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The Inflammatory Response of Respiratory System to Metal Nanoparticle Exposure and Its Suppression by Redox Active Agent and Cytokine Therapy

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1. Introduction

Engineered nanoparticles (NPs) are increasingly being developed for needs of electronic, pharmaceutical and chemical industry. Manufactured NPs as a specific subset of ultrafine particles being suspended in air may pose a hazard to human health. Exposures to nanosized airborne metals facilitate respiratory irritation and lung inflammation. The lung is the target portal of environmental and engineered nanopollutants that lead to exacerbation of respiratory diseases, increased risk of infection, allergy and cardiopulmonary mortality. Inflammatory challenge causes hypoxia of tissues. Potential risks of widespread nanoengineered products should be carefully evaluated. The new nanoproducts for therapeutic and diagnostic applications were emerged in pharmaceutical market. The safety and potential hazards of new nanomaterials are not examined properly. Revelation of indicators of pathophysiological response determinants in the reactivity of respiratory tracts is of a great significance, on one hand, for understanding the mechanisms of nanotoxicity of new ultradispersed materials and, on the other hand, for improvement of clinical-diagnostic procedures of studying lung cancer by magnetic-resonance imaging technique (MRI) (Rinck, 2001). The diagnostic efficiency of MRI in elucidation of malignant feature of lung lesion can be improved with the use of contrast agents. The variety of contrast agents for MRI involves a series of preparations on the basis of magnetic NPs of iron oxides. The iron-containing contrast agents are widely used in clinical diagnostics of liver and spleen diseases and in angioplastics due to low toxicity of iron NPs, their ability for prolonged existence in blood flow, and high nonspecific ability of reticular-endothelium system of liver to absorb the dispersed particles (Wang et al., 2001). A contrast agent for increasing contrast of lung tissue in the MRI technique can be delivered by inhalation through respiratory airways. Successful clinical trials of gadolinium chelate inhalation showed a possibility of scanning time reduction in the MRI experiment (Haage et al., 2005). Further development of inhalation procedure of contrasting is governed by the knowledge of the contra-indication pattern, doses, and the scheme of preparation administration. A wide application of iron-containing contrast agents in diagnostics of lung pathology by NMR tomography is determined by

magnetic characteristics of contrast agent (Wang et al., 2001; Gubin et al., 2005), possibility of direct clinical administration of contrast agent into lung tissue (Limbach, 2007; Oberdörster G., et al 2005), safety of contrast agent, its biological availability, and physiological response of bronchoalveolar system to the influence of dispersion agent (Li et al., 1999; Gupta et al., 2005; Oberdörster et al., 2000; Tran et al., 2000).

Pulmonary inflammation is known to be controlled by a complex network of cellular and humoral mediators that have pro-inflammatory and regulatory anti-inflammatory functions. The main cellular components in NPs injury are neutrophils and macrophages and the vast number of antibody mediators include cytokines, reactive oxygen species, electron-transport proteins and proteases. The pro-inflammatory cytokine interleukin-1 beta (IL-1β) is produced by alveolar macrophages and neutrophils in response to iron NPs challenge (Simbirtsev, 2011). This cytokine is crucial in the cytokine cascade events because it activates other inflammation stages. IL-1β promotes the movement of neutrophils by augmenting interaction between neutrophils and endothelial cells and increasing capillary leak. Another cytokine as IL-1β receptor antagonist may play an essential role in decreasing inflammatory reactions of certain respiratory states associated with acute inflammation induced by NPs. The animals which have got IL-1β intratracheally rapidly develop lung neutrophil accumulation and neutrophil-dependent acute edemateous lung leak which may be decreased by intravenous administration of IL-1β receptor antagonist. Intratracheal instillation of IL-1β receptor antagonist has been shown to lessen acute lung inflammation. Aerosol delivery of IL-1β antagonist may prevent pneumonia by impeding bacterial access and reducing inflammation phenomena in pulmonary tract. For anti-inflammatory treatment caused by impact of NPs the interleukin-1 receptor antagonist (IL-1ra) was applied. Recent studies had shown that IL-1ra may play an essential role in decreasing inflammatory reactions of certain respiratory disorders induced by administration of metal dust powder (Danilov, 2003). IL-1β may decrease inflammation in the hypoxia cases which as a rule typical for NPs aerosol exposures. Hypoxia transforms the endothelium toward a pro-inflammatory phenotype and enhance leukocyte adhesion on airway walls provoking pathological conditions of vascular remodeling and ischemia–reperfusion injury.

Efficient immunotherapy of inflammatory impact by NPs is energy consumptive procedure. Activation of alveolar macrophages and neutrophils induced by phagocytosis of NPs demands high ATP-dependent energy supply (Buttgereit et al., 2000). Specific immune functions mediated by IL-1 as migration diseases associated with infection and acute inflammation. Aerosol administration of IL-1ra may be employed for efficient delivery of IL-1ra to the bronchoalveolar space versus instillation procedure. Direct delivery of IL-1ra to the target site of lung in a high ratio of local to systemic bioavailability results in the decrease of an effective drug dose. However, the clinical efficiency of antagonist IL-1 of leucocytes, cytokinesis, phagocytosis, antigen processing and its presentation and synthesis of antibodies, cytotoxicity, regulatory functions consume large amounts of nucleoside triphosphate (ATP). Immunocompetent cells affiliated to IL-1 and its antagonist require much energy to maintain cellular integrity and basal metabolism (Dziurla et al., 2010). Most of their specific immune functions directly or indirectly use ATP or other high-energy nucleoside phosphates. Active cell respiration provides free energy in the chemical form of ATP and electrical transmembrane potential (Nicholls, 2000). Oxidative phosphorylation and glycolysis in mitochondria ensures proper functioning of immune cells as lymphocytes.

Mitochondria support the energy-dependent regulation of assorted cell functions, including intermediary metabolism, ion regulation and cell motility, cell proliferation through processes of oxidative phosphorylation and glycolysis. In active metabolic state respiratory cells derive up dominant energy through oxidative phosphorylation. But inflammation leads to decrease of main oxidative substrates in tissue, oxygen and glucose and fall down of synthesis of energy rich compounds ATP, NADH. Oxygen deficiency during inflammatory cause of NPs challenge may produce deep changes in cellular metabolism that may lead to tissue failure. Hypoxia and lack of glucose affect on cellular energy metabolism and on cytokine secretion in stimulated human CD4+ T lymphocytes (Dziurla et al., 2010). Therefore, bioenergetic aspect of cell functioning is very essential for treatment of cell injury by cytokine therapy.

The central point of energy conversion in injured cells under hypoxic stress is the transfer of electron via electron-transport chain (ETC) to final oxygen acceptor coupled with generation proton gradient across mitochondrial membrane. Transmembrane electrical potential $\Delta\psi$ is the measure of cell energetic activity (Skulachev, 1996). Study of metabolic adaptation to hypoxia stress displayed the key role of membrane potential $\Delta\psi$ as the indicator and regulator of metabolic cell activity. The absolute value of transmembrane potential $\Delta\psi$ depends on the physiology of cell and can be easily evaluated by electrode method. In respiratory systems of cells quinone can operate as donor-acceptor junction sites for transfer electrons between redox active enzymes. Quinones are membrane-entrapped redox-active entities that carry 2 electrons and 2 protons in the quinol state. Synthesized species of quinones with different electrochemical potential are able to repair the defected sites of ETC after inflammatory influence of metal NPs. The pharmaceutical “Oliphen” belongs to the class quinones with redox properties compatible to ETC. Oliphen is the mixture of poly-(2,5-dehydro-oxy-phenylene-4)-thiosulfonate sodium redox oligomers with median molecular mass 300-500 D. The trade names of medicines containing oliphen are «Oliphen», «Olifen», «Hipoxen». Redox properties of oliphen are linked with quinone and thiosulfate residues in their molecular structure. Oliphen activates ETC of cells shunting the possible molecular disorders of oxidative reactions in mitochondria. Oliphen enhances the resistance to extreme conditions such as oxygen stress and ischemia. The preparation “Oliphen” is a prescribed medicine for correcting defects of mitochondrial electron-transport chain associated with inflammatory state. The application of redox agent “Oliphen” is proposed to favour the therapy efficiency of ethiotropic preparations of receptor antagonist IL-1β. To be sure in benefits of redox curing of mitochondrial defects of ETC by oliphen in treating tissue hypoxia followed after metal NPs exposure the present mouse model study is aimed to determine antihypoxic efficacy of aerosol oliphen exposures.

Here we present the results of studying the effect of magnetic iron NPs on the bronchoalveolar system of mouse at cellular and biochemical levels in inhalation contact with mucosa (Nikolaev, 2009). Intratracheal instillation was used as well-known standard procedure for evaluation of respiratory toxicity of particles. The iron NPs for intratracheal administration were prepared by gas-phase synthesis and precipitation from suspensions. The ability of the samples for contrasting of magnetic resonance images was tested based on the line broadening of ^{1}H NMR spectra of aqueous iron suspensions in vitro.

The response of bronchoalveolar system to iron NPs was studied based on variations in some characteristics of bronchoalveolar lavage (BAL). A special attention was given to anti-

inflammatory activity of the preparation as a factor of its inhalation application. The level of anti-inflammatory activity was estimated from the protein concentration, content of neutrophils, and methabolic fingerprints of cellular respiration elucidated using ^{1}H NMR of supernatant obtained after centrifuging of BAL. The metabolic activity of immunecompetent cells in BAL was assayed by measurement of trans membrane potential $\Delta\psi$ and redox potential (ORP). The choice of these parameters for characterization of inflammation response and hypoxic state was based on the previous study of anti-inflammatory activity of intranasal instillations of bacterial lipopolysaccharides (Ischenko et al., 2007). This study showed that mouse pneumonia causes neutrophil recruitment into alveoli, an increase in the total cell content of BAL, generation of active oxygen species, and variation in the metabolic activity of bronchoalveolar lavage fluid (BALF). To study the characteristics of inflammatory response we used microscopy, high performance liquid chromatography (HPLC), gel electrophoresis, ^{1}H NMR spectroscopy, and various methods of estimating size and concentration of finely dispersed preparations.

2. Materials and methods

2.1 Animal handling and study design

The reactivity of bronchoalveolar system was studied using white outbred mice (n=20) and mice of the C57BL/6 line (n=25). As the preparation for the current study, the mice were kept in vivarium with ordinary food and water ad libitum. A single dose of inflammation inducer in the form of iron NPs (1 mg per mouse) suspended in isotonic sodium chloride solution (0.9% w/v) was administered to a group of anesthetized mice through trachea with the use of a DP-4M aerosol microsyringe (Penn Century, the United States). Before using, an aerosol nebulizer was checked for reproducibility of dosage and fractional composition of the aerosol. After intratracheal administration of iron NPs the mice were killed by cervical dislocation and the thorax was dissected. To obtain 4 ml of lavage, four 1-ml portions of 0.15 M NaCl solution were administered into the free trachea. The cellular component of BAL fluid was separated by centrifuging at 1700 rpm for 5 min and analyzed immediately after preparation. The samples for subsequent studies were kept at 4°C. The inflammatory response of bronchopulmonary system of mice was estimated within 1, 3, and 7 days after inhalation contact with iron NPs from the variation of the relative content of cellular component in BAL. The inflammatory response of mouse bronchopulmonary system was monitored after 3 days from the reliable difference in the characteristics of BAL fluids obtained from intact and infected mice.

The anti-inflammatory treatment by human IL-1ra was studied upon delivery of the aerosol formulation of the drug to mice with acute pulmonary inflammation, induced by iron NPs. IL-1ra was produced by recombinant gene technology in State Research Institute of Highly Pure Biopreparations (Federal Medical –Biological Agency, Russia). The protein IL-1ra (99% purity) had been isolated from E.coli BL21 in solution. The structural identity of IL-1ra to native protein was confirmed by gel electrophoresis and HPLC data.

Redox active drugs were delivered into upper and lower parts of respiratory tract in the form of dry and droplike aerosols. Oliphen was used in the form of preparation “Oliphen” (SRR” Oliphen”, Saint-Petersburg, № PN000125/01-2000). Its efficacy was compared with reference drugs: sodium succinate (Reachem), sodium oxybutirate (Reachem). The dosage

exposure was selected in conformity with therapeutical effective concentrations achieved by intravenous and peroral administration to humans. To get the respirable medicinal liquid aerosol the jet and ultrasonic nebulizers were applied. The particle-size analysis was monitored by photo-electric counter combined with microcomputer PDP-11. A mass median aerodynamic diameter (MMAD) of droplets was no more than 5 mkm. The aerosol concentration was determined by measuring the mass of deposited particles on the filters or microcyclones (impingers).

The acceptance and compliance of dry powder inhalations for redox agent treatments was accessed in original inhalation system. The inhalation system consisted of pneumatic glass generator (unitary dose 50-250 mg and MMAD 2.5-4 mkm). Micronized powder formulation for mice inhalation was prepared by air-jet milling with inert excipients. Aspiration doses for mice were calculated in approach with coefficient of aerosol deposition 0.88 MMAD 3 mkm and polydispersity square deviation 0.65. The mice were exposed to aerosol therapy for prechallenged terms 10, 30, 60 min. The aerosol inhalations occurred once a day during 1, 3, 5 day curse. Aspiration doses were 0,03 -0,06 mkg/mouse.

For aerosol inhalation exposure of mice by antihypoxic drugs the specially constructed dynamic and static chambers were applied. Groups of 4, 10 mice were placed in chambers, containing special cages to prevent any possibility to get drug from external hair surfaces of animals.

Survival in groups of mice after aerosol exposure to antihypoxic agents was studied in model of acute normobaric hypoxia. Outbred white mice (20-25 g) were used. Animals were placed in the transparent plastic box with volume 30 l with an inlet and outlet through which the nitrogen flowed. Hypoxia was induced by continuous flushing N_2 up to partial pressure pO_2 4.2% during 10 min. The O_2 concentration was monitored with an in-line oxygen analyzer (Clark's electrode and registration block). The final CO_2 level was 20% according measurements of IR absorption of hypoxic gas. The whole number of mice that have received an acute hypoxia challenge and aerosol therapy was 100.

2.2 Synthesis of iron magnetic NPs

Iron NPs were synthesized by two ways: continuous gas-phase synthesis (Kim et al., 2007; Vasilieva et al., 2006; Choi et al., 2002; Montagne et al., 2002) and precipitation from a suspension (Vidal-Vidal et al., 2006; Capek et al., 2004). NPs of iron oxides were synthesized by reduction of iron salts in a solution according to the procedure of suspension precipitation. NPs with an inner iron core were synthesized by pyrolysis of iron pentacarbonyl in a flow-type reactor in the medium of anhydrous carbon oxide at atmospheric pressure (Vasilieva et al., 2006; Choi et al., 2002). The fractional composition of the resulting powders was estimated using light and electron microscopy. The average particle size was approximately 30 nm. Iron NPs were sphere-shaped and formed filamentary structures due to the strong magnetic interaction between spheres. The powder sample NPs consisted of NPs with inner iron core 30 nm in diameter covered with outer Fe_3O_4 shell (2-3 nm). The physicochemical characteristics of iron NPs are given in details in (Kim et al., 2007; Vasilieva et al., 2006; Choi et al., 2002). The magnetic characteristics of iron NPs were observed from the abrupt reduction of transverse nuclear magnetic relaxation time T_2 of solvent in NPs suspension.

2.3 NMR experiment

The samples of BAL fluids were transferred to standard NMR tubes 5 mm in diameter. The ^{1}H NMR spectra of the samples of BAL fluids were recorded at room temperature on Bruker CXP 300 spectrometer operating at 300 MHz. To detect weak signals of metabolites the large signal of water was suppressed by homonuclear irradiation at the frequency of water resonance using one pulse sequence. The number of acquisition was varied from 500 to 2000. No exponential weighting function was applied to the free induction decay before Fourier transformation. Chemical shifts were measured relative to the signal of ammonium formate at 8.50 ppm; the error in determination of chemical shifts did not exceed 0.02 ppm. The 4.5-6.0 ppm spectral region was removed to eliminate water signal. The concentration of a metabolite was determined from the ratio of integral intensities of the corresponding metabolite signal and the signal of ammonium formate.

2.4 The bronchoalveolar lavage analysis by HPLC

The samples of BAL fluids from exposed mice, mouse serum and one sample of IL-1ra were prepared for HPLC. The total protein was measured by Lowry assay. The bronchoalveolar lavage analysis was realized by reversed-phase HPLC with conditions listed below: chromatograph HP-1090 Hewlett–Packard, USA; column "Jupiter" Phenomenex C-18 (5 µm, length 250 mm, internal diameter 4.6 mm), USA; solvent system gradient, 0.1 % trifluoroacetic acid/acetonitrile, 30% to 70% acetonitrile in 15 minutes; temperature 35°C; flow rate 1.5 ml/min; detection: 220, 254, 280 nm; injection volume 50 µl.

2.5 Polyacrylamide gel electrophoresis

The proteins of BALF from exposed and nonexposed mice were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). We used 5% (w/v) stacking gel in Tris–HCl buffer pH 6.8 and 12.5% (w/v) separating gel in pH 8.8 Tris–HCl buffer. The BALF samples were prepared by dilution 1:1 in Tris–HCl buffer 50 mM pH 6.8 with 70 mM sodium dodecyl sulfate (SDS), 1.4 M glycerol, 100 mM dithiothreitol and 0.01 mM bromophenol blue. After 5 min of heating at 100°C, samples were pipetted on gels. Electrophoresis was carried out in a Tris-glycine buffer, pH 8.3, containing 25 mM Tris, 250 mM glycine, 3.5 mM SDS, at a constant power of 50 W. After electrophoresis, gels were stained with Coomassie blue G-250 0.1% (w/v), for 2 h, and then de-stained with 4% (v/v) perchloric acid and 50% (v/v) ethanol solutions. Protein patterns in the gels were recorded as digitalized images and analyzed by Gel Analyzer (MediaCybernetics). Molecular mass of proteins was determined according to molecular weight marker (PageRuler™ Unstained Protein Ladder, Fermentas).

2.6 Transmembrane (Δψ) and redox (ORP) potentials

Δψ of bacterial and red blood cells were assayed by the distribution of the penetrating tetraphenylphosphonium (TPP^{+}) cation between the cytoplasm and the external solution using an ion-selective electrode. Sorption TPP+ by cells was estimated as decrease of its concentration in external media. After washing in physiological solution cell suspensions were added to Tris-HCl buffer medium containing 10 mkM TPP^{+}. Electrode readings were calibrated by TPP^{+} solutions with variable concentration. Transmembrane potential Δψ was calculated using Nernst equation (1) (Skulachev, 1996).

$$\Delta\psi = -RT/zF \times \ln(TPP_{+in}/TPP_{+ext}) \quad (1)$$

The functioning of electron transport chain (ETC) of E.coli cells and the membrane permeability of mouse blood red cells in the presence of therapeutical doses of oliphen were investigated. The estimation of bacterial respiration activity was made with the Clark oxygen electrode. Anaerobic state of cells was achieved by blowing through of nitrogen gas. In the anaerobic state no inhibition of oxidize-phosphorilation enzymes was registrated with the help of membrane potential by the mean of cation tetraphenylphosphonium absorption. Oliphen (0.01-0.1% w/v) caused the decrease of membrane potential and of the rate of cell respiration.

ORP (oxidation-reduction potential, E) of the reaction medium was measured by a platinum electrode (EPV-01, Gomel' Instrumentation Plant, Republic of Belarus') and Ag/Ag Cl reference electrode (ER-10103, Gomel' Instrumentation Plant, Republic of Belarus'). Molecular hydrogen release from bacteria was estimated by using the Orion pH electrode.

3. Results

Intratracheal iron NPs exposure provoked intense infiltration of lymphatic interstitium and blood-capillary net. Increase of alveolar-capillary permeability enhances the recruitment of immune cells (lymphocytes) to lung tissue. Iron NPs exposure of mice with the help of an aerosol microsyringe evoked a transient inflammatory increase of macrophages, neutrophils and lymphocytes number during one week. Acute inflammatory response assayed as relative percentage cell count in BAL at 1, 3, 7 day after instillation NPs is represented at Fig.1.

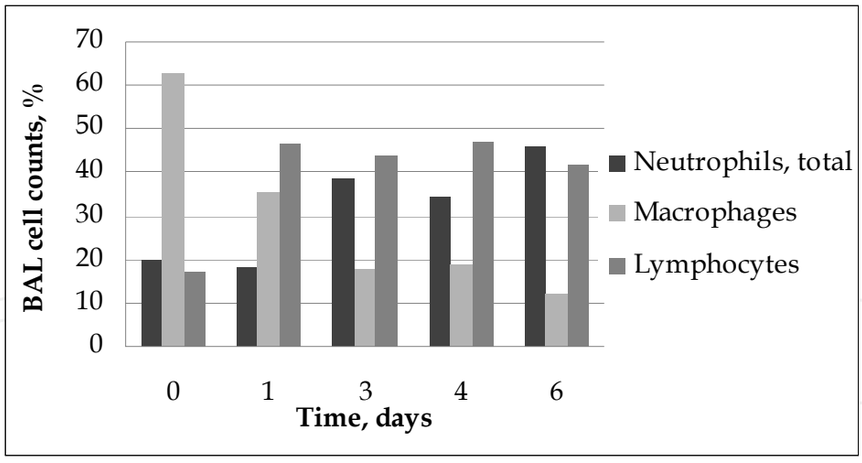

Fig. 1. Bronchoalveolar differential cell counts (macrophages, neutrophils and lymphocytes, %) following NPs challenge (day 0).

The number influx of mature neutrophils from the third day is the robust indicative of acute lung inflammation. Transient fall down of relative macrophage number is the additional evidence of curtain cytotoxicity of iron NPs for macrophages in endocytosis stage. The nanotoxicity for cells of reticulum-endothelial system is implicated with bronchotracheal pathway of delivery of nanoparticulate agent. The observed high absorption of iron NPs by alveolar macrophages is consistent with preliminary results of study in vitro. The cell

cultures of human monocytes and macrophages of inbred rat RAW264.7 were reported to uptake superparamagnetic iron oxide NPs (Muller et al., 2007; Stroh et al., 2004) . The particles uptake activates the following cascade of events as the start function of macrophagal lysosomal enzymes, generation of reactive oxidative species (ROS) and signaling system of nuclear transcriptional factor NF-kB that controls expression of a wide variety of pro-inflammatory genes. These features are known to involve in general mechanism of acute inflammation caused not only by nanoparticulate magnetic substance but other external pollutant factors. The ability of micronized particles of various chemical nature and size to induce acute lung inflammation made in some reports supports this notion (Danilov et al., 2003; Siglienti et al., 2006; Fujiia et al., 2002; Schins et al., 2004; Donaldson et al., 2002; Wong et al., 2007).

The cellular profile of BAL compared to corresponding data for whole blood may be interpreted as evidence of general immunoregulatory reaction which develops during few days after NPs delivery to trachea. The data presented in Fig.1 and Table 1 lead to conclusion that inflammation study can be able to explore right from the third day after NPs challenge.

Days after NPs challenge	**Neutrophils, mature; %**	
	Blood	**BAL**
0 (control)	25.68	4.37
3	28.30	27.20
4	36.68	16.95
7	23.13	28.00

Table 1. The relative content of granulocytes in blood and BAL at different terms of inflammation.

The inflammation level was estimated by total cell number in BAL and differential content of macrophages, lymphocytes, neutrophils (total and mature). These results are presented in Table 2.

Characteristics of inflammation	**Control**	**Inflammation**
Total cells (x106/ml BAL)	0.01±0.00	0.05±0.01
Macrophages,%	50.1±10.3	17.5±7.3
Lymphocytes,%	31.8±5.3	23.8±4.8
Neutrophils, total; %	18.1±6.3	57.4±10.8
Neutrophils, mature; %	4.0±2.3	33.5±9.7

Table 2. Inflammation response of bronchoalveolar system to inhalative challenge by iron NPs according data of cell count in BAL.

The table data after 3-days post exposure intratracheal instillation of iron NPs are appeared to display acute lung inflammation accompanied by the relative 5 times increase of total number of neutrophils and 8 times increase of number of mature lung neutrophils. Accumulation of neutrophils in the BAL is the result of a breakdown of alveolar barrier and impaired lung gas exchange function.

The possible drawbacks of inhalative delivery of redox active agent oliphen had been checked by control of cell composition of BAL, blood and morphological examination of bronchopulmonary ducts. Acute hypoxia changed the cell profile of BAL fluid. Hypoxic challenge provoked lymphocyte influx thus increasing the relative content of lymphoid cells in BAL. No inflammation response or changes in relative quantity of cellular morphological forms in BAL were recorded either for oliphen or for hydroxybutyrate lung delivery both in normoxic and hypoxic conditions. Nontoxic doses determined by separate study of dose-effect dependence in aerosol experiments on BAL data for oliphen were not more than 0.3 mg. Inhalation of oliphen in total doses higher 0.3 mg resulted in toxic accumulative effects and shortage of life-span.

3.1 Proteome analysis of BAL after iron NPs challenge by HPLC and PAAGE

Protein leakage to bronchoalveolar space was assessed by proteome analysis. The results of estimation of total protein content in BALF are presented in Table 3.

Inflammation indicator	Control	Iron NPs administration
Total protein, μg/ml	89.3±3.0	171.3±44.7

Table 3. Influence of inhalative NPs challenge on total protein content in BALF. All values represent mean ± SEM from 5 independent mice in group, for level of significance ($p<0.05$) v.s control (mice without NPs) in accordance with Manna-Whitney and Student tests.

From analysis of total protein follows that iron NPs instilled to trachea give 2-fold increase in BALF total protein content. Altered protein composition of BALF of exposed mice was investigated by HPLC and PAAGE methods. There were two groups of mice (C57BL/6 line, n=10 and outbred mice, n=10), each group contained 5 mice treated with iron NPs and 5 nontreated (control) mice. The analysis of samples of bronchoalveolar lavage supernatants with inflammation caused by NPs at dose 1 mg/mouse in 0.1 ml 0.9 % sodium chloride solution showed the altered protein composition.

The samples numbers 06, 09 from NPs-exposed animals differ from control non exposed samples most prominently for certain as there are new peaks with retention times at ~ 6.8, 7.2, 7.6 min on chromatograms of these samples (Fig. 3, 4). Spectral analysis results showed that all peaks belong to proteins by nature that correlates with data of total protein content in these samples. The chromatogram of a mouse serum sample was made for protein peaks identification.

Comparison of chromatogram of a mouse serum sample with chromatograms of samples numbers 6, 9 leads to conclusion that main peaks retention times of bronchoalveolar lavage sample with inflammation and serum sample were identical. In accordance with samples with inflammation main peaks present serum proteins peaks themselves and don't have any relation to IL-1ra (Fig. 5).

The results obtained for C57BL/6 mice are shown in Fig. 6. The similar results were obtained for outbred mice. Data for outbred mice are not shown. BALF from control and inflammatory mice contain four main spots for proteins with molecular mass 82, 72, 55, 52

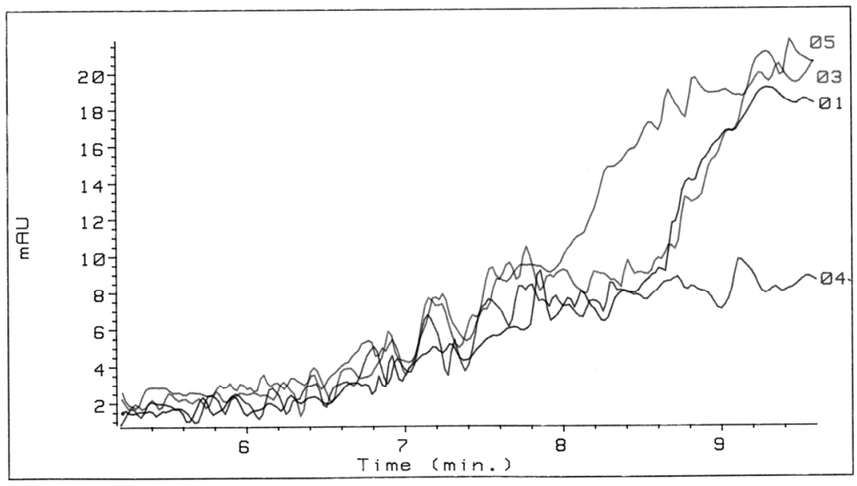

Fig. 3. Chromatograms of bronchoalveolar lavage fluids of C57BL/6 mice nontreated by NPs. 01-05 – BALF samples of nontreated mice (control); wavelength 220 nm.

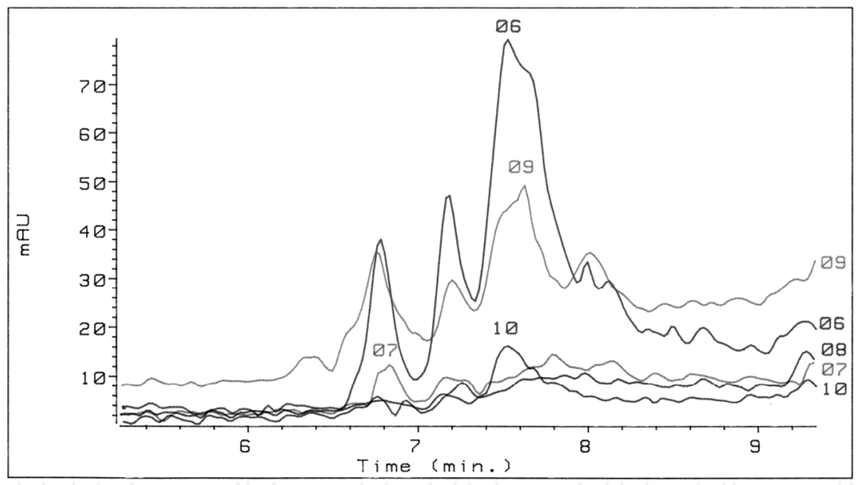

Fig. 4. Chromatograms of bronchoalveolar lavage fluid of C57BL/6 mice treated by NPs. 06-10 – BALF samples of treated mice (with inflammation); wavelength 220 nm.

kDa (Wattiez, R., 2005); furthermore, concentrations of these proteins in inflammatory samples are higher than those in control samples. In addition, one observed the presence of proteins with molecular mass 14, 25, 40 kDa in inflammatory samples. All the above proteins were found in the mouse plasma. The increase in the content of BALF proteins and emergence of low-molecular-mass proteins in BALF (in comparison with control samples) is apparently caused by the increase of capillaries permeability and passive diffusion of plasma proteins.

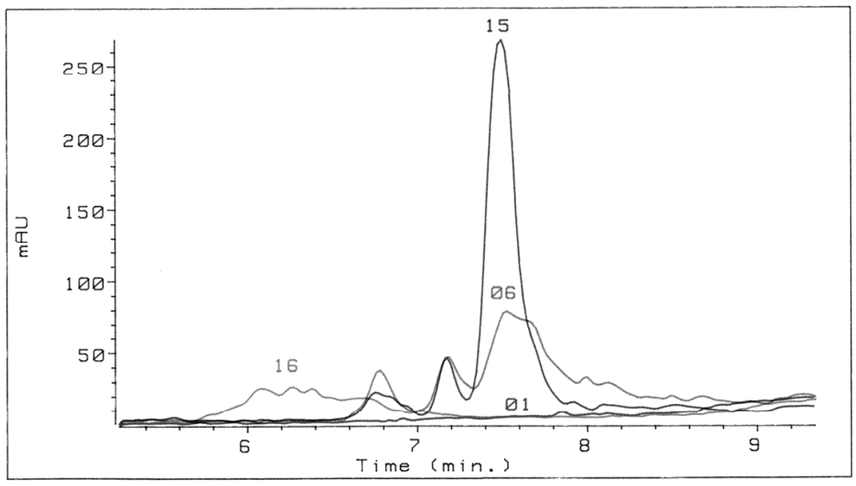

Fig. 5. The comparison of a bronchoalveolar lavage sample with inflammation and a serum sample by HPLC data. 01 - a control BALF sample; 06 - a BALF sample with inflammation; 15 - a control serum sample; 16 - sample of IL-1ra; wavelength 220 nm.

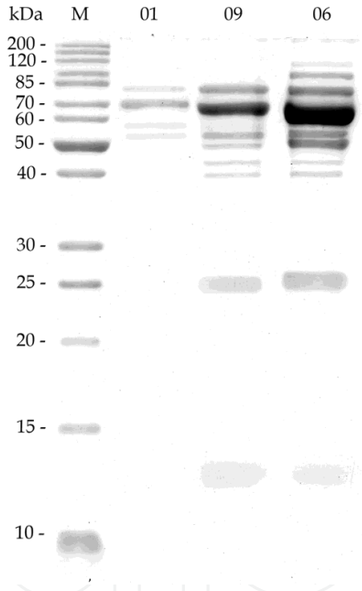

Fig. 6. Gel electrophoresis of BALF proteins obtained from C57BL/6 mice. M – protein molecular weight marker; 01 – BALF sample from nontreated mouse (control); 06 and 09 – BALF samples from mice treated by NPs 1 mg/mouse on the 3rd day after treating.

3.2 NMR analysis

In the ^{1}H NMR spectra of BAL fluids collected from the control mice there are the signals of lactate (doublet at 1.36 ppm) and acetate (singlet at 1.95 ppm) with the ratio of integral intensities approximately 6:1. In addition, low-intensity signal at 2.4 ppm assigned presumably to succinate and also a broad signal in the range 3.7-3.8 ppm were observed General view of spectra is shown in Fig.7.

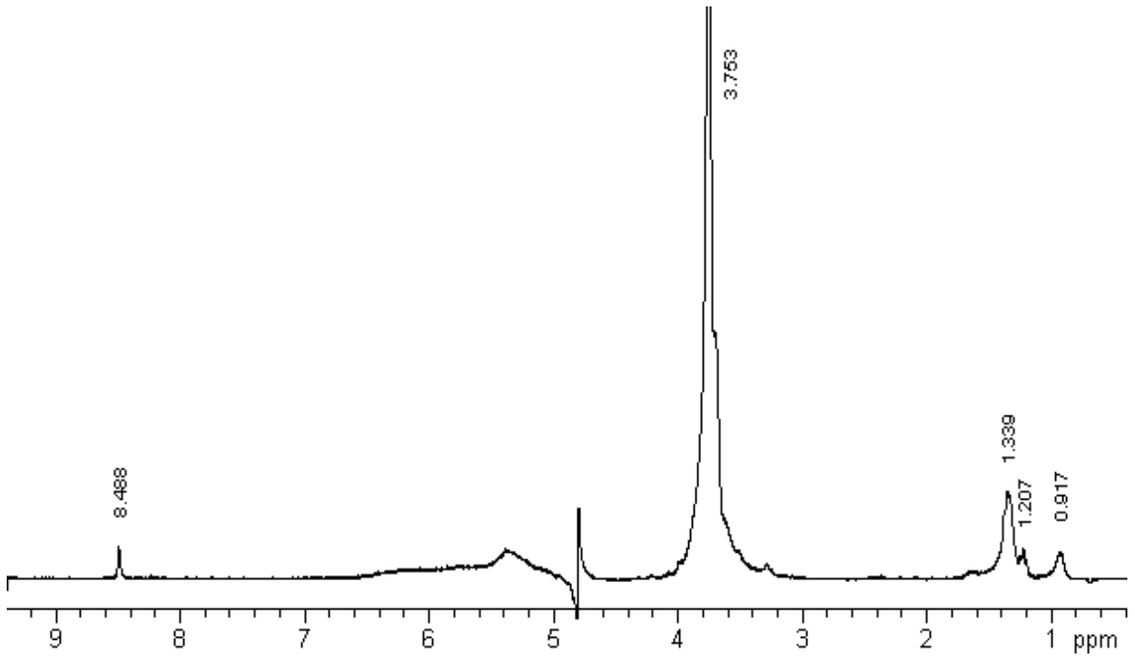

Fig. 7. ^{1}H NMR spectra of BALF of nontreated white outbred mice by NPs.

According to published data (Pottset al., 2001; Azmi et al., 2005) the latter signal was assigned to monosaccharides, particularly glucose, which is characterized by the signals at 3.83, 3.76, and 3.42 ppm. In the spectra of BAL fluids collected from the mice subjected to the action of iron NPs (Fig. 8) there are the signals of lactate and acetate but the signal at 2.4 ppm was not observed.

The main distinction in the spectra of BAL fluids collected from the mice subjected to the action of iron NPs and those collected from the control mice is the presence of a triplet at 1.22 ppm whose intensity is comparable with the intensity of the lactate signal and a multiplet at 3.3 ppm. We assigned these signals to ethanol, which is apparently one of the metabolites appearing under the action of iron NPs on the bronchopulmonary system of a mouse. This assumption was verified by addition of ethanol into the sample of BAL fluid, which resulted in increase in the intensity of the corresponding signals. At the same time, in addition of sodium propionate into the sample of BAL fluid we also observed a triplet and quartet typical for ethyl group but their chemical shifts did not coincide with the chemical shifts of the signals in hand.

The presence of lactate and ethanol in BAL fluids suggests inflammation status of mouse organism, which is accompanied by not only suppression of lung respiration but also insufficient oxygen supply into the cells, i.e. tissue hypoxia. As was noted previously (Nikolaev et al., 2003), an increased level of lactate concentration and the absence of pyruvate signals in the ^{1}H NMR spectra suggest an anoxia of cells. As an example, the ^{1}H NMR spectra of BAL collected from mice subjected to acute hypoxia and acute inflammation caused by bacterial endotoxin (Ischenko, 2007) have signals corresponding to the products of glycolysis similar to those observed in the case of cell hypoxia caused by iron NPs. The emergence of ethanol in BAL of NPs-exposed mice seems to originate from impaired glottic competency and following gastric particle deposition in bronchopulmonary ways. Gastric

particulate lung aspiration yields the partial contamination of airways by anaerobic bacteria or fungi. The colonization of the respiratory tract by microorganisms from digestive tract or upper respiratory tract (nose, throat) during inflammation may cause metabolite accumulation of end products of glycolysis in the injured lung. The production of minor quantity of ethanol in the inflamed mice with low level of its oxidation in lung results in BAL ethanol accumulation compared with control mice. A wide range of hypoxia stress tolerant animals use similar adaptive defense strategy switching over to metabolic pathways with synthesis of ketone bodies and low consumption of oxygen. As example, the appearance of ethyl alcohol as a metabolite was also noted for some deep-sea animals, for which metabolism is of anaerobic feature (Leninger, 1982).

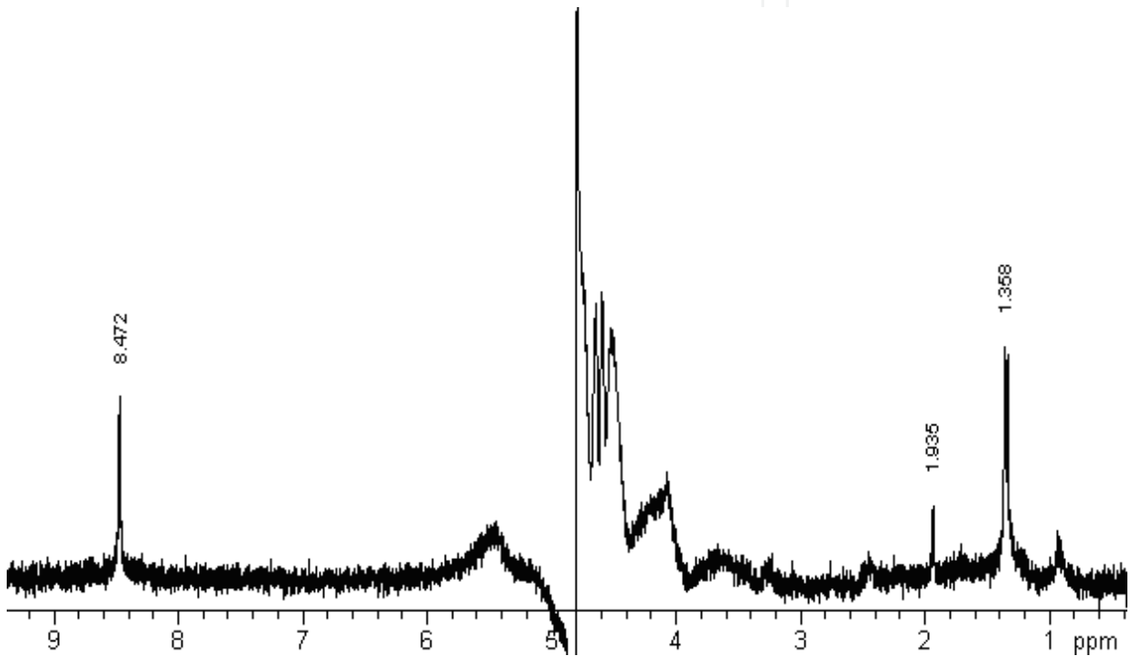

Fig. 8. ^{1}H-NMR spectrum of BALF of white outbred mice treated by NPs.

The data of lactic acidosis were also received by blood spectral analysis of animals in hypoxia stress. NMR spectra of mice blood plasma under acute hypoxia are shown on Figure 9.

The NMR spectra consist of one distorted wide line of resonance of water and groups of lines of saccharides and phospholipids. The resonance of species were assigned in a straitforward manner on the basis of multiplet structure, chemical shift and spectra of individual substances in solution.

The intense quadruplet and doublet lines are the essential characteristics of COH, CH_3 groups of lactate which have the definite position 4.1 and 1.3 ppm in spectra (Aime et al., 2002; Nikolaev et al., 1997). The spectra displayed the appearance of high lactate lines of resonance in hypoxic cases. No traces of pyruvate were observed in spectra. Integral intensity of lines was proportional to concentration of soluble lactate in plasma. Acute hypoxia induced the growth of lactate level up to 6.7 mM (normoxic value 2.3 mM).

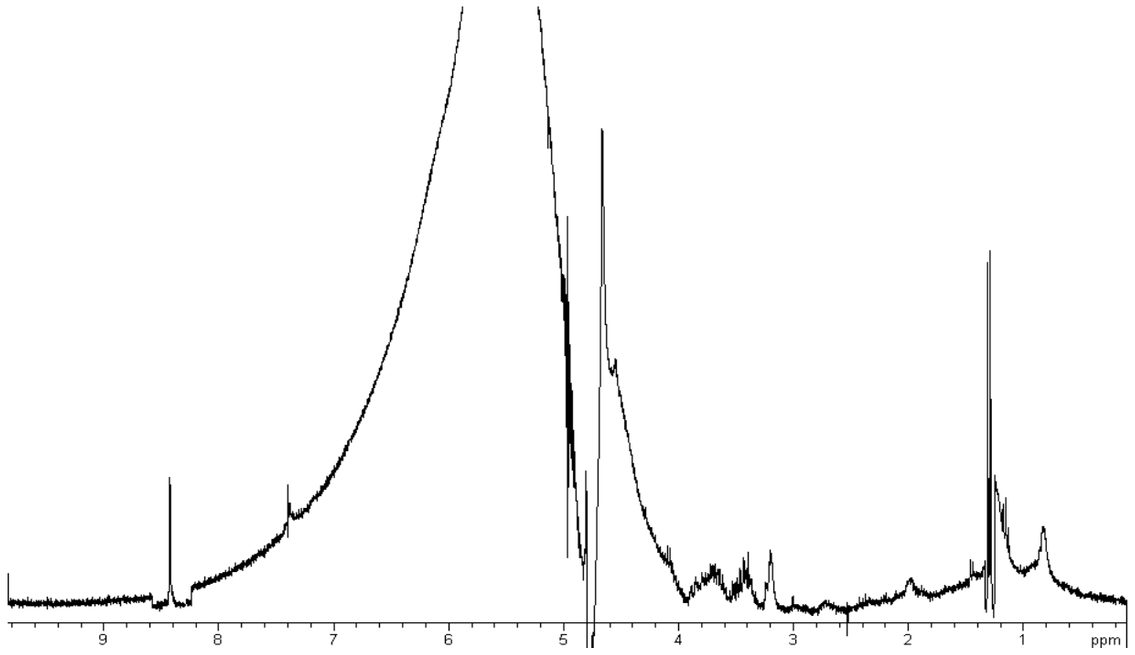

Fig. 9. ^{1}H-NMR spectrum of blood plasma of mouse after acute hypoxia at 4.2% pO_2 during 10 min.

3.3 The post-exposure treatment with IL-1ra

The anti-inflammatory activity of IL-1ra delivered as aerosol was estimated in the experiments with different doses (1, 10, 100 μg) of preparation in the cycle of three inhalations. The indicators of inflammation measured in inbred mice upon IL-1ra administration using aerosol were the percent of mature neutrophils in BAL relative to total BAL cells and the total protein in BAL fluid. The results of these measurements for different doses of IL-1ra dispersion are presented in Table 4. The experimental data show that inhalation of IL-1ra aerosol at the dose of 100 mkg per mouse in the cycle of three inhalations resulted in reduction of inflammation. IL-1ra treatment attenuated the iron NPs-induced protein increase and prevented the increase in pulmonary vascular permeability. The anti-inflammatory effect is achieved due to the 77% homology between recombinant human IL-1ra and the native murine protein. The blockage of IL-1 receptors on phagocytic monocytes, macrophages and pulmonary epithelium cells inhibits the generalization of inflammation throughout respiratory organs. The anti-inflammatory effect of IL-1ra is appeared to be of dose-dependent character.

3.4 The treatment of hypoxia by redox active agent

Anti-hypoxic action of oliphen was studied in a mouse model of acute normobaric hypoxia. Experimental study of hypoxic response of mice to normobaric acute hypoxia exposure revealed the relationship between the rate of mortality, partial pressure O_2, length of exposure and initial time of prechallenge. LD_{50} served as criteria to select partial pressure 4% pO_2 in chamber for 1 hour exposure. Hypoxic response in assigned mice groups was a function of prechallenge time. The dependence of percentage of mortality from prechallenge

time had a maximum value at 15 min. The 10 min was chosen as standard time for introduction to hypoxic state for all trials. The response of mice to acute hypoxia has appeared to be dependent on preliminary aerosol treatment by redox agent. Preliminary inhalation of nontoxic doses of oliphen prolonged the survival of mice under hypoxic conditions only at a certain dosage. The time-course of survival rate is shown in Fig.10.

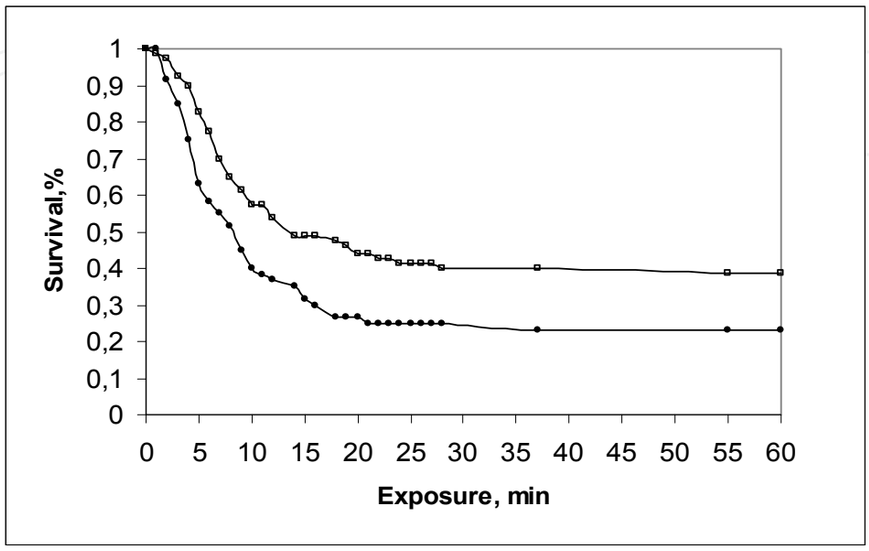

Fig. 10. Survival rate of white outbred mice treated by oliphen (upper line) as compared with control (lower line) in acute normobaric hypoxia for 60 min. Upper line – mice (60 animals) with oliphen (aspiration doses for oliphen 0.05 mg), median of survival 14 min; lower line – nontreated mice (80 animals) without oliphen, median of survival 9 min.

Oliphen prolonged the life-span of mice and increased their survival rate. The median time of life span increased from 9 min to 14 min after aerosol treatment by oliphen. Equivalent aspirated doses of oliphen in droplike and spray-dryed aerosols appeared to exert the same protective effect against acute hypoxia. Significant changes in mytosis of bone marrow cells, growth of permeability of erythrocyte membranes and their electrochemical potential $\Delta\psi$ were consistent with appreciable systemic absorption of oliphen in inhalation route. The investigation of oliphen action on mouse red blood cells by pulmonary delivery indicates that particulated oliphen regulates the permeability of erythrocytes membranes. This action is revealed as the decrease of membrane potential and the increase of the permeability for urea. So, the redox agent oliphen not only participates in the ETC functioning of prokaryotic cells but also regulates the permeability of blood cells as oxygen-transfer units. A full compensation of unsufficient supply of oxygen by physiological adaptation lasted for no more than 2-5 minutes. At late stage the symptoms of tissue hypoxia were observed. Suppressed NAD-dependent aerobic pathway of cellular oxidation switches over more rational succinate-oxidase and alternative pentosephosphate way of glucose consumption (Zarubina, 1999; Hochachka et al., 1996; Boutilier et al., 2000). Oxygen lack in blood leads to intensive high energy phosphate utilization. Severe tissue oxygen insufficiency affects oxidative phosphorylation in mitochondria, switching over the alternative glycolytic ways of respiration. The glycolytic reactions become the dominant source of energy production in

cells at oxygen lack. Elevated lactate level observed by NMR method and drop of pH in blood were the important symptoms of cell oxygen starvation (Shchukina, 1986). The common cause of lactic acidosis is tissue hypoxia and as consequence - the onset of glycolytic conversion of glucose to lactate. When there is the sufficient supply of cells by carbon substrate, glycolysis provides dramatic accumulation of lactic acid. Along with the change of pH and lactate level there were a low down shifts of hemoglobin, decrease of hematocrit and the increase of deformability of red blood cells (Table 4). The reticulocyte count in blood after hypoxic challenge diminished too.

Parameters		Respiration state	
		Normoxia (n=6)	Hypoxia, (n=5)
Hemoglobin (Hb), %		15.0±1.4	*13.4±0.4
Mean corpuscular hemoglobin (MCHb), pg/red cell		15.1±1.2	13.9±2.5
Erythrocyte sedimentation rate (ESR), mm/h		0.7±0.4	0.6±0.2
Hematocrit (Hct), %		93.8±1.6	*90,0±2,9
Whole blood pH		7.4±0.1	*6.9±0.2
Red blood cells	Red blood cells (RBC) count, mln/mm^3	9.9±0.6	9.6±1.4
	Red cell deformability (Def.), %	60.3±20.4	*98.0±4.5
	Reticulocytes, %	3.2±1.7	2.6±1.8

Table 4. Hematological parameters of mice in normoxia and hypoxia. The values represent mean±standard error of the mean.*P<0,05 in relation to the control group.

Treatment of hypoxemia by oliphen inhalations was shown to be due to systemic activation of erythropoiesis in red marrow. Short-time exposure to aerosolized oliphen initiated the proliferation of proerythroid cells immediately after challenge (Rodrhguez et al., 2000; Klausen et al., 1996). Adaptive reaction of blood system to drug challenge in normoxia have been recorded in 2 times growth of mitotic index of bone marrow cells for time 48 h. The possible synthesis of erythropoietin (EPO) de novo may be responsible for rise of oxygen capacity of blood initiated by systemic influence of oliphen. The pattern of polyxemia response has some similar features with intermittent hypoxic training, resulting in increase of hematocrit. Indeed these data are in accordance with observations on production de novo EPO for rodents after delay time 15 min and 2-6 h for humans as a result of hypoxic reaction (Klausen et al., 1996). The considerable advantage of oliphen in inhalation route of administration comprises the possibility to induce polycythemic response and to quench the reactive oxygen species of posthypoxic state at the same time (Cuzzocrea et al., 2001).

The antihypoxic action of aerosolozied oliphen in animal model may be of medical use for treatment of hypoxic syndrome caused by infections, since the severity of disorders

correlates with oxygen stress burden for tissue respiration. As shown, combined application of redox active oliphen with recombinant interferon alfa-2b in a form of respirable aerosol resulted in acceleration of normalization of main clinical functions (lung ventilation, heart output and arterial pressure) of blood parameters in complicated influenza and pneumonia (Vasilieva et al., 2002). The experimentally confirmed efficacy, the absence of adverse effects and the feasibility to achieve therapeutically effective doses in noninvasive route of administration is a prerequisite for further investigation of oliphen as a perspective antihypoxic agent.

4. Discussion

The study of the effect of magnetic iron NPs on rodent model showed that inhalation administration of iron NPs is accompanied by the general inflammatory response. Direct intratracheal administration of iron NPs results in the total inflammatory response for third day, which is reliably confirmed by the increase in the content of neutrophils, alveolar macrophages, and proteins in the bronchoalveolar lavage fluid. It was established that iron NPs synthesized by the gas-phase procedure and microemulsion precipitation method is a pro-inflammatory agent in administration via the respiratory ways. Administration of iron NPs activates macrophage nonspecific lung protection. In the BAL samples there are a large number of macrophages engulfing dispersed iron. The relative population of macrophages decreases in time after a contact with a dispersed iron sample and consequent ingesting. The latter also confirms the toxicity of iron NPs for the mucous epithelium cells. Aspiration of suspended iron NPs through a trachea is aimed for delivery of iron particles to distal area of the lung. For clinical practice ventilation of lung with aqueous aerosols of iron NPs can serve as a method of delivery of contrasting agent for MRI (Rinck, 2001; Wang et al., 2001; Gubin et al 2005; Bonnemain et al., 1998). However, in inhalation procedures a possibility of damage or, in the other words, enhancement of permeability of capillary-alveolar membrane for blood proteins should be considered. The results of studying the protein composition by the HLPC and gel electrophoresis suggest transfer of a part of plasmatic proteins including antibodies into the alveolar area, which is a part of nonspecific protection of the organism caused by administration of NPs into bronchopulmonary tract. As a whole, the pattern of inflammatory processes with initiation of synthesis of pro-inflammatory cytokins and activation of the NF-kB complex corresponds to the pattern of nonspecific response of lung to inhalation of finely dispersed aerosols (Limbach et al., 2007; Oberdörster et al., 2005; Li et al., 1999; Tran et al., 2000; Danilov et al., 2003; Siglienti et al., 2006; Schins et al., 2004; Donaldson K. & Tran Cl, 2002; Zhu et al., 2008). The toxicity of iron NPs can be caused by the chemical composition of materials, the particular features of their size, increased aspiration dose of the preparation, and physiological peculiarities of their delivery through a respiratory tract. In step-by-step estimation of the role of each factor the following circumstances should be considered. The structure of dispersed samples used in trials presents an iron core covered with oxide shell. These preparations as chemical compounds are low toxic. Intravenous injections of suspended Fe_3O_4 and Fe_2O_3 NPs as contrasting agents for MRI are widely used in clinical practice without noticeable drawbacks (Rinck, 2001; Wang et al., 2001). An excess of iron is assimilated with an organism in the form of ferritin and is removed by kidneys in the form of soluble salts. Along with this, a possibility of contamination of NPs with iron ions, which are able to initiate burst-like oxidation processes in macrophage system, should be taken in account. Generation of active oxygen

species and radical products catalyzed by the reactions of the Fenton type facilitates the pathogenesis of inflammatory response on respiratory iron NPs impact (Lay et al., 1998; 1999). Through monitoring of feasible contamination should exclude or noticeably reduce inflammatory activity of iron NPs.

The main reasons of inflammatory activity of particulate metal are governed by the spatial organization of NPs at the lung surface. It is likely that the toxicity of iron NPs observed in respiratory delivery is caused by their self-association. This self-association manifests itself in strong broadening of ^{1}H NMR signals under the action of iron NPs, which is explained by appearance of superparamagnetic state in reaching threshold particle sizes (from our estimation, approximately 30 nm) (Wang et al., 2001; Björnerud et al., 2004). Due to the strong magnetic interactions the iron NPs can form micron-sized thread-like structures, which are eliminated with macrophage cells during phagocytos. Formation of thread-like structures is may be the main factor of inflammation development, which is governed by the degree of association and the amount of aspirate in airways. The metabonomic characteristics of BAL fluid (concentrations of lactate, pyruvate and appearance of ethanol-like signal) studied by the ^{1}H NMR suggest that the inflammatory process results in the lack of oxygen and suppression of cell respiration (Nikolaev et al., 2003). As a rule the severe pulmonary tissue hypoxia follows after delivery of metal NPs. The limited time of the experiment (one week) did not allow to solve the problem of persistence of inflammation process initiated by aspiration of iron NPs into the respiratory tract and probability of formation of chronic lesion focuses.

The inflammatory response can be reduced by decrease in the single dose of iron NPs, multiple aspirations with reduced dose of NPs in diluent, modification of the surface of iron NPs with biologically adapted polymers (dextran, alginate, chitosan, etc), desintegration of associates of iron NPs formed in synthesis by mechanical action and addition of surfactants. The adverse effects of iron NPs-inhalation can be also prevented by prudent therapeutic procedures. One of the major drawbacks of iron NPs-inhalation is acute inflammatory response accompanied by hyperproduction of a pro-inflammatory cytokine mediator IL-1. As it has been shown IL-1 receptor antagonist (IL-1ra) suppresses the adverse effects of IL-1 by blocking the related cell receptors making it a promising way for treatment and prevention of a number of pathological inflammatory states (Simbirtsev, 2011). Once in this study IL-1ra was successfully used to reduce iron NPs-induced pulmonary neutrophils infiltration and other inflammation-associated processes in the mouse, the aerosol delivery of IL-1ra may be considered as medicine for treatment NPs-induced lung inflammation. To avoid inflammation effects of iron NPs for MRI assay IL-1ra may be delivered jointly with NPs in the form of aerosol or instillation. It is hopeful application of this substance for iron NPs-enhanced MR imaging of lung and magnetic field-guided drug delivery to the lung.

The therapeutic efficiency of airway administration of IL-1 receptor antagonist is reduced by tissue hypoxia caused by defects in working of ETC. The main sign of tissue hypoxia at late stage is the energetic discharge in the respiratory chain of cells. Severe hypoxic exhaustion of high energy substrates ATP, ADP, phosphocreatine resulted in membrane polarization and uncontrolled influx of calcium, being the possible reason of cell death and the following dangerous inevitable consequences for whole body (Zarubina, 1999; Boutilier et al., 2000). Acute hypoxia exerts a damaging effect on the cells, their membranes and function. According to the study of mouse normobaric hypoxia model, these defects of cell respiration

can be effectively removed by delivery of redox active agent of quinone origin into bronchopulmonary tract. Thanks to a concomitant matching between the redox potential of oliphen (0.7 V) and redox state of substrates participating in electron transfer at the NAD/NADH locus of mitochondrial ETC, oliphen performs the role of electron equivalent carrier capable to shunt the injured sites of membrane respiratory complex (Tolstoy & Medvedev, 2000). Administration of oliphen into blood circulation results in restoration of the electron transport function of mitochondrial respiration chain in NADH/NAD site subjected oxidative damage at oxygen deficit (Vinogradov et al., 1973; Smirnov et al., 1992; Popov & Igumova, 1999). The treatment by oliphen recovered the lactate level to the normoxic value and stimulated the rise of hematological characteristics: hemoglobin and reticulocyte count. In conditions of hypoxia the oliphen showed antihypoxic activity at low pO_2 without reoxygenation. The membrane potential of mice red blood cells, measured by degree of absorption of tetraphenylphosphonium cation, decreased from 2.0 to 1.4 mkM ($n=28$; $P<0.05$). The presence of exogenous oliphen in interstitial liquids maintained the adequate transmembrane potential for transfer of protons across membrane. These data are consistent with our early reported results on study of oliphen participation in anaerobic cell respiration in vitro (Yakovleva et al., 2002).

The physiological cell response to single aerosol oliphen challenge retained activity for 3-4 houres. The main mass of inhaled soluble oliphen was removed from circulation by the liver and kidney. The particulate oliphen had been withdrawn also by bronchocillary clearance system. Airway delivery of dry particulate oliphen stimulated nonspecific defence reaction. As electron microscopy data showed macrophages engulfed particulate oliphen. Phagocytosis is known to be the source of pro-inflammatory impact which easily spread over the whole body (Cuzzocrea et al., 2001). The marked absence of irritation at the site of deposited particles was associated with powerful antioxidant activity (Driscoll et al., 2002). Oliphen inhibits the pathogenetic products of oxidative burst of macrophages by scavenging active forms O_2, H_2O_2 and other reactive products into nontoxic species. Antioxidant action of oliphen prevents development of inflammation in epithelial tissue (Cuzzocrea et al., 2001). The influence of oliphen on tolerance to hypoxia was more pronounced as compared with the action analogous nootropic and actoprotective drugs such as sodium hydroxybutyrate and sodium succinate (Smirnov et al., 1992; Zarubina, 1999). Inhalation of sodium hydroxybutyrate in a form of liquid aerosol essentially reduced the life-span (resulted in death of all tested mice at 10 min of exposure) at aspiration dose of 2.0 mg, which stimulate central nervous system. High sedative aspiration doses of sodium hydroxybutyrate (9 mg) improved resistance to acute hypoxia, but its effect was less than that elicited by oliphen inhalations. Inhalation of dry powder of sodium succinate induced additional resistance of mice to acute hypoxia at high aspiration doses 0.06 mg about in two times. Human insufflations of sodium succinate had some drawbacks as cough and irritation of respiration ways, but adverse effects were not observed in mouse model. Aerosol treatment with all of these drugs 2-2.5 times increased the initial time of life. This initial time is considered to be associated with adaptation of organism via increased ventilation of lung, cardiac output, mobilizing erythrocytes depot and allocation of pathways of oxygen consumption in more rational schemes.

So, on base of our experimental study the metal NPs exposure generate inflammation, oxidative stress and hypoxia of respiratory system. The respiratory disease induced by NPs challenge may be effectively treated by airway delivery of IL-1 receptor antagonist and of

redox active preparation of oliphen. The applied IL-1ra improve the anti-inflammatory resistance of airway epithelium to iron NPs exposure. The adverse consequences of acute tissue hypoxia associated with pulmonary inflammation may be successfully overcome by inhalative application of the redox active hydroquinone oligomer in form of drug "Oliphen". The positive effect of antihypoxic treatment by oliphen is related with restoration of normal functioning of alveolar and epithelium mitochondria ETC. The combined application of recombinant antagonist IL-1 and redox active agent "Oliphen" may be the new possible strategy of therapeutic defensive measures against toxic hazards of production and application of metal NPs.

5. Conclusion

A tracheobronchial administration of iron NPs is shown to induce an acute inflammatory response in mouse model, which is similar to inflammatory pattern of inhaled endotoxin with respect to cellular and biochemical characteristics. The inflammatory response to inhalation contact with iron NPs is characterized by hypoxic secretion of partial oxidation products (ethanol and lactate) into bronchoalveolar lavage fluid. Receptor antagonist IL-1 aerosol administration may prevent the development of the acute inflammation induced by inhalation of iron NPs. Inhalation of redox agent in the form of fine respirable oliphen positively corrected the hypoxic response. Short-time exposure to aerosolized oliphen enhanced nonspecific defence against acute hypoxia through activation of mitochondrial respiration and stimulation of erythropoesis. Inhalation aerosol treatment by micronized oliphen could be realized in effective antihypoxic doses without adverse effects of inflammation and irritation of respirative tract. So, the pulmonary contact with iron NPs causes acute pulmonary inflammation, but IL-1β receptor antagonist and redox active agent oliphen may be used for it's suppression. A tracheobronchial administration of iron NPs combined with anti-inflammatory medicine IL-1 receptor antagonist is feasible for delivery into lungs as a contrasting agent for MRI.

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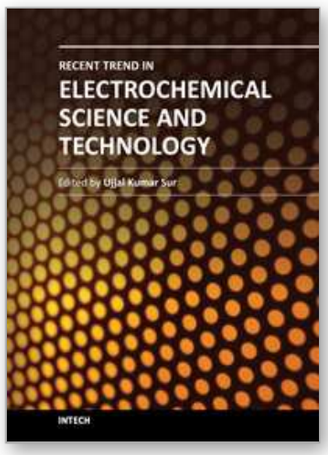

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This book titled "Recent Trend in Electrochemical Science and Technology" contains a selection of chapters focused on advanced methods used in the research area of electrochemical science and technologies; descriptions of electrochemical systems; processing of novel materials and mechanisms relevant for their operation. This book provides an overview on some of the recent development in electrochemical science and technology. Particular emphasis is given both to the theoretical and the experimental aspect of modern electrochemistry. Since it was impossible to cover the rich diversity of electrochemical techniques and applications in a single issue, the focus is on the recent trends and achievements related to electrochemical science and technology.

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