Protective Effect of Fenspiride on the Bronchi in Rats with Chronic Obstructive Pulmonary Disease

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We studied the effect of a non-steroidal anti-inflammatory drug fenspiride on contractive activity of bronchial smooth muscles on the model of chronic obstructive pulmonary disease of rats induced by 60-day exposure to nitrogen dioxide. The administration of fenspiride during the acute stage of the disease (day 15) abolished the constricting effect of the pollutant on the bronchial smooth muscles. Dilatation effect of fenspiride in a low dose (0.15 mg/kg) was mediated by its interaction with nerve endings of bronchial capsaicin-sensitive nerve C-fibers. The interaction of drug with receptors of C-fibers prevented neurogenic inflammation, which was confirmed by the absence of structural changes in the lungs typical of this pathology. The broncholytic effect of fenspiride in a high dose (15 mg/kg) was mediated by not only afferent pathways, but also its direct relaxing action on smooth muscle cells. The observed anti-inflammatory and bronchodilatation effect of fenspiride in very low doses can be used for prevention of chronic obstructive pulmonary disease in risk-group patients contacting with aggressive environmental factors.

Key Words: *chronic obstructive pulmonary disease; bronchodilatation; inflammation; non-steroidal anti-inflammatory drugs*

Chronic obstructive pulmonary disease (COPD) is one of leading causes of morbidity and mortality over the world. The key pathogenic mechanism of COPD is chronic inflammation induced by adverse environmental factors and involving all lung structures (bronchi, interstitial tissue, and alveoli). Modern pharmacological treatment of COPD, primarily corticosteroids, cannot prevent progressive impairment of external respiration. The resistance of COPD patients to anti-inflammatory effects of corticosteroids is related to a decrease in activity/expression of histone deacetylase 2 and suppression of the transcription of anti-inflammatory genes [4]. Corticosteroid treatment alleviates bronchial obstruction in only 10-30% COPD patients [3]. The resistance of COPD patients to corticosteroid therapy is a pressing problem and the search for new alternative drugs producing broncholytic and anti-inflammatory effects at all stages of the disease is necessary. Therefore, attention should be paid to a non-steroidal anti-inflammatory drug fenspiride, which dilatation effects are not studied yet [3,6,7].

Here we studied the dilatation effect of fenspiride on the bronchial smooth muscles in rats with experimental COPD induced by nitrogen dioxide inhalation.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (n=49) weighting 150-170 g. The study was conducted in accordance to the Direction of Ministry of Health and Social Development No. 708n (23.08.10) "Guidance for Laboratory Practice". Step-by-step development of COPD was modeled by inhalation administration of nitrogen dioxide [1]. The rats were put

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into a chamber assembled in a ventilation cabinet and connected via a hose to the laboratory device for nitrogen dioxide production. A mixture of nitric oxides produced in the chemical reaction between sodium nitrite and sulfuric acid was delivered to the chamber with animals via a tube. After reaction with oxygen, colorless nitric oxide was converted into more stable yellow-brown nitrogen dioxide (NO_2). NO_2 concentration estimated calorimetrically was 30-40 mg/m^3. The animals were exposed to NO_2 for 15 and 60 days (3 times a day, 30 min per session, at 30-min intervals between the sessions). Every day, the rats (n=28) received fenspiride solution (Erespal, Lab. Servier Industry) through an esophageal probe in doses of 15 mg/kg (n=14; a therapeutic dose for a patient subject to interspecific conversion) or 0.15 mg/kg (n=14) 1.5 h before inhalations of NO_2. Control rats (n=14) received 0.9% NaCl solution *per os*. Intact rats (n=7) were placed in a chamber with air. The animals were sacrificed by cervical dislocation.

The lungs for histological study were spread by infusion of 10% formaldehyde solution through the trachea. The tissues were embedded in paraffin, the sections (5-7 μ) were stained with hematoxylin and eosin. To estimate contractive activity of bronchial smooth muscles, two fragments of bronchi (segmental bronchi II-VI, 4-5 mm thickness) were taken from each animal. The preparations were equilibrated for 60 min in a temperature-controlled bath with aerated Krebs—Henseleit solution (pH=7.3-7.4, 37.0-37.5°C, 0.6 ml/min) at resting tension of 500 mg. Isometric contraction (tension in mg) of smooth muscles of isolated bronchial strips was measured with an electromechanical converter. Two series of stimulations with electric field (9 stimulations per series) were performed on each preparation. In series I, the preparations were stimulated with bursts of stimuli of 0.5 msec duration and 7 Hz frequency (stimulation of preganglionic fibers); in series II, the preparations were stimulated with bursts of stimuli of 2.0 msec duration and 30 Hz frequency (stimulation of smooth muscles). The duration of stimulation was 10 sec in each case, the amplitude of the stimulus was 20 V, and the interval between stimulations was 3 min. In each series, the mean response to the first 3 stimulations served as the control, the mean responses to the following 3 stimulations (4-6, and 7-9 stimulations) served as additional responses simulating long-term load on contractive function of the respiratory smooth muscles. In the experiments with pharmacological influences on bronchial segments from intact rats, the drugs (novocaine and capsaicin) in specified concentrations were perfused through the chamber for 8 min. The bronchial preparations were stimulated on minutes 1, 4, and 7 after substance administration into the chamber. The preparations were washed with physiological saline for 30 min between the series.

Statistical analysis was performed using Microsoft Excel 7.0 software. The mean amplitude, latent period, and contraction rate and the mean error were estimated. The means were compared using Student's t test and method for paired comparisons.

RESULTS

Addition of fenspiride (1 μg/ml) to the perfusate was followed by a decrease in the amplitude of bronchial contractions in intact rats, which was induced by electrical stimulation of preganglionic nerves (by 15.0±3.3%, p<0.05) and muscles (by 27.4±2.8%, p<0.05). Increasing the concentration of fenspiride in both cases led to a dose-dependent decrease of contraction amplitude (Table 1).

Novocaine pretreatment (1 μg/ml) abolished bronchial dilatation induced by low concentrations of fenspiride (1 and 10 μg/ml) and significantly decreased dilatation induced by 100 μg/ml fenspiride (the effect was preserved; Table 1). After addition of capsaicin (10 μg/ml) to the perfusate, the amplitude of bronchial smooth muscle contraction caused by additional preganglionic stimulation increased to 122.2±4.9% (p<0.01), while addition of fenspiride (100

TABLE 1. Effects of Fenspiride in Various Doses on Contractive Activity of Bronchial Preparations from Intact Rats

Stimulation	Amplitude of bronchial contractions, % of response before administration of fenspiride in a dose of		
	1.0 μg/m	10 μg/ml	100 μg/ml
Nerve	85.0±4.9°	77.1±4.6°	59.0±2.9*+
Muscle	72.6±3.2°	61.0±3.3*	45.8±3.8*+
Nerve under conditions of novocaine blockage	96.2±5.2	92.3±6.1	75.6±5.8*+

Note. p<0.05 in comparison with the dose of *1.0 μg/kg, +10.0 μg/kg, °before fenspiride addition to the perfusate.

TABLE 2. Effects of Fenspiride on Contractive Activity of Bronchi in Rats with COPD Induced by Control Stimulation of Preganglionic Nerve and Smooth Muscle

Stages of COPD	Amplitude of bronchial smooth muscle contractions, mg					
	nerve stimulation			stimulation of smooth muscle		
	control	fenspiride		control	fenspiride	
		0.15 mg/kg	15 mg/kg		0.15 mg/kg	15 mg/kg
15-day NO_2 exposure	248.0±21.5$^+$	169.3±18.6*	187.4±20.8*	332.0±22.4	149.1±13.3*$^+$	190.2±16.7*$^+$
60-day NO_2 exposure	237.9±24.5$^+$	228.1±22.3	183.2±17.2*	335.1±23.2	275.4±24.7	235.3±19.3*
Intact rats	181.3±12.3			294.2±20.1		

Note. $p<0.05$ in comparison with: *control (without treatment), $^+$intact animals.

μg/ml) decreased a constricting effect of capsaicin (by 72.6±6.0%), which reflected the neurogenous mechanism of fenspiride-induced bronchodilatation. It can be concluded that bronchodilatation effect of fenspiride in low concentrations is mediated by nerve endings of capsaicin-sensitive nerve C-fibers. The effect of fenspiride in a high dose is mediated by not only afferent pathways, but also its direct action on bronchial smooth muscles.

Inhalation of air–NO_2 mixture for 60 days was followed by the gradual development of functional and morphological signs of COPD starting from the acute damage reaction (15 days) to lung remodeling (60 days) [1]. Bronchial preparations from rats inhaling NO_2 for 15-60 days demonstrated enhanced response of smooth muscle to stimulation of preganglionic nerves (Table 2) with further 13-17% increase in this parameter in response to additional stimulation. Contractive response to muscle stimulation remained practically unchanged; only after 60-days exposure the amplitude of contraction in response to additional stimulation decreased by 20-22% ($p<0.05$).

After 15-day exposure to NO_2 combined with daily injections of fenspiride in low and in therapeutic doses, the contractive responses of bronchial smooth muscles to control stimulation of preganglionic nerves decreased in comparison with the control and did not differ from this parameter in intact rats. The degree of muscle contraction in bronchial preparations from rats receiving fenspiride after control stimulation of the muscle was lower than in the control and intact animals (Table 2). The responses of isolated bronchi to additional stimulation of nerves irrespective of fenspiride dose (89.8±4.0%, 0.15 mg/ml; 107.7±2.4%, 15 mg/ml) were lower than in preparations from non-treated rats (117.6±5.4%, $p<0.05$) and did not differ from those in intact animals. Bronchial responses to additional muscle stimulation in rats receiving low or high dose of fenspiride were lower (88.8±3.8 and 88.2±4.1%, respectively) than in control animals (102.9±2.6%, $p<0.05$).

After 60-day exposure to NO_2, only injection of a high dose for fenspiride was followed by a decrease in contractive response of the bronchi to control nerve and especially smooth muscle stimulation (Table 2). Additional stimulation of the preganglionic nerve was associated with an increase in contractive activity of bronchial smooth muscles in non-treated animals. However, after fenspiride treatment (in both doses), contractive activity of preparations from these rats (and preparations from intact specimens) remained unchanged. After additional muscle stimulation, the amplitude of bronchial contraction in rats receiving fenspiride in both doses was lower (70.2±3.3%, $p<0.01$) than in control (116.0±6.7%) and intact animals (101.2±3.1%).

The mechanism underlying the effect of fenspiride on the bronchial smooth muscles depends on the dose. The dilatation effect of fenspiride in a low dose observed during the acute stage of COPD (15 days) was mediated by interaction with nerve endings of bronchial capsaicin-sensitive nerve C-fibers. The absence of broncholytic action of low dose at the stage of developed COPD (60 days) can be related to the previously observed phenomenon of C-fiber inactivation after prolonged exposure to NO_2 [2]. The decrease in contraction amplitude of bronchial smooth muscles during fenspiride treatment in a therapeutic dose was more pronounced after muscle stimulation than after stimulation of preganglionic nerves. It can be suggested that in this case the broncholytic action of fenspiride is mediated by not only afferent pathways, but also its relaxing action on smooth muscle cells. It was found [5] that fenspiride in a medium concentration

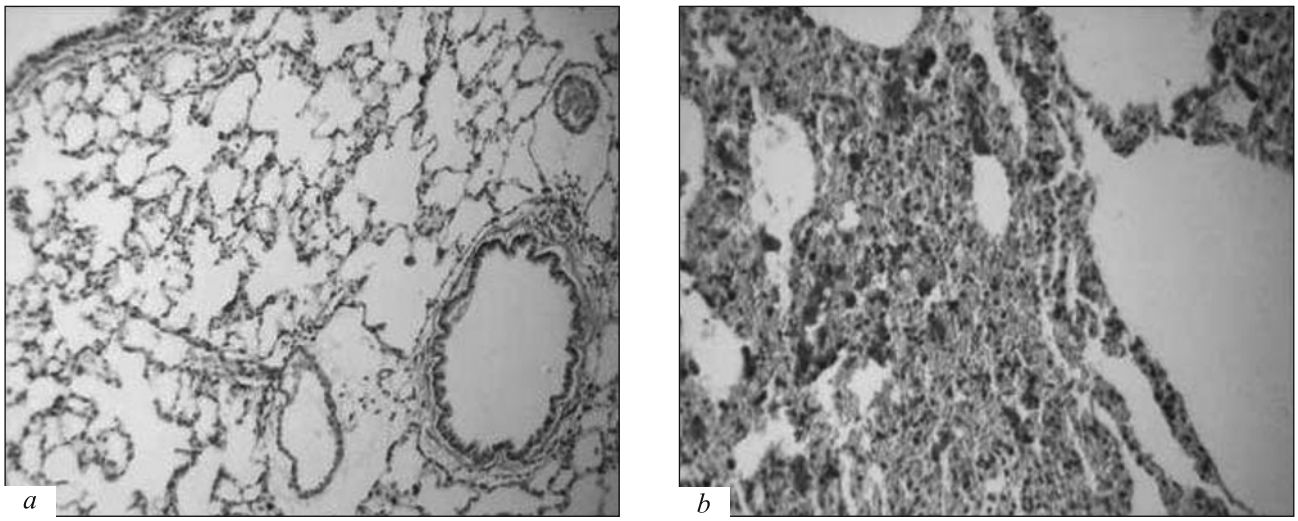

Fig. 1. Rat lung after 60-day NO_2 exposure and fenspiride administration in doses of 0.15 mg/kg (*a*) and 15 mg/kg (*b*). *a*) lung tissues is not changed, alveoli are somewhere insignificantly enlarged; *b*) edema, hemorrhage, and numerous hemosiderophages. Hematoxylin and eosin staining, ×120 (*a*), ×180 (*b*).

(10^{-6}-10^{-4} M) decreases the release of neuropeptides, including tachykinins, from presynaptic sensory nerve endings. Fenspiride in high concentrations (>10^{-4} M) produces a postsynaptic effect on bronchial smooth muscles.

The lung tissue of rats received fenspiride in a low dose was characterized by normal airiness; enlarged alveoli and insignificant cellular infiltration were rarely found (Fig. 1, *a*), *i.e.* no structural abnormalilities typical for untreated rats with COPD (emphysema, and fibrosis foci) were found [1]. It can be hypothesized that the interactions between fenspiride and C-fiber receptors not only induced broncholytic action, but also prevented neurogenic inflammation. Broncholytic effect of fenspiride in a therapeutic dose was confirmed histologically (enlarged bronchial lumen and reduced number of goblet cells). Serous fluid and partly hemolyzed erythrocytes were found in the alveoli (hemorrhages of various periods; Fig. 1, *b*). Inflammatory changes characterized by exudation and proliferation of macrophage-lymphocyte cells were observed only in the interstitial tissue. These morphological changes were not typical for COPD, and, most probably, were related to the toxic influence of NO_2.

The results of our experiments show that fenspiride can be a perspective drug for the treatment of patients with COPD and the development of scheme for prevention of this disease. It should be emphasized that the dose of fenspiride for COPD prevention is significantly lower than the therapeutic doses. This enables long-term continuous fenspiride treatment in patients with COPD contacting with aggressive inhalation factors (pollutions, occupational hazards, and cigarette smoke).

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