting the activity of free radicals.

Antihypoxant and antioxidant therapy in conditions of LP initiation produces pronounced energy correction effect on the body organs and systems. Therapeutic strategy, based on the principles of energy correction, enables to balance cell energy properties and to decrease intensity of hypoxic disorders. Today, this strategy is a highly effective method, enabling to decrease body oxygen consumption. Energy correction makes possible to activate protective mechanisms, increasing resistance to the oxidant stress through the activation of own antioxidant systems.

In recent years, close attention has been paid to the antioxidant and antihypoxant effect of succinic acid, which is a universal metabolite [17].As a derivative of succinic acid, Es reveals antihypoxicand antioxidant activity. Acting as catalysers to the Krebs cycle, succinates decrease concentrations of lactate, pyruvate, and citrate which accumulate at the early stages of hypoxia. Phenomenon of rapid succinate oxidation by succinate dehydrogenase is called

“monopolization of the respiratory chain” which biological value is involvement in rapid ATP resynthesis [18]. Antihypoxic succinate effects are associated with activation of succinate dehydrogenase oxidation and reactivation of cytochrome oxidase which is the key enzyme of respiratory chain. Succinates reduce blood concentration of lactate due to glycolysis products smoothly passing into the tricarboxylic acid cycle. Besides, they decrease blood concentrations of pyruvate and citrate, reactivate cytochrome oxidase, and normalize histamine and serotonin content by improving microcirculation.

Our positive findings, concerning oxygen and carbon dioxide metabolism, acid-alkaline balance, and decreased LP activity can be attributed to the above-mentioned mechanisms of Es therapeutic effect. Pc is both incurable and irrevocable, the list of medicines for the treatment of Pc-induced hypoxia being limited. Therapeutic application of a drug with antioxidant and antihypoxant characteristics that has not been used before is crucial and capable of improving the life quality of the patients involved. The data obtained may provide a basis for the further research on the use of the drugs with antioxidant and antihypoxant properties in the comprehensive treatment of the patients with Pc.

CONCLUSIONS

1. Simulation of pneumoconiosis in albino rats was accompanied by the development of hypoxic hypoxia, respiratory alkalosis, and increasing activity of lipid peroxidation processes that was manifested in the decline of arterial blood saturation index to 91.0%, of partial oxygen pressure in the blood – to 66.15 mmHg, and 3.05-5.3 times increase in the content of DC, TC, and MDA.
2. Application of ethylmethylhydroxypyridine succinate in a daily dose of 3.0 mg/kg for three days in the rats with experimental pneumoconiosis was found to decrease signs of hypoxia, to normalize acid-alkaline metabolism and lipid peroxidation activity that in the animals with simulated pneumoconiosis of various duration showed itself in the increase of arterial blood saturation by 1.21-3.0%, partial oxygen pressure in the blood – by 3.18-11.25 mm Hg, alongside with 1.28-1.95 times increase in base deficiency BE and 1.61-2.0 decrease in the blood content of diene conjugates and MDA.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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