Оригинальные статьи

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Hypothermia induced by bronchoalveolar lavage with perfluorocarbon liquids as a method for the treatment of the alveolar stage of toxic pulmonary edema. Experimental assessment

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Introduction. The rapid development of the chemical industry in the Russian Federation is accompanied by an increase in the production and storage of highly toxic substances belonging to the group of pulmonotoxicantsare able to induce lung injury, the most severe form of which is toxic pulmonary edema (TPE). The treatment of TPE includes medication and respiratory therapy, but such a combination turns out to be insufficient, and therefore the development of new methods of treatment of the alveolar stage is an important task of modern medicine. One such approach is the use of therapeutic hypothermia, which can reduce the severity of TPE in general and the alveolar stage in particular. Recently, the use of various options for liquid ventilation of the lungs with perfluorocarbon (PFC) liquids for the induction of therapeutic hypothermia has attracted increasing attention from specialists. This article will present the results of an experimental assessment of the effect of hypothermia induced by BAL with PFC fluids on the course of the alveolar stage of toxic pulmonary edema.

The study aims to determine the effect of hypothermia induced by bronchoalveolar lavage with a pre-cooled PFC liquid on the course of the alveolar stage of toxic pulmonary edema.

Materials and methods. The authors conducted a study on male Wistar rats aged 4 months, weighing 200–220 g. TPE was modeled by intratracheal administration of 0.1 m HCl solution at a dose of 2 ml/kg. Specialists pre-injected atropine into the animals, then anesthetized, intubated with cannulas, injected a model substance and connected to a ventilator. After that, the animals were randomized by weight into two groups of 6 individuals each. In animals of the experimental and control groups, with a decrease in oxygen saturation (SpO_2) below 80%, the researchers performed 2 procedures of bronchoalveolar lavage (BAL) with PFC liquid in a single dose of 2.0 ml / kg, with different temperatures. Perfluorodecalin was used as a PFC liquid. In the experimental group, the temperature of the PFC liquid during BAL was 0.5°C, in the control group — 38.0°C. Specialists recorded heart rate (HR), oxygen saturation, rectal temperature (T_{rect}) and duration of survival.

Results. Immediately after instillation of 0.1 M HCl solution, all animals showed a sharp decrease in SpO_2 (from 97±1.3 to 64±11.5) and heart rate (HR), (from 269±8.7 to 123±24.5). Recovery of indicators was observed after administration of atropine sulfate. Over the next 25–30 minutes, the following signs appeared and subsequently intensified: a decrease in SpO_2 below 80%, an increase in heart rate (HR) to 302±11.4 beats/min, the presence of wet wheezing in the lungs and the release of exudate from the endotracheal catheter. In response to the BAL, all animals showed an increase in SpO_2 and a decrease in heart rate. So, in the control, in response to the BAL, SpO_2 increased by 8.0±2.5%, and heart rate decreased by 21.0±5.4 beats /min. While in the experimental group there was an increase in SpO_2, which was 11.0±3.1%, and a decrease in heart rate by 57.0±10.2 beats/min. At the same time, during the experiment, the researchers observed an increase in temperature in the control group by 0.08±0.02°C, and in the experimental group — its decrease by 0.70±0.07°C. The average survival time of animals in the control and experimental groups was 64.8±2.2 minutes and 91.2±5.9 minutes, respectively.

Conclusion. *The use of BAL PFC liquids with a low temperature makes it possible to increase the survival time of small laboratory animals in the alveolar stage of TPE due to the development of local and general hypothermia. The development of local hypothermia during BAL of the lungs makes it possible to remove a significant amount of edematous fluid, which, in turn, manifests itself in less pronounced respiratory and systemic hemodynamic disorders.*

Ethics. Studies involving laboratory animals were conducted in compliance with the following regulations: Helsinki Declaration of 2000 "On humane treatment of animals", Order of the Ministry of Health of the USSR No. 755 dated 08/12/1977 "Rules for carrying out work using experimental animals", Order of the Ministry of Health and Social Development of Russia No. 199n dated 04/01/2016 "On approval of the rules of laboratory practice". The research protocol was approved by the Ethics Committee of the Izmerov Research Institute of Occupational Health (Protocol No. 4 dated May 25, 2022).

Keywords: *perfluorocarbon; perfluorodecalin; toxic pulmonary edema; hypothermia; bronchoalveolar lavage*

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Introduction. The rapid development of the chemical industry in the Russian Federation, caused by sanctions pressure from unfriendly states, is accompanied by an increase in the scale of production and storage, including highly toxic chemicals, which is a prerequisite for accidental or intentional accidents at chemical enterprises with the occurrence of mass sanitary losses among the civilian population [1]. The results of the analysis of fatal accidents at industrial chemical facilities for the period from 2012 to 2021 indicate that the main substances that caused acute poisoning were carbon monoxide and dioxide, chlorine, hydrogen sulfide, ammonia, formaldehyde, sulfur dioxide, phenol, acids, herbicides, fungicides and others [1]. Most of them belong to the group of pulmonotoxicants, severe forms of poisoning which are characterized by the development of non-cardiogenic toxic pulmonary edema (TPE), occurring in two stages — interstitial and alveolar[1].The latter is characterized by the accumulation of exudate in the alveolar space, rapid progression of acute respiratory failure and, as a rule, the development of an unfavorable outcome. This stage is difficult to treat even in modern realities, although

[1] Basharin V.A., Chepur S.V., Tolkach P.G., Vengerovich N.G., Yudin M.A., Nikiforov A.S., et al. Toxicology of pulmonotoxicants. textbook., 2021.

mortality from it has decreased somewhat recently. TPE is included in the list of occupational diseases[2].

Treatment of the alveolar stage of TPE, which is the most severe form of acute respiratory distress syndrome (ARDS), includes medication (the use of high doses of glucocorticoids and diuretics, the use of narcotic analgesics and defoamers) and respiratory therapy. Currently, respiratory therapy is the main method of treating TPE, including at the alveolar stage, and consists in performing artificial lung ventilation (ALV) in a moderate hyperventilation mode with a positive pressure at the end of exhalation (PPEE) greater than 5 cm H_2O and an oxygen content in the inhaled mixture (FiO_2) of at least 40%. However, as a rule, such therapy turns out to be ineffective, and therefore the development of new methods of treatment of the alveolar stage of toxic pulmonary edema is an important task of modern medicine.

Toxic pulmonary edema is accompanied by a diffuse inflammatory process in the pulmonary parenchyma, resulting from increased permeability of the vascular wall, migration of immunocompetent cells and the release of inflammatory mediators, the intensity of which, theoretically, can be reduced due to hypothermia.

Therapeutic hypothermia is a treatment method based on artificially lowering the patient's body temperature to achieve the necessary therapeutic effect.

This method can effectively reduce brain hypoxia, improve cellular metabolism and reduce inflammatory autoaggression of the body [2]. In clinical practice, therapeutic hypothermia is used to prevent hypoxic-ischemic encephalopathy caused by cardiac arrest, acute cerebrovascular accident, as well as spinal cord and brain injury [2]. Thus, maintaining mild hypothermia in a patient who suffered cardiac arrest with the help of chilled saline solution (32.0–34.0°C) favorably affected neurological symptoms and reduced mortality in this category of patients [3].

Recently, the use of various options for liquid ventilation with perfluorocarbon (PFC) liquids for the induction of therapeutic hypothermia has attracted more and more attention from specialists. This is due to the fact that PFCs have several orders of magnitude higher heat capacity compared to air, due to which the heat exchange process in the lungs during breathing of PFCs occurs much faster, which determines the possibility of their use in liquid respiration to create hypothermia directly in the chest organs in severe lung lesions [4, 5].

In recent years, experimental studies have been widely conducted on the use of various variants of liquid ventilation for the treatment of ARDS of various etiologies [6–9]. The combination of artificial ventilation and hypothermia is available in the publication Wei F. [10], where the authors established on the ARDS model in dogs that hypothermia caused by partial fluid ventilation (PFV), compared with artificial lung ventilation (ALV), not only significantly increased the partial pressure of oxygen in arterial blood, but also reduced the volume of lung damage, at the same time, it significantly weakened the inflammatory response, as evidenced by a significant increase in the expression of anti-inflammatory factor (IL-10), decreased expression of proinflammatory interleukin (IL-6) and tumor necrosis factor-α (TNF-α) in peripheral blood and bronchoalveolar fluid.

[2] Order No. 417n of the Ministry of Health and Social Development of the Russian Federation dated 04/27/2012 "On approval of the list of occupational diseases".

Previously published research results showed the effectiveness of bronchoalveolar lavage (BAL) with PFC liquid in the treatment of alveolar edema [11].

Based on the above, it was assumed that the induction of hypothermia due to BAL with PFC liquid may have a beneficial effect on the course of the alveolar stage of TPE.

The study aims to determine the effect of hypothermia induced by bronchoalveolar lavage with PFC liquid on the course of the alveolar stage of TPE.

Materials and methods. The study was performed on male Wistar rats aged 4 months, weighing 200–220 g (209.4±5.1 g, mean value ± standard deviation). Before starting the study, the authors kept the animals in quarantine for 14 days. To determine the number of animals in the sample and distribute them into groups, the researchers used a statistical processing recommendation[3].

For the purpose of premedication, the authors introduced atropine sulfate intramuscularly to all animals at a dose of 0.2 ml/kg to prevent reflex cardiac arrest. The animals were anesthetized by intramuscular administration of veterinary drugs "Zoletil" and "Decor" at a dose of 1 and 0.5 mg/kg, respectively. After that, the rats were intubated using a 16G intravenous catheter (Polymed, India) and connected to a ventilator for small laboratory animals (Ugo Basile, model 7125-30, Italy) with the following parameters: volume control mode (VCM), respiratory volume — 6 ml/kg, respiratory rate — 70–75 breaths./min., the concentration of oxygen in the inhaled mixture (FiO_2) is 21%. Acute lung injury was modeled by intratracheal administration of 0.1 M HCl solution at a dose of 2 ml/kg [11].

After the introduction of the model substance, the animals were randomized by weight into 2 groups, a control and an experimental group of 6 individuals each. Specialists have measured SpO_2 and heart rate in animals of both groups every 5 minutes from the start of acid administration using a portable pulse oximeter (Zoomed LLC, UT100 model, Russia).

The specialists have measured SpO_2 in animals of both groups, and also measured the heart rate every 5 minutes from the start of acid administration using a portable pulse oximeter (Zoomed LLC, UT100 model, Russia). Rectal temperature was measured throughout the experiment using an electronic thermometer (TianJin, DC-1 model, China). When signs of the alveolar stage of TPE appeared (wet wheezing in the lungs, fluid discharge from the lumen of the boat), the animals were given a BAL using PFC of liquids of different temperatures. Perfluorodecalin (PFD) produced by HaloPolymer JSC was used as a PFC liquid [11].

In the experimental group, the temperature of the PFC of the liquid during the BAL was 0.5°C, in the control group — 38.0°C. The temperature of the PFC of the liquid was measured using a TP-300 thermometer (Cuiisw, China). Prior to the BAL, perfluorodecalin was enriched with oxygen using the Mark 5 Nuvo Lite oxygenator (Nidec, Japan) up to 80 vol.%, which was determined using the Microx 4 oxygen analyzer (PreSens, Germany). Carrying out the the BAL in both groups: PFC liquid was collected using a 5 ml syringe, which was then attached to an endotracheal cannula and injected for 3–5 seconds.

[3] Recommendations for statistical processing of the results of experimental toxicological studies. Council for the Coordination of scientific research and the introduction into practice of scientific achievements of the Ministry of Health of the USSR 1965 from 15–33.

Then the animal was transferred to a ventilator for 20–30 seconds in the background, after which the aspiration of PFC and edematous fluid into a dry test tube was carried out using a syringe. The volume of aspirated PFD and edematous fluid was assessed using a measuring glass cylinder. The indication for the first BAL was a decrease in SpO_2 below 80%, the second BAL was held 15 minutes after the first. The researchers have performed an necropsy after the death of the animals to determine the pathological picture in the lungs.

The authors processed the data obtained by methods of variational statistics using the Microsoft Excel program. To analyze the date, experts used descriptive statistics: scientists calculated the average values *(M)* and standard errors of the average value *(m)*. They checked the data for the normality of the distribution using the Shapiro–Wilk criterion, analyzed the intergroup differences using parametric methods using the Student's criterion. The differences were considered significant at $p<0.05$. For data related to a distribution other than normal, the researchers used the nonparametric Mann–Whitney method.

Results. After instillation of 0.1 M HCl solution, all animals showed a sharp decrease in SpO_2 (from 97±1.3 to 64±11.5) and heart rate (from 269±8.7 to 123±24.5). We observed the recovery of indicators after the administration of atropine sulfate (i/m 0.1 ml/kg) against the background of a ventilator. During the next 25–30 minutes the appearance and subsequent increase of the following signs was noted: a decrease in SpO_2 below 80% ***(Fig. 1)***, an increase in heart rate to 302±11.4 beats/min. ***(Fig. 2)***, wet wheezing over the entire surface of the lungs and the release of foamy fluid from the endotracheal catheter. These signs indicated the development of the alveolar stage of TPE and were an indication for the BAL. In response to the BAL, all animals showed an increase in SpO_2 and a decrease in heart rate. However, the degree of severity of these changes was different in the groups. So, in the control, in response to the BAL, SpO_2 increased by 8.0±2.5%, while the heart rate decreased by 21.0±5.4 beats/min. In turn, in the experiment after BAL SpO_2 increased by 11.0±3.1%, and heart rate decreased by 57.0±10.2 beats/min. The death of animals in the control group occurred at an average SpO_2 value of 64.6±4.2% and a heart rate of 190.4±24.9 beats/min. While in the experimental group, the onset of adverse outcomes was recorded at SpO_2 equal to 71.2±5.6% and heart rate — 148.7±31.5 beats/min.

When studying the amount and composition of aspirated lavage liquid after the BAL, it was found that these indicators differed significantly depending on the group. Thus, the total volume of aspirated lavage fluid in the control was 12.2±0.5 ml/kg, of which PFD was 10.5±0.5 ml/kg, edematous fluid was 1.7±0.2 ml/kg. In turn, in the experimental group, the total volume was 9.4±0.5 ml/kg, of which PFC — 7.3±0.5, edematous fluid — 2.2±0.2 ml/kg.

When studying the rectal temperature, it was found that in the control group, in response to the PFC BAL with a liquid with a temperature of 38.0°C, the change in this indicator during the experiment was 0.08±0.02°C. In turn, in the animals of the experimental group, in response to the PFC BAL with a liquid with a temperature of 0.5°C, there was a decrease in rectal temperature by 0.4±0.1°C for 2–3 minutes, with an average decrease during the experiment by 0.70±0.07°C.

When studying the timing of death, we found that in 5 out of 6 animals of the experimental group, the survival time was more than 70 minutes, while in the control group this indicator was significantly less than the specified value ***(Fig. 3)***. At the same time, the average survival time of animals after endotracheal administration of 0.1 M HCl solution in the experimental group was 91.2±5.9 minutes, which is significantly ($p<0.05$) more than in the control — 64.8±2.2 minutes. It was also found that the survival time of rats after the appearance of signs of the alveolar stage of pulmonary edema in the control group was 27.5±1.1 minutes, which is 1.5 times lower than in the experimental group — 41.6±2.5 minutes.

As a result of necropy, we have not identified any significant differences in the macroscopic picture of the lungs of animals of the control and experimental groups. Differences were observed in lung mass coefficients. So, in

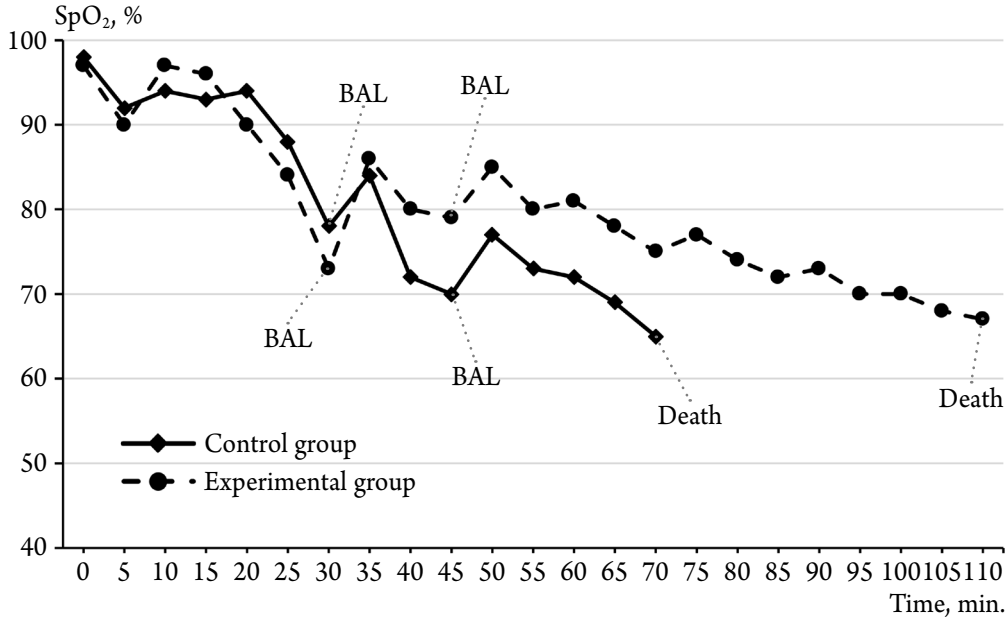

Fig. 1. Changes in oxygen saturation in animals of the control and experimental groups during the experiment

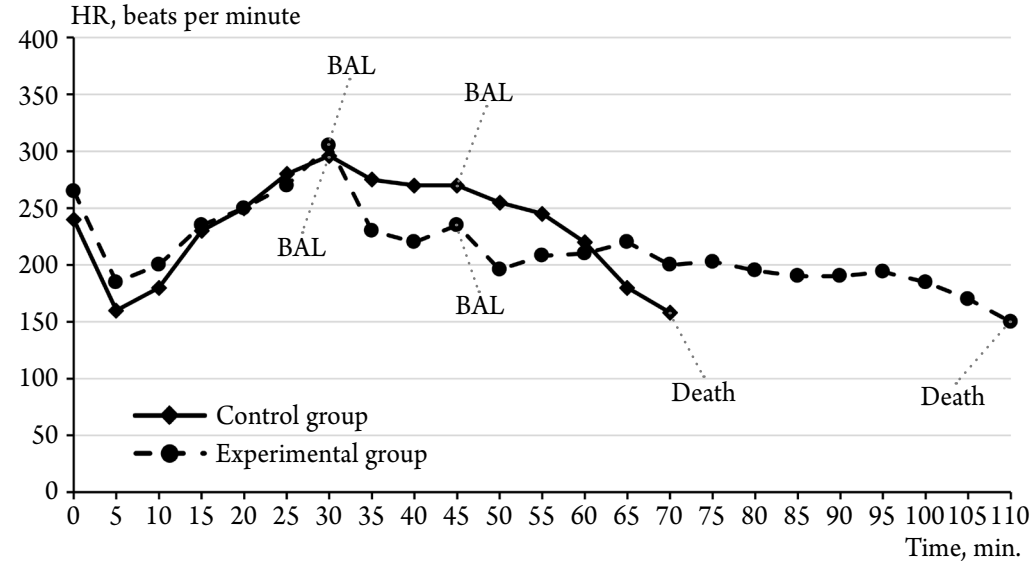

Fig. 2. Changes in heart rate in animals of the control and experimental groups during the experiment

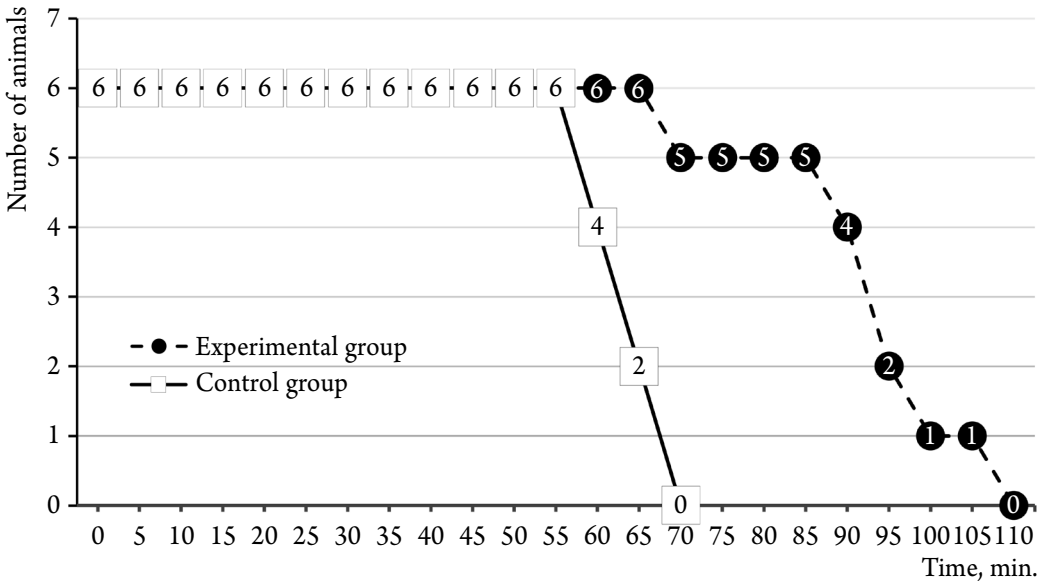

Fig. 3. Kaplan–Meyer survival curves in control and experimental animals

the experimental group, this indicator was 1.86±0.03%, which is significantly (p<0.05) less than in the control group — 2.03±0.04%, despite the greater amount of aspirated PFD at BAL in the control group compared with the experimental one, which indicated a greater amount of edematous fluid in the lungs of animals of the control group.

Discussion. The ability of therapeutic hypothermia to reduce the intensity of metabolic processes is currently widely used in clinical practice, in particular in the treatment of damage to the central nervous system [2–4].

Based on the data obtained, it can be said that a BAL using PFC with a low-temperature liquid leads to a sharp and prolonged decrease in heart rate; a short-term increase in SpO_2 with each BAL, a significant and prolonged decrease in rectal body temperature. The results obtained indicate that with a local decrease in lung temperature due to the low-temperature PFD BAL, it is possible to remove a greater amount of edematous fluid from the lungs in the alveolar stage of TPE compared with the normothermal BAL and, thereby, not only reduce the severity of respiratory and hemodynamic disorders, but also increase the duration of animal survival.

Based on the analysis of our own data and data from literary sources, we can assume the following mechanism of action of hypothermic BAL with PFC liquids during the treatment of TPE: due to hypothermia, the rate of inflammatory reactions decreases [12], which reduces the intensity of cytokine storm development; due to a decrease in the intensity of metabolism, it is possible to provide the body with oxygen and remove carbon dioxide from tissues when lower intensity of gas exchange with the environment [13]; due to hypothermic vasoconstriction [14, 15], the intensity of blood flow through damaged vessels decreases in lung tissues, which reduces the degree of blood loss.

However, it is worth noting that the method used to create hypothermia of the lungs using a BAL with cooled PFD has a short-term positive effect and does not prevent the progression of TPE. In this regard, in our opinion, hypothermia induced during a long session by total liquid ventilation of the lungs with PFC with liquids may become a promising method of treating TPE in the alveolar stage. This

will ensure a long-term controlled decrease in temperature, constant evacuation of edematous fluid, and gas exchange in the lungs when gas breathing is not effective enough.

Conclusion. *The use of BAL PFC with a low temperature liquid makes it possible to increase the survival time of small laboratory animals in the alveolar stage of TPE due to the development of local and general hypothermia. The development of local hypothermia in BAL allows the removal of a significant amount of edematous fluid, which, in turn, is manifested by less pronounced respiratory and systemic hemodynamic disorders.*

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