The Effect of Hypoxic-Hypercapnic Training on Recovery of Local Protective Lung Factors during Experimental Chronic Pneumonia

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The results of many studies testify that inhalation of hypoxic-hypercapnic gas mixtures leads to an increase of the minute volume of respiration, alveolar ventilation, and oxygenation of the blood, improves the ventilation-perfusion correlation [1,4,5], and, finally, decreases the physiological pulmonary shunt of patients with chronic respiratory insufficiency [2]. Overall, inhalation of a hypoxic-hypercapnic gas mixture enhances the organism's tolerance of extreme effects, which appears to be associated with certain changes of the immune system [5].

However, there are now data concerning the influence of hypoxic-hypercapnic training (HHT) on the immune system and on the progress of inflammation of the lungs. The study of these aspects was the aim of this investigation.

MATERIALS AND METHODS

Experiments were carried out on 24 Wistar rats. Pneumonia was induced by the introduction of kapron thread in the lumen of the trachea [3]. One month after treatment the rats were decapitated, the heart-lungs complex was isolated, and a bronchoalveolar flush (BAF) and blood were taken. The surface tension (ST) of the surfactant was determined on Wilhelm-Langmuir scales (minimal ST - ST_{min}, maximal ST -ST_{max}). The stability index (SI) was calculate after Clements [6,7].

The number and percentage composition of cells in BAF were determined by the routine method. The functional activity of BAF and blood phagocyte cells was tested by their adhesive, migrational, and rosette-forming capacities, and also by latex microsphere absorption (MA and PF), and oxygen-dependent (NBT-test) and oxygen-independent metabolism (lysozyme). Microscopic study of the slides, stained with hematoxylin and eosin, was performed to examine the morphological changes of the lung tissue.

HHT was performed in special chambers using the "recycled" respiration method. The concentration of O_2 and CO_2 at the end of training was 15% and 6% respectively. The period of treatment lasted 10 days, and the daily training lasted 10 minutes.

There were four experimental groups: untreated animals; control rats with inflammation of the lungs, placed in HHT chambers with free access to air; treated animals with pneumonia, which received

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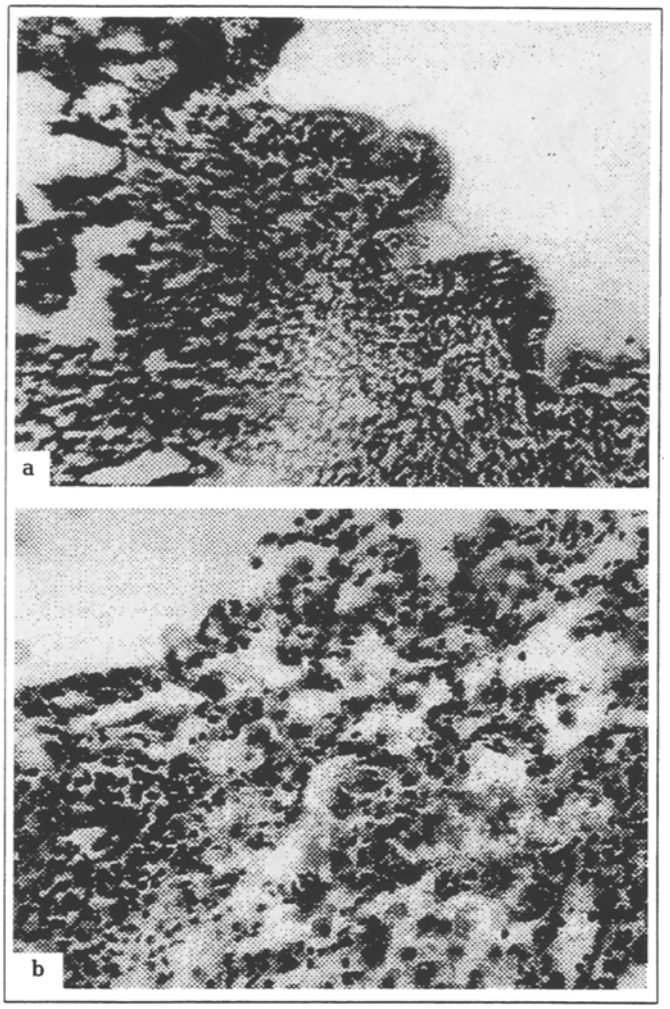

Fig. 1. Morphology of lung one month after induction of inflammation. *a*) catarrhal–suppurative bronchitis. ×160. Hematoxylin–eosin staining; *b*) serous exudate with admixture of cellular elements, primarily macrophages, in alveolar spaces; cell infiltration of alveolar septa. ×160. Hematoxylin–eosin staining.

HHT in two variants: before the induction of inflammation or one day after introduction of the foreign body in the trachea. The data were processed by statistical analysis using Student's *t* test.

RESULTS

During one-month inflammation of the bronchi, rats typically develop catarrhal sometimes suppurative (Fig. 1, *a*) bronchitis with epithelial desquamation. The alveoli respond to the inflammation mainly with an interstitial reaction (edema, round-celled infiltration of alveolar septa); only isolated cells are detected in the alveolar spaces (Fig. 1, *b*). There are small foci of swelling in the respiratory part of the lungs (dilatation of alveolar spaces and ducts).

The index of BAF surface activity becomes differ from that under normal conditions. The difference is reflected in an increase of St_{min} (38.93±2.73 versus 16.64±0.53) and St_{max} (56.12±1.96 versus 36.38±0.69) and a decrease of SI (the differences between experimental groups are reliable).

Data characterizing the number and the functional state of alveolar macrophages (AM) and neutrophils (N) of the blood are presented in Table 1. Rats with one-month inflammation presented no changes in the number of BAF cells, but the cell composition differed greatly from that of untreated animals. The number of active AM was in the normal range, but their phagocytic capacity was reduced 1.6 times; their intracellular oxygen- dependent metabolism diminished, while the oxygen-independent metabolism increased. At the same time it was possible to observe the activation of blood N phagocytic capacity, but the intracellular oxygen-dependent metabolism was decreased. Thus, the one-month inflammation of the lungs resulted in dramatic changes in functional and metabolic activity of AM BAF and blood N.

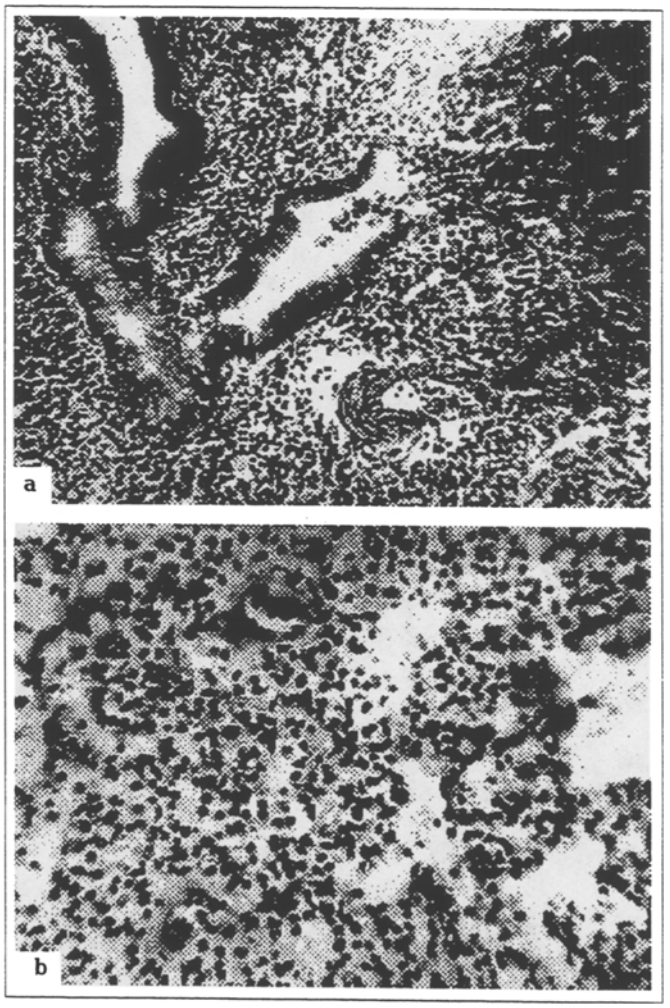

Fig. 2. Morphology of lung after HHT: *a*) suppurative bronchopneumonia. ×100. Hematoxylin–eosin staining; *b*) suppurative leukocytic exudate in alveolar spaces. ×100. Hematoxylin–eosin staining.

TABLE 1. Influence of HHT on Number, Composition, and Functional Activity of BAF and Blood Phagocytic Cells in Rats with Inflammation of Lungs.

Experimental group	Number of BAF cells $\times 10^6$	BAF cytogram, %				Functional state of N					Functional activity of AM BAF		
		AM	Mc	N	L	Adhesion, %	Migration, %	PF, %	MA, c.u.	NBT-test, %	Lysozyme, μg/ml	PF, %	MA, c.u.	NBT-test, %
HHT before induction of inflammation, n=8	4.9±0.9"	63.4±4.5"	16.5±3.4	11.3±1.4"'	8.0±1.1"	24.8±5.4	42.3±13.1"'	45.6±2.9	8.5±0.4'	55.0±2.6"'	23.1±2.8	48.1±1.9"'	8.08±1.9"'	3.2±0.4"
HHT after induction of inflammation, n=5	11.5±0.8"'	47.7±4.1"	7.7±1.8"	33.0±9.9"	8.3±2.9	32.0±5.5	26.7±2.6"'	46.3±2.7"	7.0±1.1	22.0±7.2	15.4±1.1	32.0±3.1"'	6.3±0.3"	0.4±0.2"
Control inflammation, n=5	3.0±0.7	47.3±2.4"	8.3±1.8"	32.3±4.0"	12.0±5.2	20.7±7.2	73.7±5.7	42.0±2.3	4.9±0.1"	7.33±0.9"	37.6±4.8"	5.5±3.3"	7.2±0.7"	0.8±0.3"
Untreated control, n=6	6.4±1.4	76.0±1.9	16.3±2.3	4.7±1.6	3.0±0.6	21.2±2.2	82.0±4.8	32.6±4.3	8.0±0.2	32.7±4.6	18.4±2.8	28.7±2.4	4.8±0.1	6.5±0.2

Note: ' — significant difference of ($p<0.05$) in comparison with control inflammation; " — significant difference of ($p<0.05$) in comparison with untreated control; Mc — monocytes; L — lymphocytes; c.u. — conventional unit.

The alterations in the lungs under HHT which started after introduction of the foreign body in the trachea are more pronounced than in the control group of animals with inflammation.

There is a predominance of catarrhal-suppurative bronchitis and some foci of suppurative bronchitis (Fig. 2, *a*). Signs of hyperplasia of the peribronchial lymphoid follicles and their inflammatory alteration are noted. In the respiratory part the changes consist not only of edema and cellular infiltration of the alveolar septa, but also of exudate in the alveolar spaces (Fig. 2, *b*). The exudate contains mainly polymorphonuclear leukocytes, and there are also microfoci of abscesses. Modest-size swellings, distelectases, and atelectases are observed. At the same time the effect of HHT on the animals with inflammatory lung disease is attended by an increase of the surfactant surface activity in the part of the lung free of empyesis. St_{min} decreases to 23.97±0.01, St_{max} to 35.25±1.41, SI to 0.38±0.04.

When HHT is performed before the introduction of thread in the trachea, the changes in the lungs correspond closely to those observed in the control groups, but catarrhal bronchitis is more frequent. The segmental and subsegmental bronchi are involved. There is minor hyperplasia of the peribronchial lymphoid follicles. The changes in the respiratory part are mainly similar to those in the control group, but small foci of atelectases may be seen. HHT here has a beneficial effect on the BAF surface activity (St_{min} 30.84±1.34; St_{max} 47.23±1.96; SI 0.39±0.03).

The BAF cytogram did not change under the influence of HHT after the induction of inflammation, but the effect before the induction made for a significant decrease of the number of N and an increase of the percentage of AM, although neither of these indexes reached the level in the untreated animals. The AM adhesive capacity in both cases did not suffer significantly, but the migration of AM decreased, mainly under the influence of HHT after the induction of inflammation. The percentage of BAF phagocyte cells did not change, but phagocyte activity increased to the level in the untreated rats. Under HHT after the induction of inflammation the oxygen-dependent AM metabolic activity rose to normal, whereas under HHT before the induction of inflammation the percentage of NBT-active cells was higher than in untreated rats. In both cases of training, oxygen-independent intracellular metabolism decreased to the level in untreated animals. There was a small decrease of phagocytic N. The level of intracellular oxygen-dependent metabolism of N under HHT before the induction of inflammation was within normal limits, whereas under HHT after in-

duction it remained, as in the controls, greatly diminished.

As is evident from the foregoing, the effect of HHT on rats with a one-month inflammation process depends on the treatment conditions. The action of HHT before the induction of inflammation on the whole provides a favorable effect, but the action of HHT after induction, in addition to positive changes, also yields significant negative alterations. First and foremost, there is an exacerbation of the inflammation of the lungs. Based on these data, HHT could be considered as an effective preventive factor. The possibility of its use in treatment of chronic inflammation of the lungs calls for further investigations.

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Characteristics of the Antilysozyme Activity of *Staphylococcus aureus* in Different Types of Experimental Infection

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According to the laws of classical microbiology, the development of an infection and its type depend upon the characteristics and specific interaction of three forces: pathogenic microorganism, susceptible macroorganism, and environmental conditions. Recently, a great deal of attention has been paid to the factors of microorganism persistence and their role in the formation of different types and forms of infection: lingering, chronic forms, bacteria-carrying forms, hospital infections, etc. [5,7]. The ability of bacteria to inactivate lysozyme, defined as antilysozyme activity (ALA), is considered to be a marker of the persistence of bacteria capable of intracellular parasitism [4]. It has been shown that the ALA of bacteria is a constitutive, secretional factor interacting specifically with lysozyme, which is a crucial element in the natural resistance of the organism [9]. A number of clinical and laboratory investigations have shown a direct correlation between bacterial ALA and the course of infection [6,11]. The role of ALA in the

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