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Tatiana M. Ustinova, Yury O. Konshakov, Nikolay G. Vengerovich, Georgiy S. Stepanov

State assessment of the rat antioxidant system's enzymatic link in the acute pulmonotoxic exposure modeling

State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, St. Petersburg, 195043, Russian Federation

ABSTRACT

Introduction. *Poisoning by pulmonotoxicants is an urgent problem of modern toxicology due to its frequency, severity of cases, and insufficient understanding of underlying mechanisms, which hinders effective treatment. The aim of this study is to evaluate the status of the rat antioxidant system during the modeling of acute pulmonotoxicants poisoning.*

***Material and* Methods.** *Acute poisoning by pulmonotoxic substances, perfluoroisobutylene and nitrogen dioxide in toxodoses of 1.67 mg/L·min and 5.6 mg/L·min, respectively, was in Modeled on nonlinear male rats in an inhalation chamber with a volume of 0.2 m3 for 15 minutes. Blood and lungs samples were taken 3, 6, and 24 hours after exposure to assess the activity of superoxide dismutase, catalase, and glutathione peroxidase, while plasma levels of malondialdehyde were also measured.*

Results. *In response to perfluoroisobutylene exposure, superoxide dismutase, catalase, and glutathione peroxidase activities in blood after 3 h reached minimum values – 75.6 U/mL, 229.2 and 11.1 mM/(L·min) correspondingly, with subsequent partial recovery of catalase and glutathione peroxidase activities, and compensatory increase of superoxide dismutase activity within 24 h. The lung tissue response is manifested in a decreased activity of the enzymes studied, within 24 h. Related to nitrogen dioxode exposure, the superoxide dismutase and catalase peak activities in blood were recorded after 6 h – 157.7 U/mL and 513.3 mM/(L·min), respectively, glutathione peroxidase's peak activity – after 3 h – 19.5 mM/(L·min). The enzymes' activity dynamics in lung is indicative of the superoxide dismutase level increase up to 54,5 U/mL within 24 h, while changes of catalase and glutathione peroxidase activities are characterized by minimum peak values after 6 h – 20.4 and 6.4 mM/(L·min), respectively. There was a sustainable malondialdehyde accumulation in blood plasma during the experiment, which exceeded the corresponding value in intact group of animals.*

Conclusion. *These findings suggest the significant effects of free-radical oxidation processes on the pulmonotoxic agents' toxicity mode.*

Limitation. *The experimental study was performed on white outbred male rats (n = 30, body weight 200-240 g), kept in a standard vivarium conditions, and randomly divided into groups with the exclusion of weakened and affected animals from the study.*

Keywords: *pulmonotoxic agents; oxidative stress; superoxide dismutase; catalase; glutathione peroxidase; malondialdehyde*

Compliance with ethical standards. *The study was approved by the Local Ethics Committee of the State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, Protocol No. 18 of 16.12.2023; conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123), European Union Directive 2010/63 EU of 22.09.2010 on the protection of animals used for scientific purposes.*

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For correspondence: *Tatiana M. Ustinova*, e-mail: gniiivm_5@mil.ru

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Original article

Устинова Т.М., Коньшаков Ю.О., Венгерович Н.Г., Степанов Г.С.

Оценка состояния ферментативного звена антиоксидантной системы крыс при моделировании острого отравления веществами пульмонотоксического действия

ФГБУ «Государственный научно-исследовательский испытательный институт военной медицины» Министерства обороны Российской Федерации, 195043, Санкт-Петербург, Российская Федерация

РЕЗЮМЕ

Введение. *Отравления веществами пульмонотоксического действия являются актуальной проблемой современной токсикологии из-за частоты встречаемости, тяжести случаев и недостаточного понимания механизмов действия, что затрудняет эффективное лечение.*

Цель работы заключается в оценке состояния антиоксидантной системы крыс при моделировании острого отравления веществами пульмонотоксического действия.

Материал и методы. *Моделирование острого отравления веществами пульмонотоксического действия, перфторизобутиленом и диоксидом азота в токсодозах 1,67 мг/л · мин и 5,6 мг/л · мин соответственно проводили на нелинейных крысах-самцах в ингаляционной камере объёмом 0,2 м³ в течение 15 мин. Через 3, 6 и 24 ч после воздействия токсикантами отбирали кровь и лёгкие для оценки активности супероксиддисмутазы, каталазы и глутатионпероксидазы, в плазме определяли уровень малонового диальдегида.*

Результаты. *В ответ на отравление перфторизобутиленом активность супероксиддисмутазы, каталазы и глутатионпероксидазы в крови крыс после 3 ч достигала минимальных значений — 75,6 U/мл, 229,2 и 11,1 мМ/(л · мин) соответственно. В последующем наблюдали частичное восстановление активности каталазы и глутатионпероксидазы и компенсаторное повышение активности супероксиддисмутазы в течение 24 ч. Реакция лёгочной ткани проявляется снижением активности исследуемых ферментов в течение суток. На фоне отравления диоксидом азота пиковые значения активности супероксиддисмутазы и каталазы в крови регистрировали через 6 ч [157,7 U/мл и 513,3 мМ/(л · мин) соответственно], активности глутатионпероксидазы — через 3 ч [19,5 мМ/(л · мин)]. Динамика активности ферментов в лёгких свидетельствует о повышении уровня супероксиддисмутазы в течение суток до 54,5 U/мл, в то время как изменение активности каталазы и глутатионпероксидазы характеризуется минимальными пиковыми значениями через 6 ч — 20,4 и 6,4 мМ/(л · мин) соответственно. В течение эксперимента регистрировали стабильное накопление малонового диальдегида, превышающее значение показателя в интактной группе животных.*

Ограничения исследования. *Экспериментальное исследование выполнено на белых беспородных крысах-самцах (n = 30, масса тела 200–240 г), содержавшихся в стандартных условиях вивария, распределённых на группы методом рандомизации с исключением из исследования ослабленных и больных животных.*

Заключение. *Полученные результаты свидетельствуют о значимом влиянии процессов свободнорадикального окисления на механизм токсического действия пульмонотоксических веществ.*

Ключевые слова: *вещества пульмонотоксического действия; оксидативный стресс; супероксиддисмутаза; каталаза; глутатионпероксидаза; малоновый диальдегид*

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Для корреспонденции: *Устинова Татьяна Михайловна*, e-mail: gniiivm_5@mil.ru

Участие авторов: *Устинова Т.М.* – концепция и дизайн исследования, написание текста, статистическая обработка данных; *Коньшаков Ю.О.* – написание текста; *Венгерович Н.Г.* – редактирование; *Степанов Г.С.* – сбор материала, обработка данных. *Все соавторы* – утверждение окончательного варианта статьи, ответственность за целостность всех её частей.
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Introduction

Poisoning by pulmonotoxicants is an urgent medical and biological problem that arises primarily in emergency situations at chemically hazardous facilities, as well as in industrial and domestic fires during combustion of residential components. The significance of the problem is due to the high frequency of occurrence and severity of clinical manifestations of such poisonings, making them a subject of special attention in modern clinical toxicology [1, 2]. Pulmonary toxic effects are characteristic of substances capable of causing structural and functional damage to the alveolar membranes of the lungs. Such agents include phosgene, perfluoroisobutylene (PFIB), chlorine, nitrogen and sulfur oxides, ammonia, and others. Signs of poisoning are due to the pronounced irritant, inflammatory, and cytotoxic effects of these compounds. Exposure to low concentrations of some of these substances typically results in upper respiratory tract irritation, while inhalation of high doses is associated with the development of life-threatening toxic pulmonary edema (TPE) [3, 4].

PFIB, a pyrolysis product of polytetrafluoroethylene (PTFE) formed as a result of fire accidents at fluoropolymer industry facilities, is a highly toxic, colorless gas that reacts with virtually all nucleophiles. Due to its hydrophobicity, upon inhalation, PFIB rapidly penetrates deep into the respiratory tract, reaching the blood-air barrier. It is partially hydrolyzed by mucous membrane fluid to hydrofluoric acid, which causes localized pH changes and degradation of the microenvironment (proteins, phospholipids, etc.), resulting in the initiation of inflammatory processes [5].

Nitrogen dioxide is a gas that, when introduced into the body, can directly activate free radical oxidation reactions. When interacting with the fluid phase covering the respiratory epithelium, as well as with the membranes of epithelial cells, it initiates the local formation of reactive oxygen species (ROS). This leads to damage of cellular structures and the development of an inflammatory response, accompanied by bronchospasm and TLE [6].

Despite the differences in the mechanisms of toxic action of PFIB and nitrogen dioxide, both compounds initiate the formation of superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and other ROS. The accumulation of these highly reactive products leads to oxidative damage to cellular structures and, consequently, to the development of TLE [7]. The main components of the enzymatic antioxidant defense system of the lungs are superoxide dismutase (SOD), catalase and glutathione peroxidase (GP). SOD is involved in the dismutation reaction of superoxide anion radicals into hydrogen peroxide and molecular oxygen. In the presence of excess H_2O_2, it undergoes alternating divalent oxidation and reduction in its active site. Catalase, an enzymatic antioxidant, is the first element in the intracellular defense against ROS, preventing their accumulation in cells by decomposing hydrogen peroxide to form water and oxygen. Although catalase is the main destroyer of hydrogen peroxide molecules, it is not capable of processing large molecular peroxides, which include lipid peroxides. GP is an enzyme that uses glutathione as an electron donor and catalyzes the reduction of peroxides, including hydrogen peroxide and organic peroxides, to the corresponding alcohols, thereby playing a key role in the antioxidant defense of the cell [8]. Malondialdehyde (MDA) is formed as a result of the breakdown of unsaturated fatty acid peroxides and is the final stable product of lipid peroxidation. It is used as a marker in determining the rate of oxidative damage to lipids [9]. Despite extensive pulmonotoxicant research, the pathogenesis of intoxication with some compounds remains unclear, complicating the development of effective treatment strategies. While studies on the mechanisms of action of individual pulmonotoxicants and their impact on the body's antioxidant system are available in the literature, these data are fragmentary and are gradually being expanded. Analysis of free radical oxidation indicators during exposure to pulmonotoxicants allows for a more complete characterization of one of the key components of the complex pathological process leading to the development of TLE[10].

The aim of this study is to evaluate the enzymatic status of the rat antioxidant system during the modeling of acute severe pulmonotoxicants poisoning.

Material and methods

The study was conducted on 30 albino outbred male rats (8–12 weeks old) weighing 200–240 g and obtained from the 'Rappolovo Laboratory Animal Nursery' of the National Research Centre 'Kurchatov Institute' (settlement Rappolovo, Leningrad Region). The animals were kept under standard vivarium conditions in accordance with

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the sanitary and epidemiological requirements for the design, equipment, and maintenance of experimental biological clinics[1], under controlled environmental conditions (temperature 19–25 °C, relative humidity 30–70%), with a 12-hour lighting cycle. LBK-120 food pellets used as diet (Tosnensky feed mill, Leningrad regiion)[2]. Animals were divided into groups (at least 6 individuals) by stratified randomization with stratification by body weight (±10%). The experimental animals were divided into the following groups:

- Group 1 – intact group (6 individuals), served as a reference value for antioxidant enzyme activity. Animals in this group were not exposed to inhalation of pulmonotoxicants.
- Group 2 – experimental group (18 individuals). Animals in the experimental group were exposed to inhalation of pulmonotoxicants, followed by euthanasia of 6 animals at 3, 6, and 24 hours, with collection of biological material to determine the dynamics of oxidative stress. Euthanasia of animals performed in accordance with the Recommendations of the Board of the Eurasian Economic Commission[3].

PFIB and nitrogen dioxide, which differ in the mechanisms of toxic effects on lung tissue, were used as model pulmonotoxicants. PFIB exerts its damaging effect primarily through direct chemical damage to the alveolar-capillary barrier, while nitrogen dioxide exerts its damaging effect through a pronounced prooxidant effect with the generation of reactive oxygen species Both compounds are capable to cause acute inhalation lung injury with the development of toxic edema and impaired oxidative status, which allows them to be used for comparative assessment of antioxidant system responses.

Modelling of acute inhalation poisoning realized by the static method in a sealed and chemically resistant polymethyl methacrylate chamber with an internal volume of 0.2 m^3, equipped with an internal gas-air mixture recirculation system. The animal exposure time was 15 minutes, which is necessary to reproduce acute inhalation exposure sufficient to produce a pronounced pulmonary toxic effect without a lethal outcome in the early stages of observation and is consistent with the accepted approach for modeling poisoning at a dose of 1 LCt_{50}. The exposure toxic dose was 1.67 mg/L · min and 5.6 mg/L · min for PFIB and nitrogen dioxide, respectively.

Concentrations of PFIB and nitrogen dioxide in the exposure chamber were determined using real-time FTIR spectroscopy based on characteristic absorption bands, ensuring the stability of the specified toxicant concentrations throughout the entire inhalation exposure period.

Animals were inhaled in clean, dry chambers without bedding. Placed the animals after inhalation in temporary chambers for 1.5–2 hours to allow degassing and removal of residual toxicant from their fur [11]. Biological samples (blood and lungs) were collected at 3, 6, and 24 hours after poisoning. Animals sacrificed by decapitation. Blood samles collected in vacuum tubes containing lithium heparin for enzyme analysis and potassium EDTA (MiniMed, Russia) for assay of MDA in plasma after centrifugation (ELMI CM-6M, Latvia; 5 min, 2500 rpm). Removed lungs were rinsed with 0.01 M phosphate-buffered saline, permeated with 9 parts of 0.01 M Tris-HCl buffer (pH 7.4) cooled to 1 ± 3 °C, and homogenized at 15,000 rpm (Heldolph SilentCrusher M, Germany). The supernatant, diluted 100-fold with distilled water, was used to determine SOD, while lung tissue homogenate from experimental animals was used to determine catalase and GP [12].

Antioxidant protection status evaluated by changes in SOD, catalase, and GP activity in the blood and lung tissue of experimental animals exposed to inhalation of model pulmonotoxicants.

Antioxidant protection status evaluated by changes in SOD, catalase, and GP activity in the blood and lung tissue of experimental animals exposed to inhalation of model pulmonotoxicants. Autooxidation of 0.015 % quercetin was performed in 0.02 M phosphate-buffered saline (pH 7.2–7.4) with the addition of 0.08 mM ethylenediaminetetraacetate (EDTA) and 13.3 mM tetramethylethylenediamine. Reaction was activated by adding 0.1 ml of a quercetin solution in dimethyl sulfoxide to the incubation medium, and recorded the absorbency at λ=406 nm immediately and after 20 min [13]. Activity of catalase determined by the rate of decrease in the hydrogen

[1] Resolution of the Chief State Sanitary Doctor of the Russian Federation dated August 29, 2014, No. 51, 'On Approval of SP 2.2.1.3218–14 'Sanitary and Epidemiological Requirements for the Design, Equipment, and Maintenance of Experimental Biological Clinics (Vivariums)'.

[2] GOST 34566–2019 Full-sized compound feed for laboratory animals. Technical conditions.

[3] Recommendations of the EEC Board dated November 14, 2023, No. 33 'On Guidelines for Working with Laboratory.'

peroxide concentration in the incubation medium [14]. Hydrogen peroxide concentration was measured using reaction with a 10% ammonium molybdate solution to form a stable colored complex (λ=410 nm). GP activity determined by a standard method based on the ability of reduced glutathione to form a colored complex with Ellman's reagent. Reaction in 0.143 M phosphate buffer with EDTA with the addition of reduced nicotinamide adenine dinucleotide (NADPH) and reduced glutathione. Incubated the reaction mixture after adding Ellman's reagent for 1 minute, then determined the absorbency at λ = 405 nm [15]. Estimated the content of malondialdehyde (MDA) using thiobarbituric acid at high temperature in an acidic medium. Measured the absorbency at λ = 515 nm and λ = 532 nm [16].

Determined enzyme activity by photometry using a T8DCS UV/Vis spectrophotometer (Persee, China).

Statistical processing of the obtained data performed using Microsoft Office Excel 2013 and StatSoft Statistica 10.0.1011.0. Described the set of central values as the median, 25th and 75th quartiles – *Me* [Q_{25}; Q_{75}]. Tested the normality of the quantitative value distribution using the Shapiro-Wilk test. The significance of differences estimated by comparing data for two independent samples in the Mann-Whitney test. Differences considered significant at $p < 0.05$.

Results

The study demonstrated the effect of inhalation exposure to pulmonotoxicants on the antioxidant status of rats by assessing enzymatic activity in both the blood and lung tissue during the first 24 hours after poisoning. The results revealed a markedly variability of trends in the activity of the antioxidant enzymatic system components during the development of signs of poisoning induced by inhalation exposure to PFIB and nitrogen dioxide. **Table** presents data on changes in the activity of SOD, catalase, and GP, as well as MDA levels, in the blood and lung tissue of rats after inhalation exposure to PFIB at a dose of 1 LCt_{50}.

SOD activity in the blood reached its minimum values 3 hours after exposure to the toxicant, while in lung tissue, the level was statistically significantly higher than in the intact group, amounting to 112.3 U/mL versus 32.1 U/mL ($p < 0.05$). Six hours after PFIB poisoning, a significant increase in SOD activity was recorded in the blood by 41.4% and in lung tissue by 149.2% compared to control values ($p < 0.05$). After 24 hours, elevated SOD activity kept both in the blood and lung, and significantly higher than that of the intact group.

The most pronounced decrease in catalase activity in the blood was observed 3 hours after inhalation poisoning, while in lung tissue, minimal levels were detected after 24 hours. GP activity in the blood after 3 hours was 11.1 mM/(L · min), which is 2.5 times lower than that of the intact group. At the same time, the activity of this enzyme in lung tissue at this point reached its maximum value of 17.0 mM/(L · min), which is 3.7 times higher than that of the intact group. Elevated GP activity in the lungs persisted throughout the observation period.

Along with changes in antioxidant status in animals exposed to pulmonotoxicants, activation of lipid peroxidation (LPO) processes was observed, reflected in increased MDA concentrations in both the blood and lung tissue homogenate. Six hours after exposure, MDA levels statistically significantly increased by 155.9% and 61.1%, respectively, and by 24 hours, by 194.2% and 65.3%, respectively, compared to levels in the intact group ($p < 0.05$).

A significant decrease in catalase activity, coupled with an increase in LPO products, as well as different trends in dynamics of other antioxidant enzymes activities indicate stress on the body's antioxidant system and depletion of its adaptive potential. [17].

Table 2 presents changes in antioxidant enzyme activities and MDA levels in the blood and lung tissue following nitrogen dioxide poisoning at a dose of 1 LCt_{50}.

In cases of inhalation poisoning of nitrogen dioxide, SOD activity significantly differed from that of the intact group both in the blood and lung tissue homogenate. SOD activity decreased in blood by 62.8% after 3 hours, followed by a 36.9% increase after 6 hours compared to that of the intact group. One day (24 h) after poisoning, SOD activity was 26.2% lower compared to that of the intact group.

Activity of SOD in the lung homogenate decreased by 41.1% after 3 hours, then increased up to 69.5% after 6 hours, and remained at the same level by 24 hours, indicating that the characteristic had reached a plateau.

In the blood samples catalase activity increased significantly up to 54.4% after 6 hours and 24 hours later corresponded to the intact group levels. In lung homogenate, a significant increase in catalase activity

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Change in the enzymatic component of the antioxidant system in the blood and lung tissue of rats following inhalation poisoning with PFIB at a dose of 1 LCt_{50} and nitrogen dioxide at a dose of 1 LCt_{50}, n = 6, *Me* [Q_{25}; Q_{75}]
Изменение ферментативного звена антиоксидантной системы в крови и лёгочной ткани крыс на фоне ингаляционного отравления ПФИБ в дозе 1 LCt_{50} и диоксидом азота в дозе 1 LCt_{50}, n = 6, *Me* [Q_{25}; Q_{75}]

Исследуемая ткань Studied tissue	Время регистрации Registration time	Активность СОД, U/мл SOD activity, U/mL	Активность каталазы, мМ/(л · мин) Catalase activity, mM/(L · min)	Активность ГП, мМ/(л · мин) GPx activity, mM/(L · min)	Содержание МДА, нМ/г Concentration of MDA, nM/g
Перфторизобутилен / Perfluoroisobutene					
Кровь Blood	Интактная группа Intact group	115,2 [76,8; 121,9]	332,4 [324,2; 337,9]	27,6 [23,5; 33,3]	1,7 [1,6; 1,9]
	3 ч | h	75,6 [64,9; 82,2]	229,2 [224,1; 294,1]*	11,1 [9,5; 13,2]*	2,1 [1,5; 2,5]
	6 ч | h	162,9 [149,9; 166,7]*	250,8 [236,0; 269,0]*	12,5 [9,9; 14,7]*	4,4 [4.0; 5.0]*
	24 ч | h	157,1 [147,9; 171,6]*	267,3 [179,3; 306,7]*	16,2 [15,6; 16,4]*#	5,0 [4,1; 5,7]*
Лёгкое Lung	Интактная группа Intact group	32,1 [30,1; 32,7]	38,6 [19,5; 66,0]	4,6 [3,2; 7,3]	7,2 [6,9; 7,4]
	3 ч | h	112,3 [109,3; 122,1]*	22,1 [21,5; 23,5]	17,0 [16,2; 17,5]*	8,2 [7,6; 8,9]
	6 ч | h	80,0 [73,7; 84,3]*	17,3 [9,1; 22,8]	13,7 [13,5; 14,6]*	11,6 [11,0; 12,5]*
	24 ч | h	74,1 [66,0; 80,1]*	11,1 [10,0; 13,5]*	10,2 [9,8; 12,6]*	11,9 [11,1; 12,1]*
Диоксид азота / Nitrogen dioxide					
Кровь Blood	Интактная группа Intact group	15,2 [76,8; 121,9]	332,4 [324,2; 337,9]	27,6 [23,5; 33,3]	1,7 [1,6; 1,9]
	3 ч | h	42,9 [17,9; 58,3]*	356,0 [288,0; 404,0]	19,5 [15,4; 20,5]	2,5 [2,4; 3,2]
	6 ч | h	157,7 [143,0; 158,7]*	513,3 [507,9; 539,9]*	15,1 [14,3; 15,7]*	5,5 [4,9; 6,1]*
	24 ч | h	85,0 [63,8; 86,2]*#	342,2 [320,4; 349,3]#	11,7 [10,7; 12,8]*#	4,5 [3,8; 4,8]*#
Лёгкое Lung	Интактная группа Intact group	2,1 [30,1; 32,7]	38,6 [19,5; 66,0]	4,6 [3,2; 7,3]	7,2 [6,9; 7,4]
	3 ч | h	18,9 [5,9; 21,8]*	57,6 [51,4; 83,1]	17,8 [15,4; 18,8]*	8,5 [7,5; 9,4]
	6 ч | h	54,4 [40,9; 63,3]*	20,4 [15,1; 23,7]	6,4 [5,1; 8,9]*	11,0 [10,4; 11,3]*
	24 ч | h	54,5 [48,6; 67,0]*	113,0 [99,7; 142,9]*	16,4 [12,8; 18,1]*	11,0 [10,3; 11,5]*

Note. * – change value is statistically significant compared to the values of the intact group, at $p < 0.05$ (according to the Mann–Whitney *U*-test); # – change value is statistically significant compared to the values of the "after 6 hours" group, at $p < 0,05$ (according to the Mann–Whitney *U*-test).

Примечание. * – изменение значения статистически значимо по сравнению с показателями интактной группы при $p < 0,05$ (по *U*-критерию Манна – Уитни); # – изменение значения статистически значимо по сравнению с показателями группы через 6 часов при $p < 0,05$ (по *U*-критерию Манна – Уитни).

was observed only after 24 hours, reaching 192.7% compared to the intact group.

GP activity in blood early after nitrogen dioxide inhalation exposure tended to decrease and was not significantly different from the intact group values after 3 hours. After 6 and 24 hours, GP levels became significantly lower than control by 45.3% and 57.6%, respectively.

In lung tissue homogenate, glutathione peroxidase activity was phasic and significantly different from the intact group at all observation points. Three hours after exposure, enzyme activity was increased up to 3.9-fold.

After 6 hours, parameter decreased by 2.7 fold compared to the 3-hour level, but activity remained significantly higher than in the intact group by 1.4

fold. One day (24 hours) later, activity of GP increased again and exceeded the intact group values by 3.6 fold.

Experimental data obtained during inhalation poisoning of rats with nitrogen dioxide demonstrated a significant increase in MDA levels at 6 and 24 hours after exposure compared to the intact group. Blood MDA levels were increased by 2.7–3.3 fold, while in lung tissue homogenate, increase reached 1.5 fold.

Discussion

Considered pulmonotoxicants demonstrate different mechanisms of toxic action, but both induce oxidative stress, accompanied by activation of lipid peroxidation processes and an imbalance in the enzymatic component of the antioxidant system. Considered pulmonotoxicants demonstrate different mechanisms of toxic action, but both induce oxidative stress, accompanied by activation of lipid peroxidation processes and an imbalance in the enzymatic component of the antioxidant system.

Changes in enzymatic activity in the blood under the influence of both PFIB and nitrogen dioxide were similar in the direction of their destructive action but differed in the degree of severity and dynamics. Meanwhile, changes in lung tissue were multidirectional, indicating different localization and intensity of the damaging effects of the toxicants.

The mechanism of action of PFIB is related to its ability to acylate biomolecules and trigger a cascade of reactions leading to the formation of ROS. As a result, the pool of antioxidant enzymes is depleted and the function of the body's redox system, including the glutathione system, is impaired.

Severe PFIB poisoning was characterized by a sharp decrease in SOD activity in the animals' blood within the first 3 hours (to 65% of control values), which may be due to overload of the superoxide radical detoxification system. However, by 6 hours, a compensatory increase in this enzyme activity was observed (41% higher than control), indicating the mobilization of the body's defense mechanisms. Moreover, in lung tissue, SOD activity after 3 hours exceeded control values by more than 3 times, indicating a pronounced local tissue response to oxidative stress.

Catalase activity dynamics a during PFIB poisoning showed marked tissue differences. A progressive decrease in this enzyme activity was observed in the blood (to 69% of control values after 3 hours), while a significant decrease in lung tissue was only observed after 24 hours (to 29% of baseline). This difference may reflect different rates of enzymatic reserve depletion of in the systemic circulation and directly in the affected organ. Simultaneously, GP activity in lung tissue increased 3.7-fold within 3 hours of poisoning and remained elevated for 24 hours, emphasizing the key role of the glutathione system in protecting lung tissue from damage.

Nitrogen dioxide also induces oxidative stress, but its action is associated with the formation of nitric and nitrous acids in the respiratory tract, leading to a decrease in pH and the progression of tissue hypoxia associated with high ROS generation. This results in activation of the enzymatic activity of the antioxidant system both in the local (tissue) and systemic levels. A 37% increase in SOD activity was observed in the blood after 6 hours, but by 24 hours this parameter had decreased by 26% relative to control values. In lung tissue, SOD activity also peaked after 6 hours (a 70% increase), but, unlike in the blood, remained elevated 24 hours after exposure to the toxicant. A sharp increase of catalase activity in the lung homogenates 24 hours after poisoning (almost 3 times higher than the control) may considered as sign of compensatory response to the accumulation of peroxide compounds.

MDA levels, as a marker of lipid peroxidation, increased significantly with both toxicants, reaching a maximum after 6–24 hours. With PFIB poisoning, MDA levels in the blood increased by 2.9 times, and in lung tissue by 1.7 times by 24 hours. For nitrogen dioxide, these values were 3.3- and 1.65-fold 6 hours after exposure in blood and in lung homogenates, respectively, then MDA levels subsequently reaching a plateau and remaining stable by the 24th hour in the lung homogenates. These changes indicate significant oxidative damage of cell membranes and correlate with data from other studies describing the role of peroxidative processes in the pathogenesis of toxic pulmonary edema [18].

Limitations of the study The experimental study was performed on albino outbred male rats (body weight 200–240 g), kept in a standard vivarium condition, and randomly divided into groups with the exclusion of weakened and affected animals from the study.

Conclusion

The study performed demonstrates that both investigated pulmonotoxicants induce significant

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changes in the antioxidant system, although the nature of these changes differs somewhat. PFIB leads to significant inhibition of enzymatic activity both in the systemic circulation and in lung tissue. Nitrogen dioxide causes a rapid but short-term increase in antioxidant enzyme activity in the blood, followed by a decrease, while changes in lung tissue are more persistent.

These findings highlight the importance of considering the specific effects of various pulmonotoxicants to develop therapeutic approaches to poisoning treatment.

Differences in the nature and dynamics of changes in the antioxidant system indicate the need for a differentiated approach to correcting oxidative stress depending on the nature of the toxic agent. These results provide the basis for further research aimed at developing effective pharmacological treatments for disorders that arise during toxic lung injury.

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(п.п. 2, 4, 6, 8, 9, 11 см References)

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INFORMATION ABOUT THE AUTHORS

Tatyana M. Ustinova *– Candidate of Sciences in Biology, senior researcher, State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, 195043, St. Petersburg, Russian Federation, https://orcid.org/0000-0001-9579-9190 E-mail: gniiivm_5@mil.ru*

Yury O. Konshakov *– Candidate of Sciences in Medicine, senior researcher, State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, 195043, St. Petersburg, Russian Federation, https://orcid.org/0000-0001-8596-6469*

Nikolaj G. Vengerovich *– Doctor of Sciences in Medicine, Professor, Head of department in State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, 195043, St. Petersburg, Russian Federation, https://orcid.org/0000-0003-3219-341X*

Georgij S. Stepanov *– junior researcher, State Research and Testing Institute of Military Medicine of the Ministry of Defense of the Russian Federation, 195043, St. Petersburg, Russian Federation, https://orcid.org/0000-0001-7959-8559*

СВЕДЕНИЯ ОБ АВТОРАХ

Устинова Татьяна Михайловна *– кандидат биологических наук, старший научный сотрудник, ФГБУ «Государственный научно-исследовательский испытательный институт военной медицины» МО РФ, 195043, Санкт-Петербург, Россия. E-mail: gniiivm_5@mil.ru*

Коньшаков Юрий Олегович *– кандидат медицинских наук, старший научный сотрудник, ФГБУ «Государственный научно-исследовательский испытательный институт военной медицины» МО РФ, 195043, Санкт-Петербург, Россия.*

Венгерович Николай Григорьевич *– доктор медицинских наук, профессор, начальник отдела, ФГБУ «Государственный научно-исследовательский испытательный институт военной медицины» МО РФ, 195043, Санкт-Петербург, Россия.*

Степанов Георгий Станиславович *– младший научный сотрудник, ФГБУ «Государственный научно-исследовательский испытательный институт военной медицины» МО РФ, 195043, Санкт-Петербург, Россия.*