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Effect of Xe/O_2 Inhalation on Hemostasis in Experimental Thromboplastin Pneumonitis

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We studied the effectiveness of Xe/O_2 mixture inhalation (30% Xe and 70% O_2, 20 min for 5 days) in a model of experimental thromboplastin pneumonitis. Inhalation of the studied mixture decreased the intensity of the inflammatory process in the lung tissue assessed by the temperature response of animals, changed lung weight and lung weight coefficient. At acute stage of pneumonitis, an increase in xenon consumption was recorded due to its retention in the gas exchange zone and a natural decrease in oxygen consumption due to partial alveolar/capillary block. The formation of pneumonitis was accompanied by a pronounced procoagulant shift in the regulation system of the aggregate state of blood. The Xe/O_2 inhalations ensured physiologically optimal levels of prothrombin and activated partial thromboplastin time against the background of a moderate decrease in fibrinogen level throughout the experiment. At the same time, the activity of the natural anticoagulant antithrombin III increased from day 5 to day 14.

Key Words: *xenon; thromboplastin; lungs; pneumonitis; hemostasis*

The lungs play a major role in immunoprotection, implementation of complex inflammatory reactions, and in the exchange of biologically active substances such as serotonin, bradykinin, histamine, prostaglandins, and surfactant [1-3]. Moreover, lung megakaryocytes are considered sites of active thrombocytopoiesis [2,4]. In turn, the activated platelets are the connecting link between hemostasis and immunity, participating in the development of diffuse alveolar damage and acute respiratory distress syndrome [2]. This fact enriches and updates information on the pathogenesis of lung damage in viral pneumonitis when the integrity in the lung microvascular endothelium and the blood–air barrier is violated by endogenous and exogenous aggression factors accompanied by permanent thrombotic complications [5-7].

Thromboplastin model of pneumonitis is the optimal choice for searching and evaluating the effective therapeutic interventions to relieve and correct hemostasis disorders in viral lung injury [8]. This model is the strictest one in relation to the formation of a procoagulant status. Inhalation of a xenon and oxygen mixture (Xe/O_2) demonstrated high efficiency in restoring of lung pneumatization and anti-inflammatory activity [9-11].

The purpose of this study was to evaluate the antithrombotic effects of Xe/O_2 inhalations in a model of thromboplastin pneumonitis.

MATERIALS AND METHODS

Experiments were carried out on female CD-1 mice (n=75) weighing 18-25 g obtained from the nursery of

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the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (health certificate available). Mice were kept in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The maintenance of animals and the design of experiments were approved by the Bioethics Committee of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (Protocol-Application No. 197a042022 of April 11, 2022).

The animals were divided into 3 groups (25 animals in each): control (intact), the model of thromboplastin pneumonitis (PT), and the PT model with Xe/O_2 inhalations (PTI). To simulate pneumonitis, mice were intravenously injected with a 0.8% thromboplastin solution in a volume of 300 μl [8]. The study was carried out 1, 3, 5, 7, and 14 days after administration of thromboplastin solution. The temperature was additionally measured on days 8 and 11 without sacrificing of the animals.

Inhalation of a Xe/O_2 mixture in a ratio of 30%/70% was carried out in a sealed inhalation chamber with composition monitoring using a GKM-03 gas analyzer (INSOVT) [12]. To evaluate the consumption of Xe and O_2 during the experiment, these parameters were digitized in the chamber during the procedure. Excess of CO_2 was removed by a soda lime sorbent (Alba Healthcare). Inhalations were carried out from day 1 to day 5, the duration of the procedure was 20 min.

The first inhalation was performed 1 h after PT modeling. The weight of the animals and the temperature were measured once a day. Body temperature was measured with a TME 2000 rectal thermometer. At the terminal stages of the experiment, the lungs were weighed and the lung weight coefficient was calculated as the ratio of the weight of the organ to the weight of the animal (in %). Lung damage was assessed on standard histological preparations stained with hematoxylin and eosin light microscopy. Prothrombin time, activated partial thromboplastin time (APTT), fibrinogen concentration, and antithrombin III (AT-III) activity were determined on a HelenaC4 semi-automatic analyzer (Helena BioSciences Europe). The blood was collected from the heart into test tubes with sodium citrate 3.8% in a ratio of 1:9.

The results obtained were statistically processed using a StatPlus 7.6.5 software (AnalystSoft, Inc.). The arithmetic mean (M) and the error of the mean (m) were calculated for each sample. Normality of distribution was tested using standardized coefficients of skewness and kurtosis. When the distribution did not correspond to the normal law of distribution, the nonparametric Mann–Whitney test was used. The significance level was set at 1 and 5%.

RESULTS

According to routine histological study performed at the terminal stages of the experiment, the morphological picture of the lungs in the PT and PTI groups was almost identical. The changes characteristic of this model were as follows: thrombosis on day 1 (obstructive and mural blood clots in the lung vessels), necrosis on day 3 (foci of necrosis and hemorrhages in the pulmonary parenchyma), inflammation on days 5-7 (edema and inflammatory infiltration of the lung parenchyma), and fibrosis on day 14 (blood clot formation in the vessels and foci of fibrosis). The severity degree and prevalence of lung damage was estimated by the dynamics of temperature, body and lung weights, and the nuances of consumption of inhaled gases.

Regarding the consumption of the inhaled mixture, it should be noted that O_2 consumption substantially reduced (by 15-20% from day 1 to day 7; $p<0.05$) and Xe consumption increased by 20-25% ($p<0.05$) compared with control. The explanation for this phenomenon is seen in the intense binding of xenon to phospholipids of the damaged surfactant in the gas exchange zone and a natural decrease in oxygen consumption due to a partial alveolar-capillary block.

Monitoring the temperature of the animals showed that body temperature in the PT group statistically significantly increased from day 1 until the end of the study ($p<0.05$ in comparison with the control; Fig. 1, *a*). In the PTI group, the body temperature increased from day 7 in comparison with the control ($p<0.05$) but was lower than in PT group ($p<0.01$).

In the experimental groups, changes in lung weight and lung weight coefficient were similar and generally reflected the course of the inflammatory process manifested by hemorrhages, infiltration, and development of serous pulmonary edema, leading to an increase in organ weight (Fig. 1, *b*, *c*). In the PT group, the increase in lung weight and lung weight coefficient was statistically significant ($p<0.05$) compared to the control throughout the study. At the same time, despite histologically confirmed tissue damage in the PTI group, lung weight and lung weight coefficient did not significantly increase, probably, due to the xenon-induced restoration of the functional activity of pulmonary surfactant that provides increased pneumatization of lung tissue [9-11].

The regulation of hemostasiological balance between the pulmonary and systemic circulation is a non-respiratory function of the lungs. In response to a thrombogenic stimulus in the pulmonary circulation, blood coagulation activity increases leading to microcirculatory thrombosis and disseminated intravascular coagulation [13-15]. In our case, pneumonitis modeled

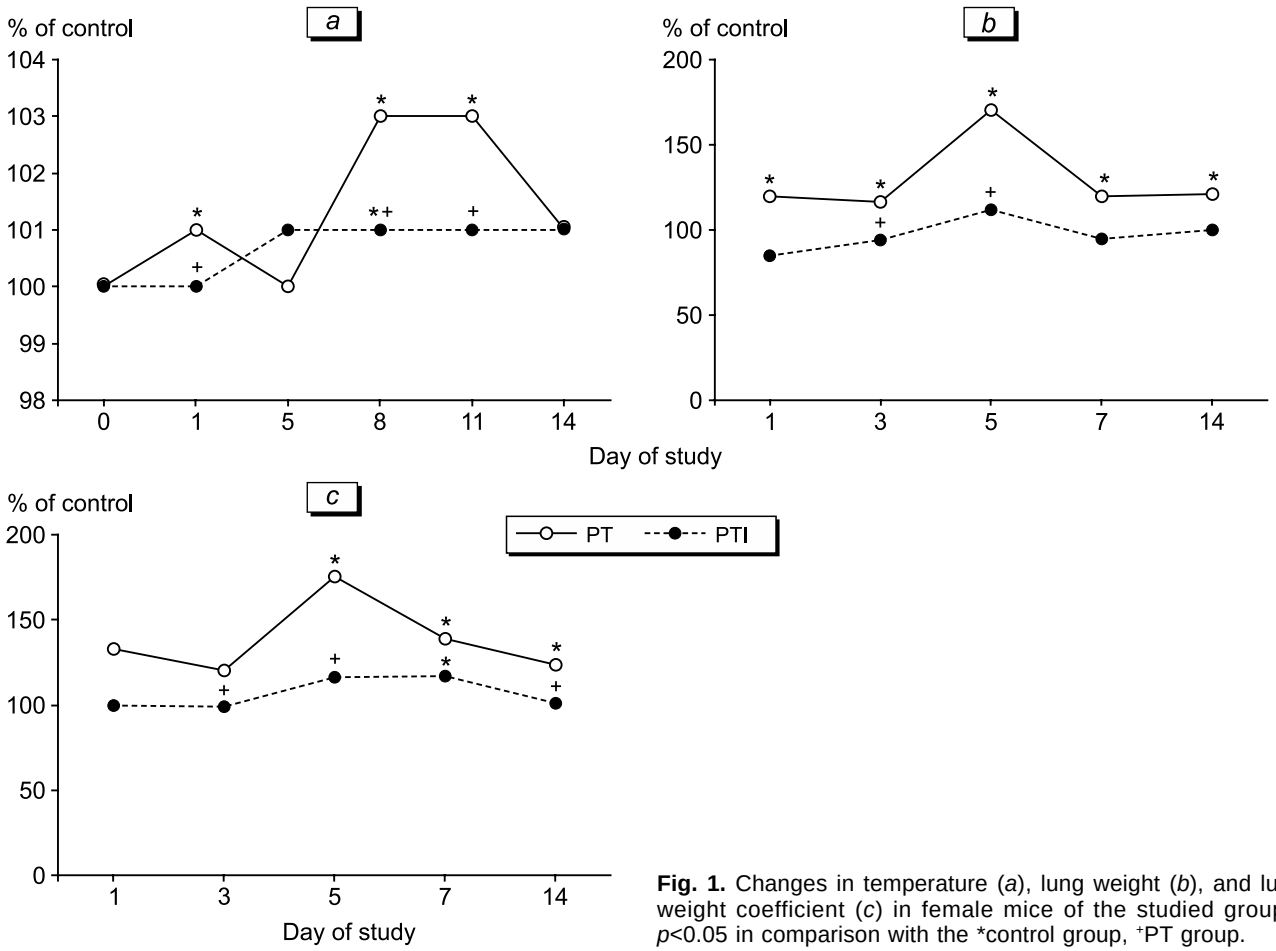

Fig. 1. Changes in temperature (*a*), lung weight (*b*), and lung weight coefficient (*c*) in female mice of the studied groups. $p<0.05$ in comparison with the *control group, $^{+}$PT group.

by thromboplastin damage of the pulmonary vascular bed did not achieve coagulopathy (increase in fibrinogen content during all observation periods for the PT group; Fig. 2, *a*) and led to the formation of a procoagulant status (decrease in prothrombin time ($p<0.05$) and APTT ($p<0.01$) compared to the control; Fig. 2, *b*, *c*). Moreover, in the PTI group, the results obtained by clotting methods and the concentration of fibrinogen did not differ from the control throughout the study. The dynamics of AT-III concentration indicates better disseminated intravascular coagulation developed in response to a thrombogenic stimulus. Thus, the AT-III concentration significantly decreased in the PT group ($p<0.05$ compared to the control) from day 3 until the end of observation (Fig. 2, *d*). In the PTI group, the elements of the complex anti-inflammatory action of xenon ensured a normocoagulation status of the hemostatic potential throughout the observation, and by day 14 the concentration of AT-III significantly increased compared with the control indicating the angioprotective effects of the course of inhalations.

Thus, the results obtained on an extremely "hard" model of PT demonstrated the positive effect of xenon on the regulation system of the blood aggregate state, realized through the well-known mechanism of restoring the function of the air–blood barrier [11].

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Conflict of interest. The authors have no conflicts of interest to declare.

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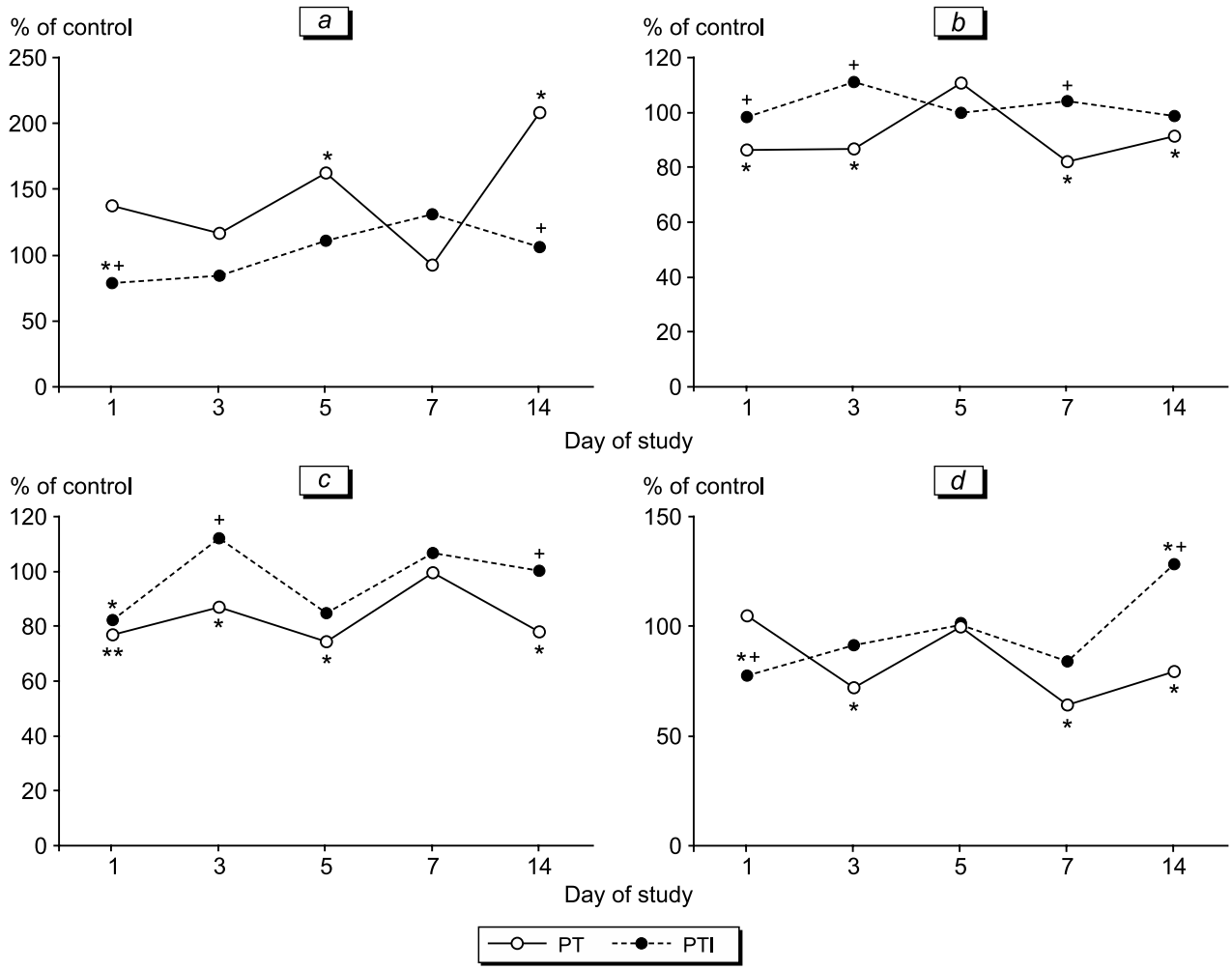

Fig. 2. Changes in fibrinogen concentration (*a*), prothrombin time (*b*), APTT (*c*), and AT-III activity (*d*) in female mice of the studied groups. **p*<0.05, ***p*<0.01 in comparison with the control group; ^{+}p<0.05 in comparison with the PT group.

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