GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Antifibrotic and Anti-Inflammatory Activity of a Neuroleptic Drug on the Model of Pulmonary Fibrosis

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The effect of course treatment with neuroleptic haloperidol on the inflammatory response and state of the connective tissue in the lungs of C57Bl/6 mice was studied on the model of toxic pulmonary fibrosis induced by intratracheal administration of bleomycin. This neuroleptic decreased the inflammatory response and reduced the growth of the connective tissue in the lungs. The anti-inflammatory effect of haloperidol is related to a decrease in activity of bone marrow hemopoietic stem cells and committed hemopoietic precursors. The antifibrotic effect of this drug is associated with inhibition of mesenchymal precursor cells.

Key Words: *bleomycin; pulmonary fibrosis; neuroleptic drug; stem cells; committed hemopoietic and mesenchymal precursors*

In contrast to acute inflammatory reactions, pulmonary fibrosis is a typical chronic process that persists over several months; inflammation, tissue reconstruction, and reparative processes occur simultaneously in this condition [15]. Despite much progress in drug treatment of this severe and socially important disease, complete recovery of patients is not achieved. The etiological and pathogenetic factors of this disease are poorly understood [4].

Recent studies elucidated the role of serotonin in the progression of fibrotic changes in the lungs. For example, the intensity of collagen production by lung fibroblasts and synthesis of antifibrotic factors depend on the concentration of serotonin and expression of $5\text{-HT}_{2A,2B}$ receptors in the lungs [9,13]. The lung tissue contains considerable amounts of dopamine and $D_{1\text{-}4}$ dopamine receptors [6]. Dopamine D_1 and D_2 receptors are present in pulmonary vessels [8,12]. These data illustrate the existence of a peripheral dopaminergic system in the lungs. However, the role of this system under normal and pathological conditions remains unknown.

Here we studied the effect of a neuroleptic on the inflammatory response and growth of the connective tissue in the lungs after intratracheal administration of bleomycin.

MATERIALS AND METHODS

Experiments were performed on 2-2.5-month-old C57Bl/6 mice weighing 20 g (n=70). The mice (class I conventional strain) were obtained from the nursery of the Institute of Pharmacology (certified animals).

Pulmonary fibrosis was induced by an intratracheal administration of bleomycin (Bleomycetin, Lensfarm Company) in a single dose of 80 μg in

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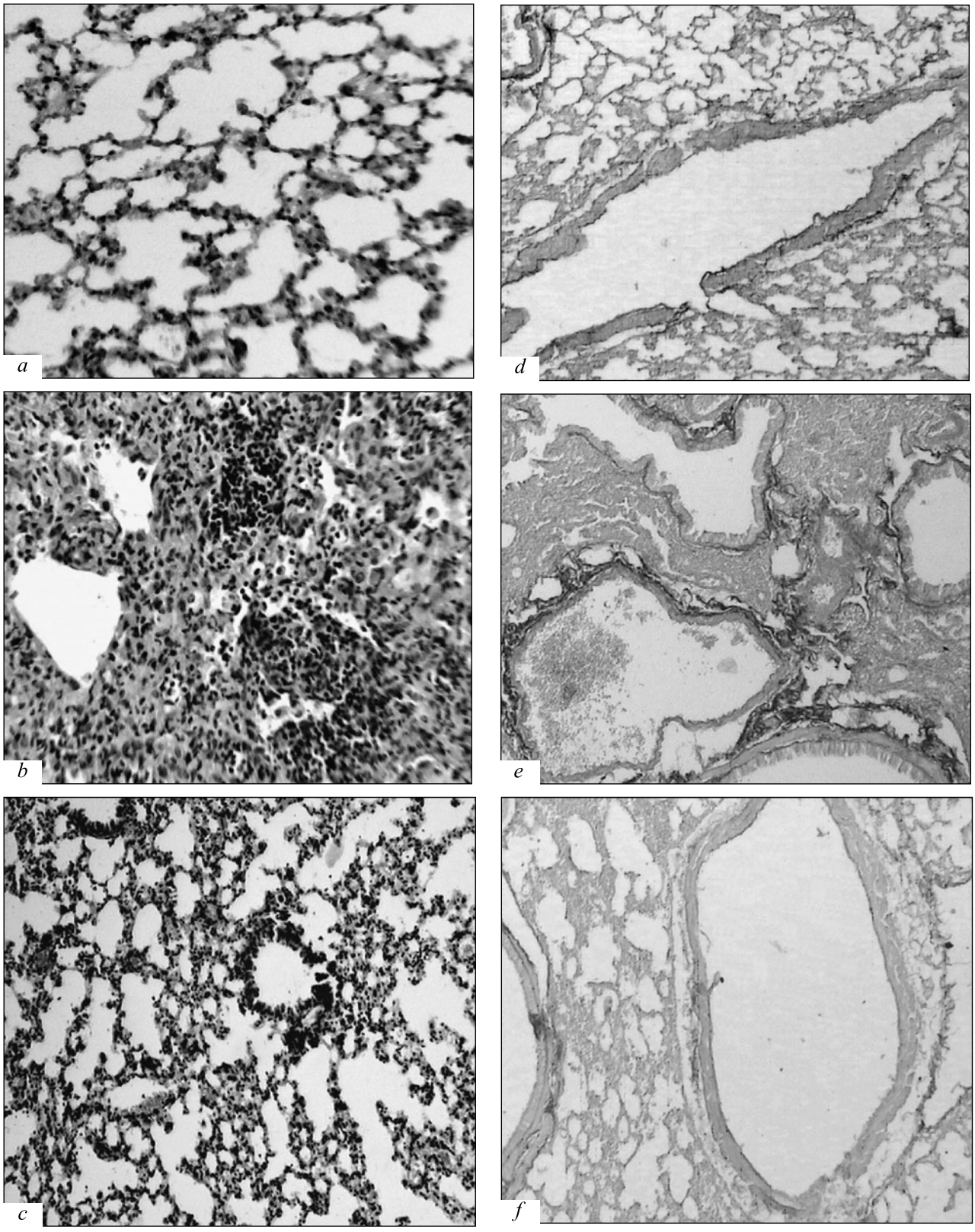

Fig. 1. Morphology of the lungs in C57Bl/6 mice after intratracheal administration of bleomycin and neuroleptic treatment. Lung of the control mice (*a*, *d*); lung of the bleomycin-receiving mice (*b*, *e*); lung of the mice with experimental pulmonary fibrosis after neuroleptic treatment (*c*, *f*). Staining with hematoxylin and eosin, ×300 (*a*-*c*); staining with picrofuchsin by van Gieson's method, ×150 (*d*-*f*).

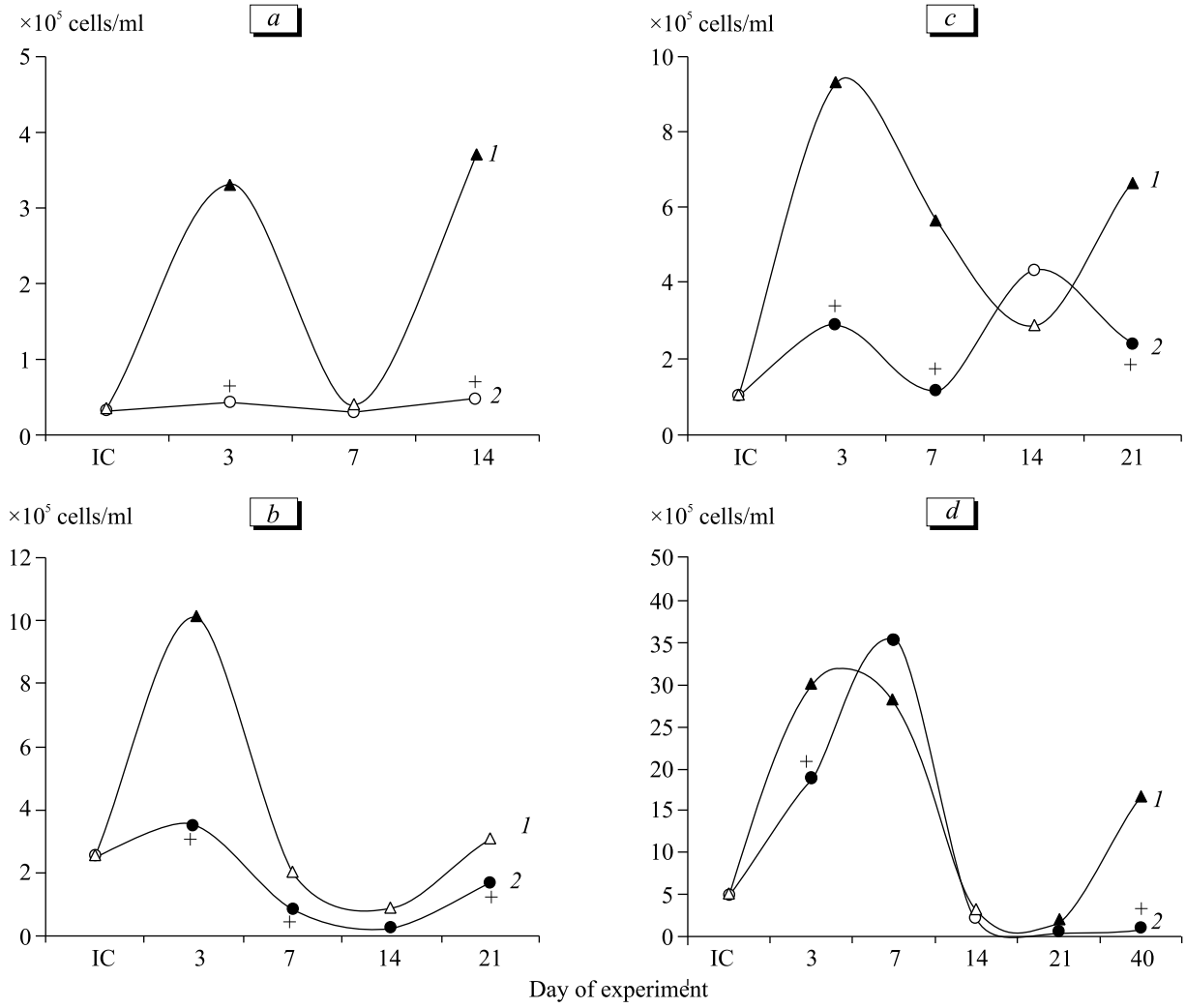

Fig. 2. Content of CFU-U (*a*), CFU-GEMM (*b*), CFU-G (*c*), and CFU-F (*d*) in the culture of bone marrow cells from C57Bl/6 mice after intratracheal administration of bleomycin and neuroleptic treatment. Here and in Fig. 3: intact control (IC); bleomycin control (*1*); haloperidol treatment after intratracheal administration of bleomycin (*2*). $p<0.05$: dark symbols, compared to IC; +compared to bleomycin control.

30 μl physiological saline. A neuroleptic haloperidol (Gedeon Richter A.O.) in a daily dose of 2 mg/kg was injected intraperitoneally on the next day and over 21 days after the surgery. The volume of a solution was 200 μl. Intact animals served as the control (intact control).

The total content and differential leukocyte count in the peripheral blood was measured routinely on days 3, 7, 14, 21, 25, 40, and 60 after bleomycin administration. The mice were euthanized with CO_2 overdose. Morphological characteristics of the lungs were evaluated. We estimated the number of bone marrow cells and content of morphologically discernible cells of the granulocyte and lymphoid hemopoietic lineages [1]. For histological study, the lung tissue samples were fixed in 10% formalin. The samples were treated by standard histological methods and embedded into paraffin. Histological sections (5 μ) were prepared. Deparaffinized sections were stained with hematoxylin

TABLE 1. Content of Connective Tissue (% of tissue area) in the Lungs of C57Bl/6 Mice after Intratracheal Administration of Bleomycin and Neuroleptic Treatment for Experimental Pulmonary Fibrosis (*M*±*m*)

Period	Bleomycin	Bleomycin+ haloperidol
Intact control	1.03±0.20	
Day 7	1.98±0.58	1.35±0.20
Day 14	3.09±0.23*	2.18±0.12*+
Day 21	3.02±0.44*	2.17±0.21*
Day 25	5.45±0.74*	2.88±0.16*+
Day 40	3.47±0.21*	2.48±0.11*+
Day 60	2.77±0.25	1.48±0.12+

Note. $p<0.05$ *compared to the intact control, +compared to bleomycin.

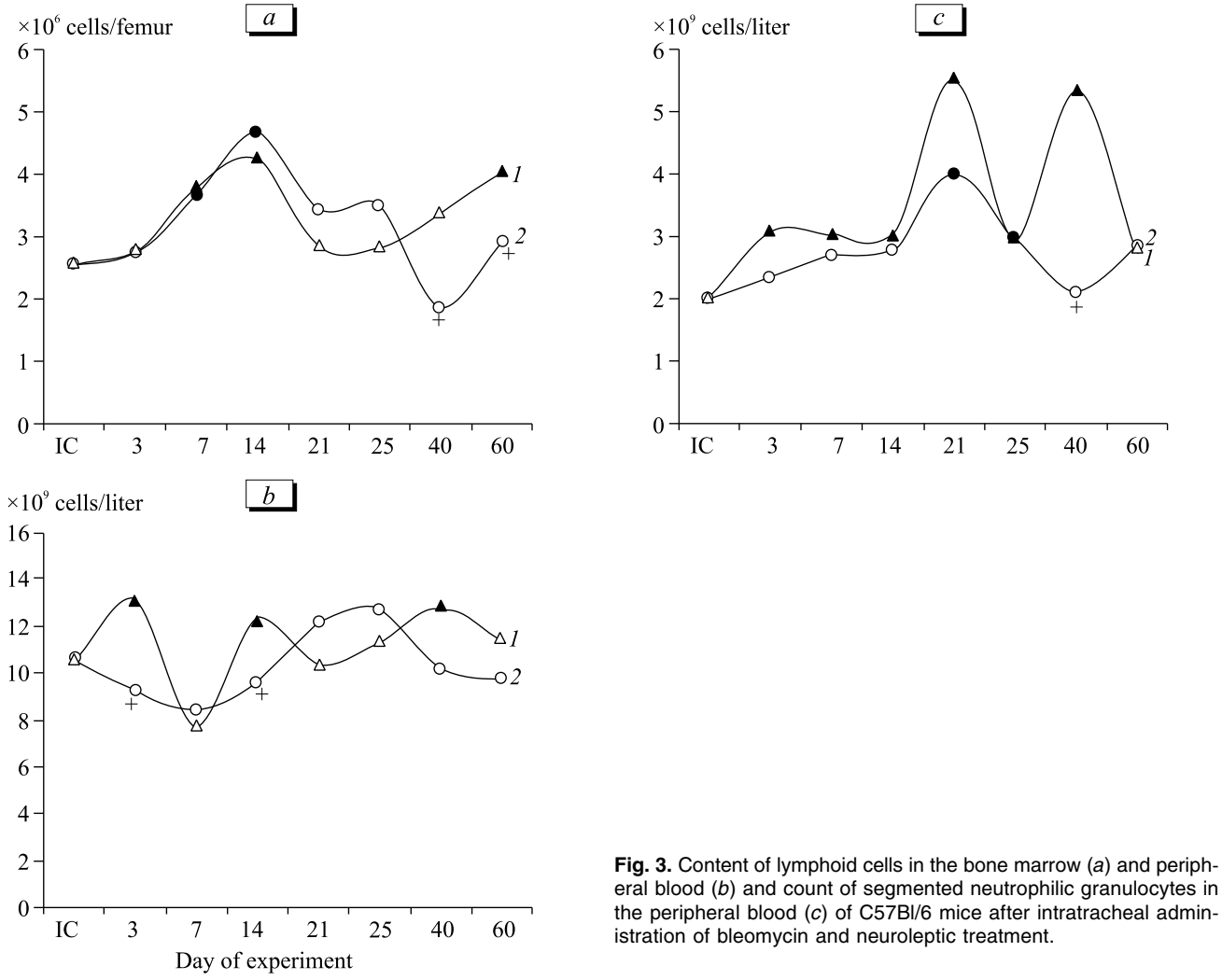

Fig. 3. Content of lymphoid cells in the bone marrow (*a*) and peripheral blood (*b*) and count of segmented neutrophilic granulocytes in the peripheral blood (*c*) of C57Bl/6 mice after intratracheal administration of bleomycin and neuroleptic treatment.

and eosin and by van Gieson's method (for connective tissue) [5]. The area of collagen fibers in the lung tissue was measured on the standard area of histological sections by computer graphic analysis. The ratio of these parameters (relative to the standard section area) was calculated.

The formation of granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) and granulocyte colonies (CFU-G) by nonadherent nucleated bone marrow cells was studied by the culture method. The growth of fibroblast colonies (CFU-F) was evaluated in culture of adherent nucleated bone marrow cells [1,3]. The content of multipotent hemopoietic precursors in the bone marrow was estimated by the method of limiting dilutions. Culturing of these cells is followed by the formation of colonies that consist of undifferentiated hemopoietic cells (CFU-U) [3].

The results were analyzed by standard methods of variation statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Mann–Whitney *U* test.

RESULTS

Bleomycin-induced damage to the lung tissue develops in stages. Infiltration of the alveolar interstitium and alveolar ducts with inflammatory cells (lymphocytes, macrophages, neutrophils, and plasma cells; Fig. 1) was observed on day 3 after cytostatic treatment. These changes were accompanied by venous plethora and hemorrhages in the alveolar wall. Previous studies showed that this period is characterized by apoptosis of epitheliocytes, massive infiltration of the lung tissue, and release of mediators and cytokines [14]. The growth of connective tissue was observed on days 7-60. The severity of pulmonary fibrosis was maximum on day 25 after bleomycin administration (Table 1).

Our results indicate that single intratracheal administration of bleomycin causes a strong inflammatory response, which is followed by the development of fibrosing alveolitis.

The signs of bleomycin-induced alveolar infiltration with lymphocytes, neutrophils, and plasma cells

were reduced on day 3 of neuroleptic treatment (Fig. 1). The growth of the connective tissue in the lungs was abolished on day 7 of haloperidol injection. This effect persisted on days 14, 25, 40, and 60 (Table 1). Antifibrotic activity of the drug was maximum on day 25.

Our previous studies showed that the blood system plays an important role in the inflammatory response during pulmonary fibrosis [2]. Studying the effect of this neuroleptic on hemopoiesis revealed a decrease in the content of multipotent bone marrow hemopoietic cells (days 3, 7, and 14) and CFU-GEMM and CFU-G (days 3, 7, and 21) in mice of the treatment group (as compared to untreated animals; Fig. 2). The inhibitory effect of haloperidol on hemopoietic stem cells and committed hemopoietic precursors contributes to a decrease in the count of peripheral blood neutrophilic granulocytes on days 3, 21, and 40 (Fig. 3). Apart from inhibition of granulocytopoiesis, haloperidol decreased the number of lymphocytes in the peripheral blood and bone marrow.

Damage to the alveolar-capillary membrane and myofibroblasts of the respiratory epithelium and release of blood cells and plasma into the pulmonary interstitium are followed by the accumulation of circulating fibrocytes in the focus of inflammation [10,15]. Studying the pool of committed fibroblast precursors after administration of bleomycin showed that the neuroleptic prevents CFU-F growth in the culture of adherent bone marrow cells (Fig. 3).

We conclude that neuroleptic treatment after an intratracheal administration of bleomycin prevents the development of inflammation and connective tissue growth in the lungs. The effects of haloperidol are associated with inhibitory influence on hemopoietic stem cells and committed hemopoietic and mesenchymal precursors.

Our results demonstrate the existence of a dopaminergic mechanism for pulmonary fibrosis. This mechanism is realized via the blood system and regulation of collagen fiber synthesis. 5-HT neurons innervate a variety of dopamine-containing regions in the brain (*e.g.*, substantia nigra, striatum, and nucleus accumbens). Dopamine produces a tonic inhibitory effect on serotoninergic neurons of the caudate nucleus [11]. Chronic administration of haloperidol (for more than 2 weeks) is followed by a decrease in the content not only of dopamine, but also of serotonin and their metabolites [7]. Taking into account the dependence of collagen production by lung fibroblasts on serotonin [9,13], it cannot be excluded that the anti-inflammatory and antifibrotic effects of this neuroleptic are associated with inhibition of the serotoninergic system.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
2. A. M. Dygai, E. G. Skurikhin, T. V. Andreeva, *et al*., *Byull. Eksp. Biol. Med*., **152**, No. 8, 132-136 (2011).
3. A. M. Dygai, E. G. Skurikhin, O. V. Pershina, *et al*., *Ibid*., **148**, No. 4, 400-404 (2010).
4. *Clinical Recommendations. Pulmonology*, Ed. A. G. Chuchalin [in Russian], Moscow (2007).
5. G. A. Merkulov, *Course of Pathohistological Technique* [in Russian], St. Petersburg (1969).
6. F. Amenta, A. Ricci, S. K. Tayebati, and D. Zaccheo, *Ital. J. Anat. Embryol*., **107**, No. 3, 145-167 (2002).
7. M. Bishnoi, K. Chopra, and S. K. Kulkarni, *Neurochem. Res*., **33**, No. 9, 1869-1880 (2008).
8. P. Bruzzone, V. D'Andrea, C. Motta, and C. Cavallotti, *Pulm. Pharmacol. Ther*., **15**, No. 4, 393-398 (2002).
9. A. Fabre, J. Marchal-Somme, S. Marchand-Adam, *et al*., *Eur. Respir. J*., **32**, No. 2, 426-436 (2008).
10. W. D. Hardie, S. W. Glasser, and J. S. Hagood, *Am. J. Pathol*., **175**, No. 1, 3-16 (2009).
11. P. Jenner, M. Sheehy, and C. D. Marsden, *Br. J. Clin. Pharmacol*., **15**, Suppl. 2, 277S-289S (1983).
12. Y. Kobayashi, A. Ricci, F. Amenta, *et al*., *J. Vasc. Res*., **32**, No. 3, 200-206 (1995).
13. M. Königshoff, R. Dumitrascu, S. Udalov, *et al*., *Thorax*, **65**, No. 11, 949-955 (2010).
14. D. J. Welsh, M. Harnett, M. MacLean, and A. J. Peacock, *Am. J. Respir. Crit. Care Med*., **170**, No. 3, 252-259 (2004).
15. M. S. Wilson and T. A. Wynn, *Mucosal Immunol*., **2**, No. 2, 103-121 (2009).