COMPARATIVE ASSESSMENT OF TOXIC PULMONARY EDEMA CAUSED BY POISONING WITH CARBONYL CHLORIDE AND FLUOROPLASTIC THERMAL DEGRADATION PRODUCTS

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Poisoning with acylating pulmonary toxicants results in toxic pulmonary edema (TPE), the approaches to treatment of which are limited. The lung injury similar to poisoning with acylating pulmonary toxicants can be simulation through body's exposure to the fluoroplastic thermal degradation products containing perfluoroisobutylene. The study was aimed to compare toxic pulmonary edema manifestations in the laboratory animals poisoned with an acylating pulmonary toxicant (carbonyl chloride) and fluoroplastic thermal degradation products. Animals (male rats, *n* = 78) were divided into three groups: controls; Poisoning 1, where the animals were exposed to carbonyl chloride; Poisoning 2, where the animals were exposed to the fluoroplastic thermal degradation products. The animals' lung/body ratio was determined and the partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) was assesed 10 min, 1, 3, 6, 24, and 48 h after the exposure. Histological examination of lung tissue was performed 3 and 6 h after the exposure. The increase in the lung/body ratio, decrease in PaO_2, and increase in $PaCO_2$ relative to controls were revealed 3, 6, 24, and 48 h after the exposure to carbonyl chloride and fluoroplastic thermal degradation products. The signs of the interstitial toxic pulmonary edema phase were detected 3 h after the exposure to the studied toxicants, and the signs of alveolar phase were revealed after 6 h. Similar changes were identified in animals of the experimental groups. The findings have shown that the exposure to carbonyl chloride and the fluoroplastic thermal degradation products containing perfluoroisobutylene lead to similar changes in the early post-intoxication period.

Keywords: carbonyl chloride, perfluoroisobutylene, toxic pulmonary edema, fluoroplastic, combustion products, acylating agents

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Compliance with the ethical standards: the study was approved by the Ethics Committee of the Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation (protocol No. 288 dated 20 February 2024). The research procedure was guided by the requirements of the regulatory legal acts on conducting animal experiments, including humane handling of animals (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes).

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СРАВНИТЕЛЬНАЯ ОЦЕНКА ТОКСИЧЕСКОГО ОТЕКА ЛЕГКИХ, ВЫЗВАННОГО ИНТОКСИКАЦИЕЙ КАРБОНИЛХЛОРИДОМ И ПРОДУКТАМИ ТЕРМИЧЕСКОГО РАЗЛОЖЕНИЯ ФТОРОПЛАСТА

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Интоксикация ацилирующими пульмонотоксикантами приводит к формированию токсического отека легких (ТОЛ), подходы к лечению которого ограничены. Поражение легких, сходное с интоксикацией ацилирующими пульмонотоксикантами, может быть смоделировано посредством воздействия на организм продуктов термодеструкции фторопластов, содержащих перфторизобутилен. Целью исследования было сравнить проявления токсического отека легких у лабораторных животных при интоксикации ацилирующим пульмонотоксикантом (карбонилхлорид) и продуктами термического разложения фторопласта. Животных (крыс-самцов, *n* = 78) разделили на три группы: контроль; «интоксикация 1», где животных подвергали воздействию карбонилхлорида; «интоксикация 2», где их подвергали воздействию продуктов термического разложения фторопласта. Через 10 мин, 1, 3, 6, 24 и 48 ч после воздействия у животных определяли легочный коэффициент, анализировали парциальное давление кислорода (PaO_2) и диоксида углерода ($PaCO_2$) в артериальной крови. Через 3 и 6 ч после воздействия проводили гистологическое исследование тканей легких. Через 3, 6, 24 и 48 ч после воздействия карбонилхлорида и продуктов термодеструкции фторопласта были обнаружены увеличение легочного коэффициента, снижение PaO_2 и нарастание $PaCO_2$ по сравнению с контролем. Через 3 ч после воздействия исследуемых токсикантов были выявлены признаки интерстициальной, а через 6 ч после воздействия — альвеолярной фазы токсического отека легких. Выявленные изменения были схожи у животных экспериментальных групп. Результаты исследования показали, что воздействие карбонилхлорида и продуктов термодеструкции фторопласта, содержащих перфторизобутилен, приводят к сходным изменениям в раннем постинтоксикационном периоде.

Ключевые слова: карбонилхлорид, перфторизобутилен, токсический отек легких, фторопласт, продукты термодеструкции, ацилирующие агенты

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The range on substances capable of causing toxic pulmonary edema (TPE) when inhaled is extremely diverse. Pulmonary toxicants with the acylating mechanism of action, carbonyl chloride and perfluoroisobutylene, should be treated as a separate group [1, 2].

Carbonyl chloride and perfluoroisobutylene are used in various sectors of industry. Thus, carbonyl chloride is used as a source component for synthesis of pesticides, plastics, dyes, isocyanates, etc. About 12 million tons of carbonyl chloride is produced annually for the needs of industry [3, 4]. Perfluoroisobutylene is used for synthesis of various fluoroplastic types. Furtermore, it is produced by thermal degradation of various fluoropolymers [2, 4]. The most likely situations associated with the carbonyl chloride and perfluoroisobutylene poisoning can arise during accidents at appropriate chemically hazardous objects [1, 3, 5], including in cases of terrorist acts and sabotage attacks on such objects [6].

Inhalation poisoning with carbonyl chloride and perfluoroisobutylene leads to toxic pulmonary edema, the mechanism underlying the development of which is currently poorly understood [1, 2]; there are no effective approaches to treatment of toxic pulmonary edema [1, 2, 7]. According to the literature, pathological changes observed in the laboratory animals poisoned with carbonyl chloride and perfluoroisobutylene have much in common, which suggests that these toxicants have common mechanisms of action [4].

Today, the literature reports approaches to simulation of toxic pulmonary edema caused by the exposure to the chemically pure carbonyl chloride and perfluoroisobutylene [1, 4, 8]. It is well known that thermal degradation of fluoroplastic yields perfluoroisobutylene [2] that represents a primary cause of toxicity of the resulting thermal degradation products [9]. Given the fact that fluoroplastic itself does not require any specific storage conditions and the fluoroplastic thermal degradation products can be obtained *ex tempore*, the model of toxic lung edema caused by the exposure to the fluoroplastic thermal degradation products can be used to search for agents for etiotropic and pathogenetic therapy of poisoning with the acylating pulmonary toxicants.

The study was aimed to compare manidestations of toxic pulmonary edema in the laboratory animals poisoned with an acylating pulmonary toxicant (carbonyl chloride) and the fluoroplastic thermal degradation products containing perfluoroisobutylene.

METHODS

The experiments involved mature outbred male rats with the body weight of 180–200 g obtained from the Rappolovo breeding nursery (n = 78). Animals were divided into groups (six animals per group): controls; Poisoning 1, where rats were exposed to carbonyl chloride; Poisoning 2, where rats were exposed to the fluoroplastic thermal degradation products. Sedation, analgesia and withdrawal of animals from the experiment were accomplished by using appropriate doses of the tiletamine–zolazepam solution (Zoletil 100, Virbak; France).

Static inhalation carbonyl chloride poisoning of the rats was accomplished in the chamber with the volume of 0.25 m^3. The rats' exposure to the products of thermal degradation of the heat-treated granular fluoroplastic-4 (hereinafter, fluoroplastic) was simulated in the original unit [10]. The temperature of thermal degradation was 320–650 °C, and the thermal exposure duration was 3 min.

Static inhalation poisoning of the rats with carbonyl chloride and the fluoroplastic thermal degradation products in the average lethal concentrations was simulated; the exposure time was 15 min. The carbonyl chloride concentration in the inhalation chamber was determined using the PortaSens II gas analyzer (ATI; USA). Qualitative assessment of perfluoroisobutylene in the gas/air mixture was performed by gas-liquid chromatography–mass spectrometry (Agilent 7890B chromatography system with the Agilent 240 MS mass selective detector (Agilent; USA)). The concentrations of carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2) in the inhalation chamber were determined using the Avtotrest-02.02 gas analyzer (Meta; Russia).

Laboratory animals were withdrawn from the experiment 10 min, 1, 3, 6, 24 and 48 h after the exposure. The lung/body ratio was determined; partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), and the arterial blood pH were estimated using the i-STAT biochemical analyzer (Abbott; USA). The ribbon lung sections were cut with the PFM Slide 2003 sliding microtome (PFM Medical GmbH; Germany). The resulting slides were stained with hemotoxylin and eosin and placed on the glass slides. Histological examination was performed using the Leica DM2000 microscope (Leica Microsystems; Germany). Images were captured using the Olympus LC35 camera (Olympus Scientific Solutions; Japan).

The experimental data obtained were expressed as the median, first and third quartiles (Me [Q_1; Q_3]). The Kruskal–Wallis test was used to compare two or more independent groups; the Newman–Keuls method was used for multiple pairwise comparisons. The intergroup differences were considered to be significant at $p < 0.05$.

RESULTS

When simulating poisoning of animals with carbonyl chloride and the fluoroplastic thermal degradation products, the analysis of the gas/air mixture in the inhalation chamber was performed. The concentration of carbonyl chloride was 68 ppm, the concentration of carbon dioxide was 472 ppm, and the oxygen concentration was 20.8%. Perfluoroisobutylene, carbon monoxide (780 ppm), carbon dioxide (1120 ppm), and oxygen (concentration 20.4%) were determined in the inhalation chamber after the end of thermal degradation.

No signs of the irritant effect were revealed when simulating poisoning of animals with carbonyl chloride and the fluoroplastic thermal degradation products. The condition of the animals after retrieval from the inhalation chamber was the same as that of the control group.

The dynamic changes in the rats' lung/body ratio are provided in Fig. 1. The lung/body ratio of the experimental group animals measured 10 min and 1 h after the exposure did not differ from the values of the control group animals. A significant increase ($p < 0.05$) in the lung/body ratio relative to controls was determined 3, 6, 24, and 48 h after the exposure in the experimental group animals, however, there were no significant differences between animals of the experimental groups (Fig. 1).

Histological examination revealed no abnormalities in the rat lungs obtained 1 h after the exposure to the studied toxicants. The increase in the lung/body ratio 3 h after the exposure was accompanied by the emergence of microscopic changes in the lung tissues (Fig. 2). Thickening of the interalveolar septa, impregnation of those with neutrophils and erythrocytes, vascular congestion, and the emergence of single erythrocytes in the alveolar cavity were revealed in the histology slides 3 h after the exposure to carbonyl chloride and the fluoroplastic thermal degradation products. Alternation of the

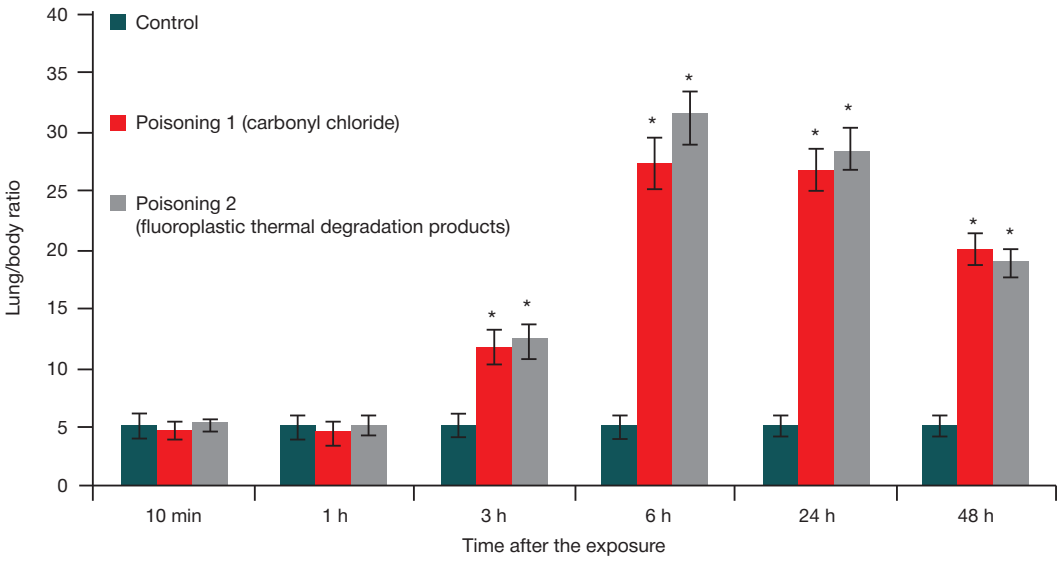

Fig. 1. Dynamic changes in the rat lung/body ratio after the exposure to carbonyl chloride and fluoroplastic thermal degradation products (relative units (Me [Q_1; Q_3]). * — significant differences from controls; six animals per group

emphysematous dilated areas and edematous alveoli filled with the effusion containing fibrin threads, segmented neutrophils, and single erythrocytes was determined in the slides 6 h after the exposure. Some alveoli were enlarged, the alveolar septa were thinned or sometimes absent. Bronchial lumens contained desquamated epithelium. Lymphoid infiltration was visible in the perivascular and peribronchial tissue. The histological alterations identified suggest the development of toxic pulmonary edema (Fig. 2).

The arterial blood gas analysis was conducted for indirect estimation of gas exchange in the lungs. A significant decrease in PaO_2 ($p < 0.05$) and a significant increase in $PaCO_2$ ($p < 0.05$) were reported as early as 1 h after the exposure to the studied toxicants. Significant hypoxemia (decreased PaO_2) and hypercapnia (elevated $PaCO_2$) were observed in blood of the rats poisoned with both carbonyl chloride and the fluoroplastic thermal degradation products 3, 6, 24, and 48 h after the exposure (Fig. 3). Accumulation of carbon dioxide in the rat blood resulted in the pH decrease. Thus, blood pH dropped to 7.22 [7.19; 7.29] and 7.18 [7.11; 7.23] (Poisoning 1 and Poisoning 2 groups, respectively) 6 h after the exposure.

The changes in the arterial blood gas composition of the rats poisoned with carbonyl chloride and the fluoroplastic thermal degradation products identified during the studied period were similar (Fig. 3).

DISCUSSION

Perfluoroisobutylene, which is primarily responsible for toxicity of the resulting gas/air mixture, was determined in the inhalation

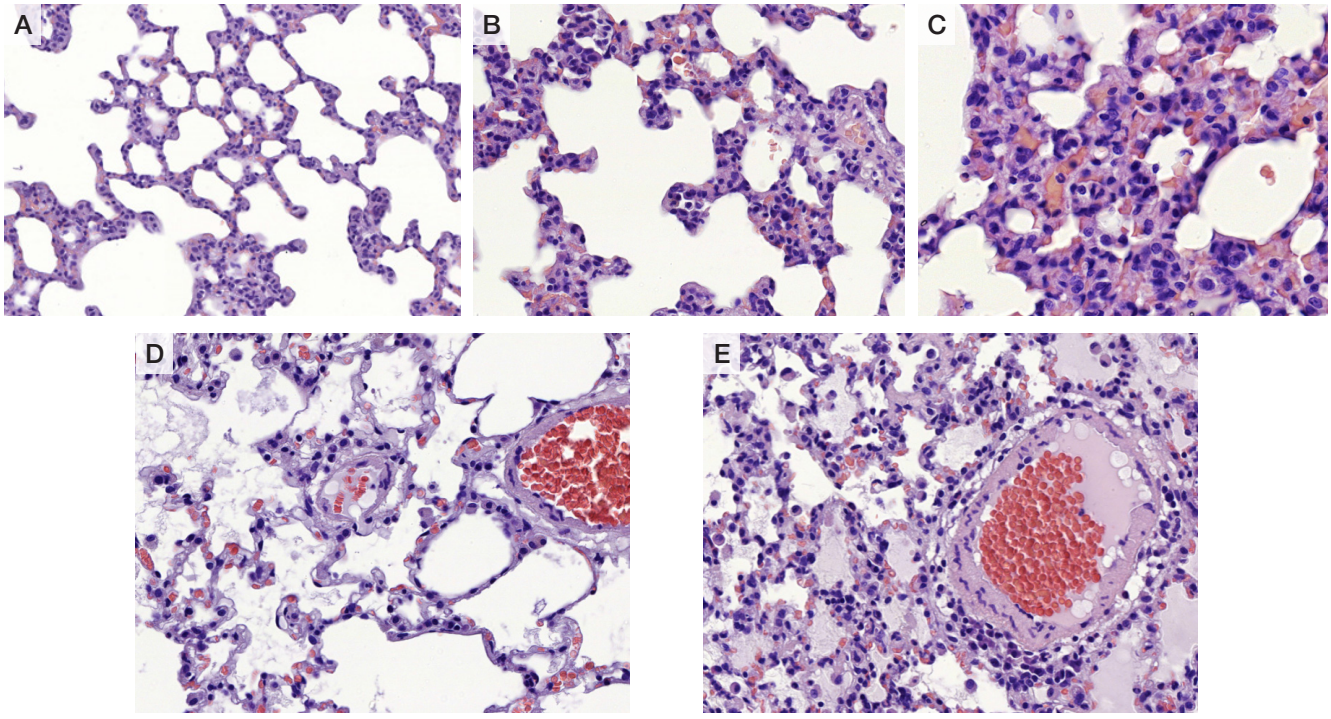

Fig. 2. Histological changes in the rat lung tissues 3 and 6 h after the exposure to carbonyl chloride and fluoroplastic thermal degradation products (hematoxylin and eosin, 50× magnification). **A**. Control. **B**. Poisoning 1 (carbonyl chloride), 3 h. **C**. Poisoning 2 (fluoroplastic thermal degradation products), 3 h. **D**. Poisoning 1 (carbonyl chloride), 6 h. **E**. Poisoning 2 (fluoroplastic thermal degradation products), 6 h

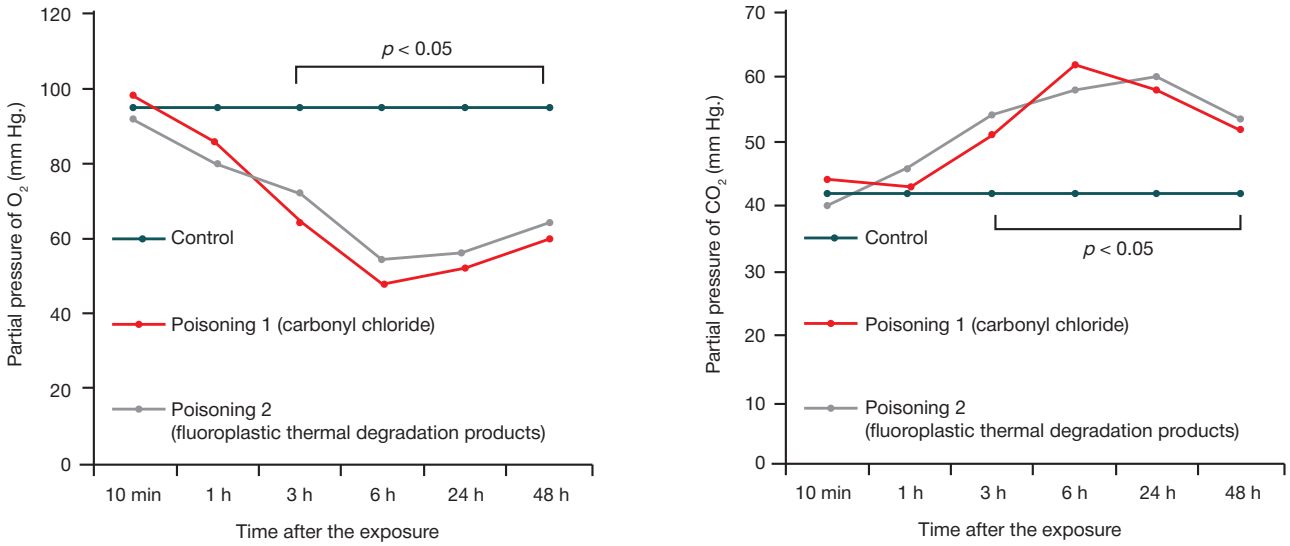

Fig. 3. Dynamic changes in the rat arterial blood partial pressure of oxygen (left) and carbon dioxide (right) at different times after the exposure to carbonyl chloride and fluoroplastic thermal degradation products (mm Hg (Me)). $p < 0.05$ — significant differences from the groups Poisoning 1 (carbonyl chloride) and Poisoning 2 (fluoroplastic thermal degradation products)

chamber during thermal degradation of fluoroplastic [9]. The carbon monoxide concentration in the inhalation chamber corresponded to 0.1 LC_{50} (for rats exposed for 15 min) [11]. The oxygen concentration did not drop below 20.4%. Thus, the animals' condition severity cannot be associated with hypoxic and/or hemic hypoxia.

In our study, lesions in the lung tissues of the rats exposed to the studied toxicants were detected 3 h after the end of exposure. The increase in the lung/body ratio was revealed, which indirectly indicated accumulation of extravascular lung water [12]. As toxic pulmonary edema manifestation progressed (6 h after the exposure), an even higher lung/body ratio was determined, which remained elevated 24 and 48 h after the exposure. The lung/body ratio increase was accompanied by the emergence of mixroscopic changes in lung tissues. The signs of the interstitial toxic pulmonary edema phase were detected 3 h after the exposure to the studied toxicants, and the signs of alveolar phase were revealed after 6 h.

Disruption of the blood-air barrier structure was accompanied by disturbed gas exchange. Thus, the most prominent decrease in PaO_2 and increase in $PaCO_2$ of arterial blood were determined 6 h after the exposure. Such changes are associated with abnormal gas diffusion caused by thickening of the blood-air barrier due to extravascular fluid accumulation [12]. Accumulation of carbon dioxide in arterial blood and disturbance of the aerobic oxidation processes associated with arterial hypoxemia resulted in the altered acid-base condition of blood manifested by mixed acidosis.

The experimental data obtained suggest that severity of the condition of the laboratory animals poisoned with carbonyl chloride and the fluoroplastic thermal degradation products results from respiratory hypoxia. Among respiratory hypoxia manifestations, arterial hypoxemia and altered acid-base condition caused by disruption of the blood-air barrier and disturbed gas exchange in the lungs were reported. It is important to note that in the studied period manifestations of toxic pulmonary edema were similar in rats exposed to both carbonyl chloride and the fluoroplastic thermal degradation products containing perfluoroisobutylene.

CONCLUSIONS

Manifestations of toxic pulmonary edema (arterial hypoxemia and hypercapnia, morphological changes in the lung tissues, lung/body ratio) caused by the rat exposure to the fluoroplastic thermal degradation products containing perfluoroisobutylene were similar to that observed under exposure to the chemically pure carbonyl chloride in the early post-intoxication period. Given the fact that fluoroplastic is chemically, physically, and biologically inert and does not require specific storage conditions [9], the fluoroplastic thermal degradation products obtained ex tempore can be used for modeling toxic pulmonary edema in animals, as demonstrated in our study. Thus, the model reported allows one to adequately simulate toxic pulmonary edema in rats, similar to that resulting with poisoning with the acylating pulmonary toxicants. This makes it possible to conduct experimental studies focused on further investigation of the development mechanism and the search for treatment options for toxic pulmonary edema.

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