The Development of Free Radical Processes in Experimental Bronchospasm. Effect of the Bronchodilator Preparation Troventol

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The development of bronchospasm is shown to be accompanied by lipid peroxidation (LPO) activation; 3-fold and 8-fold rises of malondialdehyde concentration are found in homogenate of lung from sensitized animals and from animals provoked with egg albumin antigen, respectively. The use of luminol-dependent chemiluminescence (CL) reveals that in sensitized rats the production of oxygen free radicals is increased by alveolar macrophages activated with phorbol myristate acetate. Troventol at 10^{-3} mg/ml inhibits the CL response of phagocytes both in intact and in sensitized rats and lowers the level of Fe^{2+}-induced LPO in lung tissue but not in the liver of intact animals.

Key Words: *experimental bronchospasm; lipid peroxidation; alveolar macrophages; bronchodilators; antioxidant properties*

Respiratory disorders are accompanied by a marked inflammatory reaction. The aggravation of inflammation is now thought to be related to the boost of free radical processes (FRP) in the lungs [3,5]. The reactive oxygen species (ROS) produced by phagocytic cells play a key role in this process [11]. The oxidative metabolism of phagocytes may be altered significantly under pathological conditions, leading to uncontrolled generation of ROS [2,4,12] that trigger the "vicious cycle" of inflammation. The ROS may switch on the primary processes of bronchospasm by themselves due to their high toxicity. In this connection it is important to monitor the effect of bronchodilating drugs on the intensity of FRP when their mechanisms of action are studied.

The aim of the present investigation was to study the oxidative metabolism of phagocytes and LPO in sensitization and experimental bronchospasm, as well as the effect of the new Russian bronchodilating drug troventol on FRP. Troventol, iodomethylate of tropine ether α-oxymethyl-α-phenyl butyric acid, selectively blocks the muscarinic cholinoreceptors in bronchial smooth muscle and decreases reflex bronchoconstriction, being a competitive antagonist of acetylcholine [8]. The study was performed in comparison with the anticholinergic preparations atropine sulfate and ipratropium bromide (atrovent).

MATERIALS AND METHODS

For the study male albino rats weighing 180-200 g were maintained on a standard vivarium diet. The animals were divided into 3 groups as follows: the 1st group was the control; the 2nd comprised the sensitized animals; the 3rd consisted of the rats with experimental bronchospasm. Each group consisted of 6 animals. Sensitization of the rats

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was induced by i.p. injection of egg albumin (1% aqueous solution) for 3 weeks. The model of bronchospasm was performed by having preliminarily sensitized rats inhale an effective dose of egg albumin 4 h prior to the experiment. A bronchoalveolar flush (BAF) was obtained as follows: under hexenal anesthesia rats were tracheotomized and 10 ml Hanks solution (37°C) was administered through a special cannula introduced into the trachea. After two slow irrigations of the lungs, the obtained BAF was filtered through two layers of nylon gauze [10]. Alveolar macrophages were obtained via centrifugation of BAF (OPN-3 centrifuge) at 400 g for 10 min [10]. After two washings the precipitate was resuspended in Hanks solution and stored at 4°C. Cell viability was determined with the trypan blue test and suspensions containing no less than 80% viable cells were used [5]. After the first centrifugation supernatant was taken for determination of the protein content after Lowry. After the BAF had been obtained, the lungs and liver were perfused with saline to remove any blood. The tissues were then minced and homogenized. The level of LPO was determined in lung and liver homogenates according to the production of malondialdehyde (MDA) as described elsewhere [9]. For the study of the effect of the drug on the level of LPO in $FeSO_4$ induction the latter was caused by introducing 200 μM $FeSO_4$ into a test tube after the addition of the appropriate amount of drug to the incubation mixture [15]. The production of ROS by BAF cells was determined by the chemiluminescence technique. The measurements were performed with an LKB 1251 chemiluminometer in an automated regime and with monitoring of the recording conditions and process. Hanks solution without dye was introduces into a 1000 μl cuvette, after which luminol (10^{-4} M, Sigma) and 10^6 BAF cells were added and the intensity of spontaneous CL was recorded. The CL response of stimulated cells was recorded following the addition of 10^{-6} mg/ml phorbol myristate acetate (PMA, Sigma). For a study of the effect of the compounds on the CL response of phagocytes the cells were preincubated with 10^{-3} mg/ml of the test substance for 10 min. All measurements were performed in a thermostatically controlled regime at 37°C with continuous stirring. Troventol, atrovent, and atropine were made available by G. Ya. Shvarts (Ordzhonikidze All-Russia Research Chemical-Pharmaceutical Institute). The other research-grade reagents used were of Russian manufacture. All organic solvents were distilled twice. Statistical treatment of the results was performed using the Student t test.

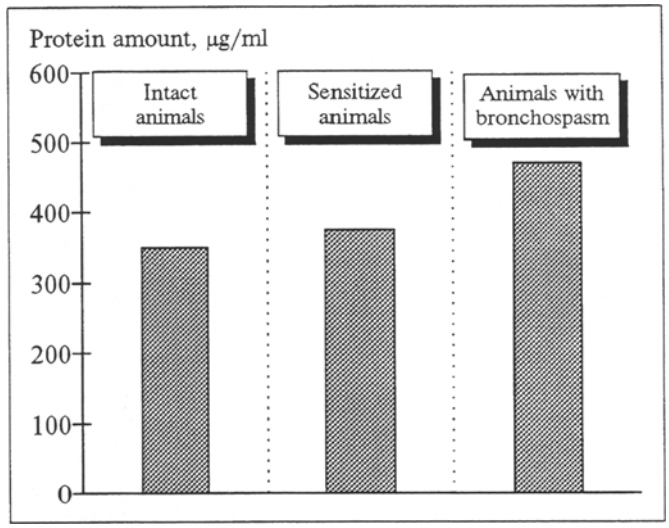

Fig. 1. Determination of protein escape to BAF.

RESULTS

An elevated concentration of free protein in the inflammatory focus is a characteristic indicator of the inflammatory reaction, stemming from the change taking place in the membranes followed by cell destruction and discharge of the cell contents into the intercellular space [14]. Therefore, the protein concentration in BAF had to characterize the severity of the inflammatory reaction in the lungs. The results of measuring the protein in BAF obtained from intact and sensitized animals as well as from rats with experimental bronchospasm are depicted in Fig. 1. Sensitization of rats did not result in a significant increase of BAF protein, although the visual picture of lung tissue was markedly changed. Characteristic white and bluish spots indicated heterogeneity in the lung tissue. On the other hand, the induction of bronchospastic syndrome by intoxication resulted in a marked protein increase in sensitized rats that attested to a

TABLE 1. Effect of Troventol, Atrovent, and Atropine on Fe^{2+} – Induced LPO in Lung Tissue ($M \pm m$)

Preparation		LPO level, μmol MDA/g tissue	Effect, %
Control		1.0±0.3	
Control + Fe^{2+}		6.0±0.5	100.00
Troventol	10^{-3}	5.1±0.3*	84.4
	10^{-6}	5.5±0.4	91.0
Atrovent	10^{-3}	5.3±0.4	87.7
	10^{-6}	5.7±0.5	94.3
Atropine	10^{-3}	5.0±0.3*	83.0
	10^{-6}	5.8±0.4	96.0

Note. Here and in Table 2 asterisk – $p<0.05$ in relation to control + Fe^{2+}.

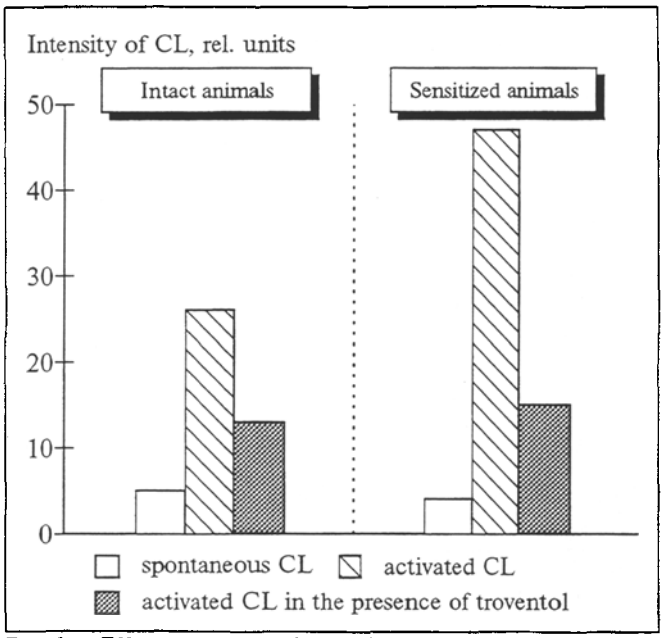

Fig. 2. Effect of troventol on CL response of macrophages of intact and sensitized animals.

stimulation of inflammatory processes. In addition, the number of BAF cells in rats with bronchospasm was 3-5-fold higher than in intact and sensitized ones.

Thus, the development of experimental bronchospasm is accompanied by a marked inflammatory reaction in rats.

Since the alveolar macrophages play an active role in the inflammatory damage of lung tissue [12,13], their oxidative metabolism was studied along with the effect of troventol on this process in intact and sensitized rats, because it was difficult to obtain a pure fraction of alveolar macrophages from BAF under bronchospasm conditions due to intensified escape of blood cells into lung lumen. It was found that activation of PMA cells resulted in a 3-fold increase of the CL burst in comparison with the spontaneous one in intact rats and in a more than 6-fold increase in sensitized rats (Fig. 2), attesting to the intensification of oxidative metabolism of alveolar macrophages. The new Russian bronchodilator troventol at 10^{-3} mg/ml lowered the level of the CL response both in intact and in sensitized rats by 43.0±7.3 and 63.0±11.0%, respectively (Fig. 2). Therefore, troventol reduces ROS production by alveolar macrophages, the effect in phagocytes of sensitized rats being markedly more pronounced. In this case atrovent and atropine sulfate, which were studied for comparison, did not have a noticeable influence on this index. The level of FRP including LPO is known to be stepped up in inflammation [2,4,13]. The sensitization of rats resulted in a more than 3-fold increase of LPO (3.7±0.5 μmol MDA/g tissue) in lung homogenates in comparison with those in intact animals (0.9±0.3 μmol MDA/g tissue). Provocation of bronchospasm with an effective dose of antigen induced an additional 2-fold rise of the LPO level (7.5±0.7 μmol MDA/g tissue) as related to that in sensitized rats, which is consistent with reported data [3,6].

TABLE 2. Effect of Troventol, Atrovent, and Atropine on Fe^{2+}-Induced LPO in Liver Tissue ($M \pm m$)

Preparation		LPO level, μmol MDA/g tissue	Effect, %
Control		3.5±0.7	
Control + Fe^{2+}		40.0±2.5	100.00
Troventol	10^{-3}	38.8±1.7	97.0
	10^{-6}	43.7±2.0	109.0
Atrovent	10^{-3}	35.6±0.8*	89.0
	10^{-6}	42.2±2.2	105.5
Atropine	10^{-3}	39.0±2.1	97.5
	10^{-6}	42.1±1.8	105.2

The antioxidant properties of the preparations were assessed in the system of Fe^{2+}-induced LPO in lung and liver tissues of intact animals. Such a test system is able more adequately and precisely to assess the ability of preparations to inhibit FRP [1]. The liver tissue was used both for comparison of the properties of the preparations and for a study of their possible specificity. As is shown in Table 1, troventol at 10^{-3} mg/ml reliably decreased the level of Fe^{2+}-induced LPO in lung homogenates by 16%, while at 10^{-6} mg/ml the decrease was only 9% vis-a-vis the control. Atrovent (10^{-3} mg/ml) possessed a weaker inhibitory effect, whereas atropine sulfate in the same concentration exhibited an effect similar to troventol. Hence, troventol possesses more marked antioxidant properties than atrovent in lung tissue. The study of the influence of the preparations on Fe^{2+}-induced LPO in liver tissue did not, however, reveal significant differences in effect either between the drugs or on LPO itself (Table 2). Thus, troventol possesses specificity precisely for lung tissue, as was noted previously [8].

Thus, the level of LPO rises in lung tissue in rats subjected to sensitization and experimental bronchospasm. ROS production by macrophages is markedly boosted in sensitized rats. The bronchodilating preparation troventol lowers ROS production by activated alveolar macrophages of intact and sensitized animals, decreases LPO in lung tissue, and has a specificity for lung cells and tissues, which are evidently responsible for its bronchodilating and anti-inflammatory properties.

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