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Mechanisms of Pulmonary Toxicity of Perfluoro-n-Alkane Pyrolysis Products with Consideration of the Structural Features of the Blood—Air Barriers

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Perfluoroisobutylene a is pulmonotoxic chemical generated during pyrolysis of perfluoro-n-alkanes (polytetrafluoroethylene). The mechanisms of acute pulmonary toxicity induced by perfluoroisobutylene have not been studied yet. The analysis of tissues of brown frogs showed that the products of polytetrafluoroethylene pyrolysis induce typical inflammatory response in the lungs (fluid accumulation, erythrocyte stasis, desquamation of the epithelium, and capillary plethora in lung septa) and oropharyngeal cavity (degeneration of ciliated epithelium, hyperemia of underlying vessels with plasmatic imbibition of the connective tissue, and margination of segmented leukocytes and monocytes). The absence of surfactant is a specific feature of the blood—air barrier of the oropharyngeal cavity in frogs compared to the lungs. It can be hypothesized that toxic effects of perfluoroisobutylene are determined by its influence on epithelial (pneumocytes and cells of nonkeratinized stratified ciliated epithelium) and endothelial cells. Even though the effects of the agent on surfactant cannot be excluded, they do not determine the probability of development of inflammatory response.

Key Words: *perfluoro-n-alkanes; perfluoroisobutylene-induced acute lung injury; frog; blood—air barrier*

Perfluoro-n-alkanes, a class of fully fluorinated compounds with unique properties (chemical stability, thermostability, low toxicity, and low electric constant) that determine their successful application in electronic, medical, and chemical industry. Perfluoro-n-alkanes are widely used as substances preventing burning, heat transfer agents, aerosol solvents, monomers for Teflon production, etc. [8]. Perfluoro-n-alkanes have low toxicity, but their thermal disintegration is accompanied by the production of highly toxic substances. For instance, pyrolysis of polytetrafluoroethylene (PTFE) at temperatures >600°C is accompanied by generation of tetrafluoroethylene (C_2F_4), hexafluoroethylene (C_3F_6), and highly toxic perfluoroisobutylene (C_4F_8) [7,8]. Perfluoroisobutylene (PFIB; $(F_3C)_2C{=}CF_2$) is a highly toxic gas inducing acute lung injury (ALI) upon inhalation and lethal outcome. No effective causal and pathogenetic therapy of PFIB intoxication exists and little is known about the pathogenesis of PTFE-induced ALI. It was found that PTFE exhibits acylating properties and participates in the reactions of nucleophilic addition and substitution with macromolecules carrying NH_2-, SH-, and OH- groups. This results in modification of the macromolecule structure and triggers oxidative stress reactions and proinflammatory cascade leading to impairment of the integrity of the blood—air barrier (BAB) and ALI development [9]. PTFE enters the body only via inhalation pathway and interacts with BAB components. However, it is not clear which component of the BAB (surfactant, alveocytes, or endothelial cells) are the primary target

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of PTFE. Electron microscopy showed ultrastructural changes in alveolocytes and endothelial cells of capillaries in rats as soon as in 5 min after PTFE poisoning [5]. Some authors believe that surfactant is the primary target of PTFE and that products of surfactant degradation (lysophosphatidylcholine) exhibit proinflammatory properties [6]. However, most researchers do not distinguish the primary target of PTFE and hold that it affects all BAB elements [7,9].

The BAB of various types of animals, *e.g*. frogs, can be used to identify the primary target of PTFE. Frogs have several organs for gas exchange (lungs, skin, and oropharyngeal mucosa) [1]. The lungs of frogs are paired organs with thin walls. The epithelium of the respiratory area consists of pneumocytes located between blood capillaries. Pneumocytes and endothelial cells of blood capillaries form the BAB [2]. Frog lungs contain surfactant, but in lower amount compared to mammalian lungs [4]. Lung breathing in frogs is low efficient and oxygen and carbon dioxide mainly diffuse through wet abundantly vascularized surfaces (skin and oropharyngeal mucosa). Frog skin consists of the epidermis containing non-squamous stratified epithelium. The system of subepidermal capillaries is located under the epidermis. Frog skin is permeable for oxygen and carbon dioxide and forms a kind of BAB. The oropharyngeal cavity is lined with stratified ciliated epithelium contacting with dense capillary system and functions as a respiratory organ performing gas transfer [3].

Thus, three types of the BAB presented by different components can be distinguished in frogs: lung BAB (surfactant—simple squamous epithelium—endothelial cell), skin BAB (stratified ciliated epithelium—endothelial cell), and oropharyngeal BAB (stratified ciliated epithelium—endothelial cell).

Our aim was to check the hypothesis that surfactant is the primary target of PTFE in the BAB. If our suggestion is correct, PTFE inhalation will cause injury only in the lung BAB.

MATERIALS AND METHODS

Brown frogs *Rana temporaria* (*Anura*, *Ranidae*) were used for the experiments. The frogs were divided into 2 representative groups: control (frogs placed into the inhalation chamber and exposed to atmosphere air for 15 min) and intoxication (frogs exposed to inhalation of the product of PTFE pyrolysis for 15 min) groups.

Thermal decomposition of PTFE was performed in a chamber at 440-750°C. Pyrolysis products enter inhalation chamber with frogs by natural convection. The analysis of the gas-air mixture was performed by gas-liquid chromatography with mass-spec detection (Agilent 7890B with the Agilent 240 MS mass-selective detector). After exposure, the frogs of both groups were placed in water. In 6 h, the frogs were sacrificed by destruction of the brain and spinal cord. For histological analysis, the lungs and fragments of the skin and oropharyngeal cavity were fixed with 10% neutral formalin, routinely prepared histological samples were stained with hematoxylin and eosin and then examined and photographed under a MIKMED-6 light microscope (Analit-Neva).

RESULTS

After heating the pyrolysis chamber to 440°C and more, white smoke containing toxic products of PFTE pyrolysis (pyrolysis products) entered the inhalation chamber. Gas chromatography with mass-spectrometric detection showed the presence of toxic fluoroolefin substances in the gas-air mixture. The comparison of typical ions of mass-spectrum of the analyzed sample with NIST MS Search 2.2 database showed coincidence with PFIB spectrum (content in the mixture 85.9%).

No signs of irritative effects of pyrolysis products were observed in frogs during poisoning. The conditions of the animals in both groups did not differ within 6 h after the exposure.

Microscopy showed that the lungs in control group frogs were free, pneumocytes had normal shape (flat and transition cells), lung capillaries were not dilated and filled with erythrocytes. Signs of injury were observed in frogs exposed to intoxication. Subtotal fluid accumulation, diapedesis of erythrocytes and polymorphonuclear leukocytes, accumulation of desquamated epithelium, and pronounced capillary plethora in lung septa were observed in these animals (Fig. 1).

The oropharyngeal mucosa of control group frogs was presented by stratified ciliated epithelium with goblet cells. Moderate amounts of erythrocytes and leukocytes were located in the center of the capillaries and blood vessels. Flat elongated endothelial cells were adjacent to each other. The exposure to pyrolysis products resulted in epithelial cell degeneration of different degree (from damage to apical cytoplasm to extensive desquamation of the ciliated epithelium up to the basal layer). Underlying vessels were sharply dilated and plethoric. Loosening and moderate contraction of muscle fibers was accompanied by plasma imbibition of the connective tissue.

Margination of segmented leukocytes and monocytes and erythrocyte conglomerates of various shapes and sizes were seen (Fig. 2). In 6 h after poisoning, no pathological changes were observed in frog skin (data not presented). The changes in the lungs and oropharyngeal mucosae observed after the exposure

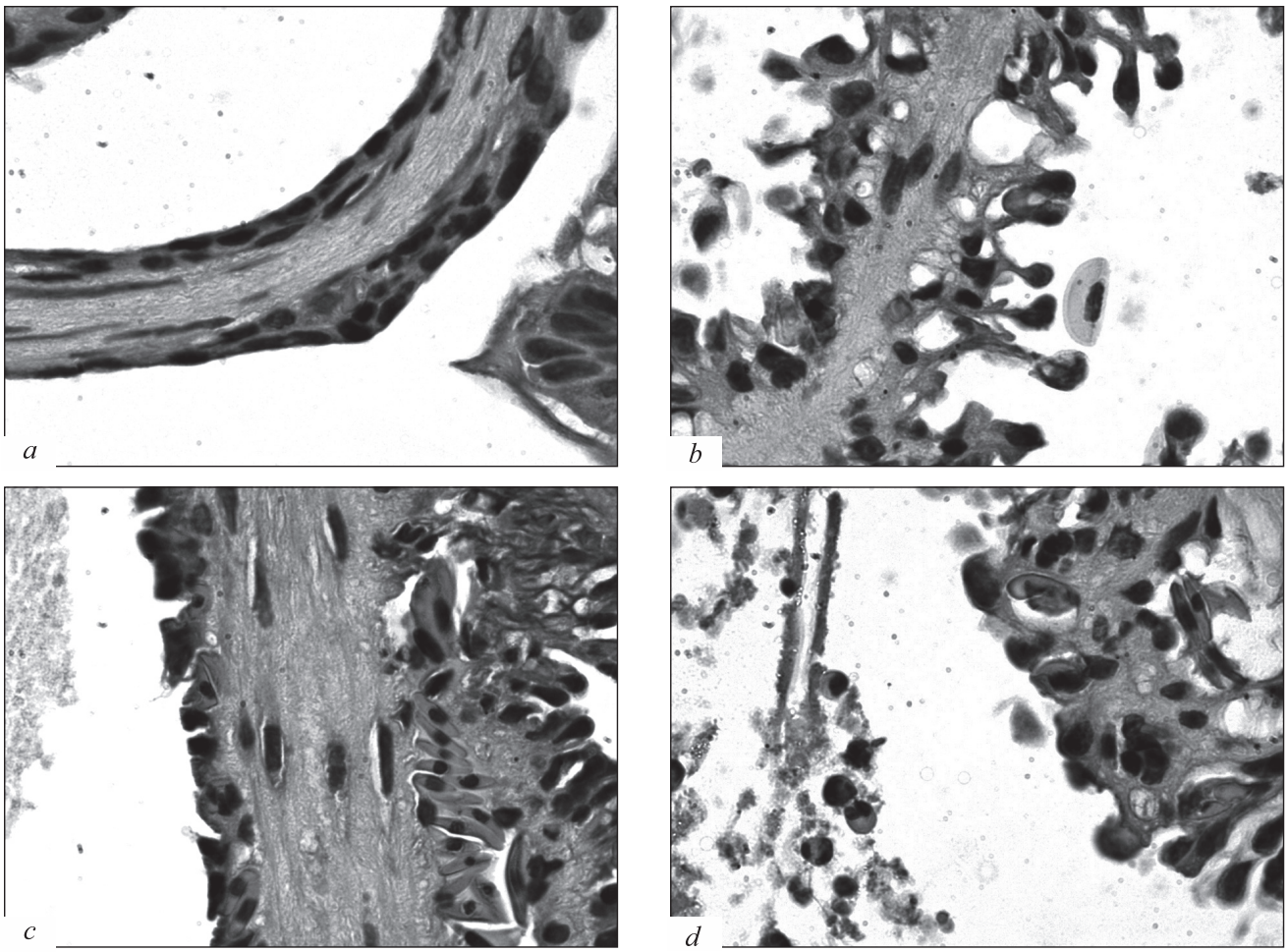

Fig. 1. A fragment of lung parenchyma in control (*a*) and experimental frogs in 6 h after exposure to pyrolysis products (*b*-*d*). Hematoxylin and eosin staining, ×100 (oil immersion). Acinar septum lined with simple squamous epithelium (*a*) with plasmolemma and hemal subepithelial capillaries. Protein-containing exudate in lung lumen (*a*) with leukocytes (*b, d*) and erythrocyte diapedesis (*b*), epithelial cell pyknosis in the transition epithelium and karyorrhexis (*b*-*d*), pronounced capillary congestion (*c*).

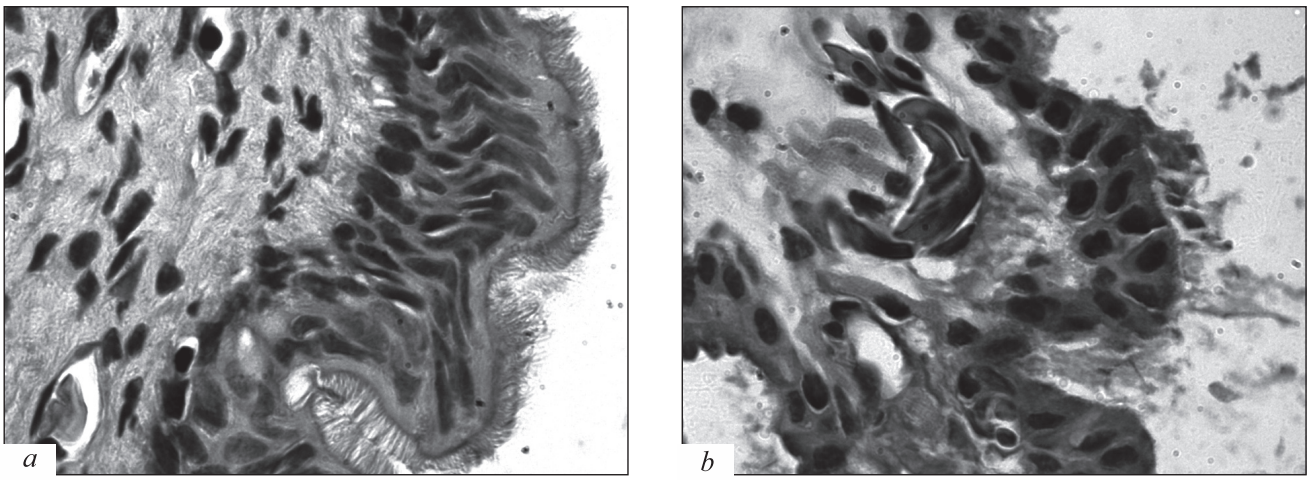

Fig. 2. A fragment of the oropharyngeal mucosa. Hematoxylin and eosin staining, ×40 (*a*), ×100 (*b*) (oil immersion). *a*) Unchanged stratified ciliated epithelium and single empty blood vessels in the submucosa, normal structure of the collagen matrix of the connective tissue of a control frog. *b*) Desquamation of apical fragments of ciliated epithelial cells and cell layers, the underlying vessels are sharply dilated, plethoric, with margination of polymorphonuclear leukocytes, plasma imbibition of the basic substance of the connective tissue in 6 h after exposure to pyrolysis products.

to pyrolysis products were typical of the inflammatory process.

PFIB intoxication can develop during thermal decomposition of perfluoro-n-alkanes in emergency situations accompanied by fires in industrial facilities [8]. The absence of effective therapy for PFIB-induced ALI can be explained by the absence of clear understanding of the mechanisms of its toxic effects [9]. We used a non-standard model for our toxicological study to identify the primary target of PFIB, inhalation exposure of frog AHBs to PFTE pyrolysis products containing PFIB.

The BAB of the lungs in frogs has similar features with BAB of mammalian lungs [2,4]. Inhalation of pyrolysis products results in the injury to the lungs. Microscopic investigation revealed typical signs of inflammation (plethora, appearance of transudate and blood cells in the lung lumens, erythrocyte stasis, and desquamation of epithelial cells). These changes reflected the development of ALI after the exposure to pyrolysis products. As the BAB of frog lungs consists of surfactant [2] and epithelial and endothelial cells [4], it can be hypothesized that all these components can be the primary targets of pyrolysis products.

The absence of surfactant and the presence of other type of epithelial cells (stratified ciliated epithelium) [3] instead of simple squamous or prismatic transition epithelium [2] in the lungs is a specific feature of BAB of the oropharyngeal cavity. If surfactant is the primary target of pyrolysis products in the BAB, application of pyrolysis products to the oropharyngeal mucosa should not induce pathological changes. However, microscopy showed pronounced signs of inflammation accompanied by epithelial damage and edema of the connective tissue of the submucosa. These changes can result from the effects of pyrolysis products on epithelial or endothelial cells.

The absence of changes in the skin after exposure to pyrolysis products can be explained by the fact that frogs breathe through the skin only in water [3]. Hence, the skin did not participate in gas exchange during the exposure and pyrolysis products did not penetrate the skin BAB.

Thus, the results obtained on this tissue model suggest that surfactant (even in case of its acylation) does not determine the probability of ALI development after exposure to PFIB. The mechanism of toxic effects of PFIB is mediated by the cell structures of the BAB. The heterogeneity of the metabolic components of these structures can help to elucidate the mechanism of toxic pathology and to create effective therapeutic approaches. Thus, identification of the cell targets of chocking gases is a difficult task, but clarification of this issue might allow to reduce the risk of mortality during intoxication with the substances of this group.

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