EFFECT OF INHALATION OF BIOLOGICALLY ACTIVE PREPARATIONS ON LUNG SURFACTANTS

V. A. Berezovskii, N. S. Pilipchuk,
R. G. Protsyuk, and V. Yu. Gorchakov

UDC 612.212.014.1.94.462.8.014.46:
615.451.35

KEY WORDS: streptomycin, isoniazid, tuberculosis, surfactants.

Surface-active substances of the lung, often called surfactants, play an important role in external respiration. They participate in this process mainly by reducing surface tension at the gas—liquid phase boundary in the lung [13]. Many pathological processes reduce the synthesis and secretion of surfactants in the lungs. If surfactant synthesis is deficient or surfactants are inactivated, certain pathological processes may develop in the lung: ateleetasis, effusion of fluid into the lumen of the alveoli, obstruction of the blood flow through the alveolar capillaries [6], and disturbance of gas diffusion through the air—blood barrier [2]. Disturbance of surface activity on the phase boundary in the lungs develops because the work of the surfactant system of the lung (SSL) is thrown out of balance. By SSL is meant certain cellular structure and the extracellular lining complex [9]. The principal cells are type II alveolocytes (AII), responsible for surfactant synthesis, and alveolar macrophages, which participate in the removal of surfactants from the alveolar surface. The lining complex includes the hypophase and the surfactant monolayer [14]. An increase or decrease in the supply or utilization of surfactants may lead to disturbance of the surface actvity of the lining complex. That is evidently why the SSL is highly sensitive to many factors of endogenous and exogenous nature. The endogenous factors include disturbance of the hemodynamics, ventilation, innervation, and metabolism in the lungs and chronic inflammatory processes and states connected with surgical operations on the thoracic and abdominal organs. Exogenous factors include changes in the partial pressure of oxygen in the inspired air, chemical and dust pollutants of the inspired air, and certain drugs. Inhalation of various drugs and, in particular, aerosols of streptomycin and isoniazid for the treatment of turbuculosis [3, 7, 8], are widely used in medical practice and may have an influence on the SSL.

The aim of this investigation was to study the effect of long-term inhalation of streptomycin and isoniazid on the state of the SSL.

EXPERIMENTAL METHOD

Sexually mature male albino rats weighing 150-200 g were used as experimental animals. Solutions of the drugs were dispersed by means of a TUR USI-50 ultrasonic inhaler. An aerosol of streptomycin and isoniazid was injected into a special chamber made of transparent plastic, divided into 12 compartments (20 × 10 × 15 cm), in which the animals were placed. The daily dose of streptomycin was 20 mg/kg and of isoniazid 15 mg/kg. For aerosol dosimetry under experimental conditions, data on the respiratory minute volume (RMV) of the animal [12] were used and the volume of air inhaled by the animals per minute during the inhalation was calculated by the formula $V = 0.5 \times W$, where 0.5 is a constant and W the animal's body weight (in kg). Knowing the animals weight it was possible to calculate RMV, and if the concentration of the drug was known, the quantity of it inhaled by the animal during the inhalation could be determined. Using Heubner's formula [12], RMV for rats (average weight 150 g) was 75 cm^3. An example of a calculation may be given. Under assigned conditions of inhalation (streptomycin concentration 25 mg/ml of inhaled solution), 1 ml of solution was converted into an aerosol in the course of 1 min. During this time 12 liters of air passed through the spray, and consequently each liter of air contained 25:12 = 2.08 mg, and 1 cm^3 contained 0.00208 mg streptomycin. During the inhalations, it must be assumed that absorption of the

A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Kiev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 8, pp. 185-189, August, 1986. Original article submitted March 28, 1985.

0007-4888/86/0009- 1081$12.50

TABLE 1. Effect of Ultrasonic Inhalations of Streptomycin and Isoniazid on Surface Tension of Lung Extracts

Number of inhalations	Streptomycin					Isoniazid				
	surface tension, mN/m									
	static	maximal	minimal	IS	n	static	maximal	minimal	IS	n
Control	35,9±2,4	49,7±1,4	18,6±1,0	0,99±0,04	10	—	—	—	—	—
1	43,2±0,77	50,08±1,10	22,8±0,4	0,73±0,02	10	36,0±0,91	49,2±1,3	19,7±0,9	0,85±0,08	10
15	37,0±0,73	50,9±1,10	20,1±0,72	0,87±0,02	10	37,3±1,00	49,6±1,1	19,9±0,9	0,84±0,02	10
30	40,7±0,80	51,2±1,07	21,6±0,79	0,79±0,04	10	34,4±2,24	48,7±2,86	18,6±1,08	0,87±0,09	10
60	40,6±0,79	49,3±0,9	22,8±1,00	0,73±0,09	10	40,0±3,43	52,3±3,20	20,0±1,30	0,89±0,07	10
90	41,9±1,60	46,0±0,95	25,6±0,39	0,57±0,01	10	49,3±3,15	57,8±1,47	26,4±1,70	0,76±0,04	10
90+interval of 7 days	45,9±1,12	53,8±0,60	26,0±1,70	0,72±0,07	10	39,0±1,00	53,6±1,10	21,3±0,70	0,87±0,06	10
90+interval of 14 days	41,9±2,01	48,4±2,73	17,2±2,41	0,95±0,06	10	36,3±0,80	49,4±0,70	19,3±1,00	0,99±0,05	10

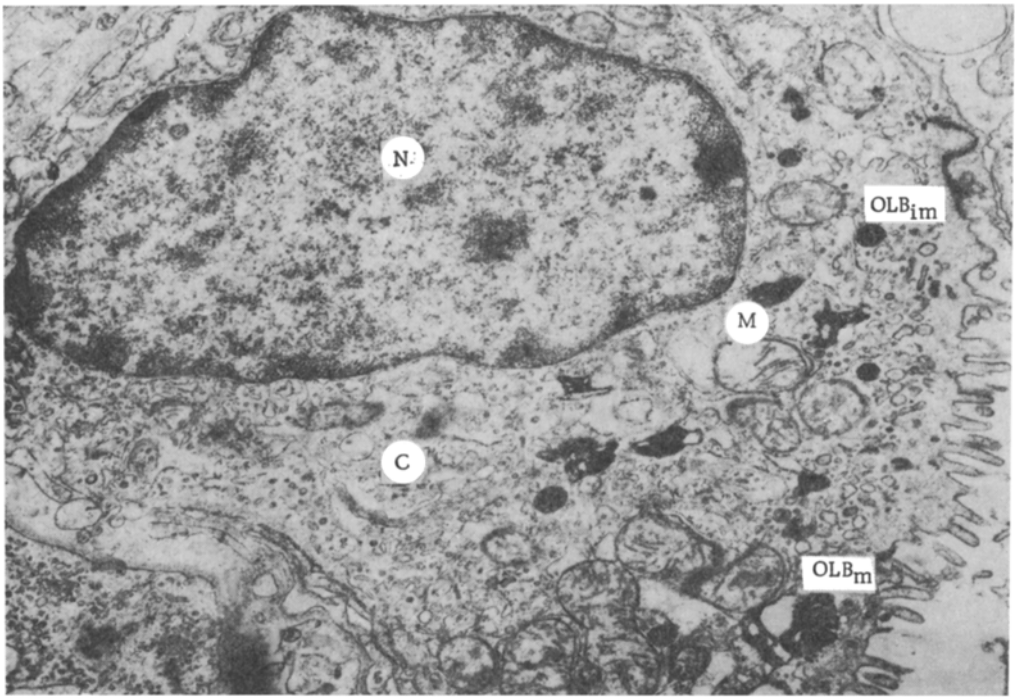

Fig. 1. Large AII with few OLB: immature (OLB_{im}) and mature (OLB_m). Mitochondria (M) have a translucent matrix and a few cristae. N) Cell nucleus. Three months of USI with streptomycin. 13,700×.

drug in the lungs amounts to 30-40% of the inhaled dose [10]. Allowing for the inhalation time, it is possible to calculate the total inhaled dose. The inhalations were given daily and each one lasted 30 min. After an assigned course of inhalations, the animals were decapitated next day. To study the physicochemical properties of the lung surfactants an extract was made from 100 mg of lung tissue and its surface activity was measured by the method described previously [2, 4, 5]. The static surface tension and the maximal and minimal surface tensions during contraction and stretching of the monolayer were recorded. On the basis of the results an index of stability (IS) — an integral value serving as measure of surface activity of the surfactants — could be calculated. IS was calculated as described previously [11].

EXPERIMENTAL RESULTS

The minimal surface tension of lung extracts from the control rats was 18.6 ± 1.0 mN/m, and the maximal 48.7 ± 1.4 mN/m. IS was 0.99 ± 0.04, evidence of the normal physiological state of the SSL. Investigation of the effect of streptomycin aerosols showed that the surface activity of the surfactants began to decrease immediately after the first session of

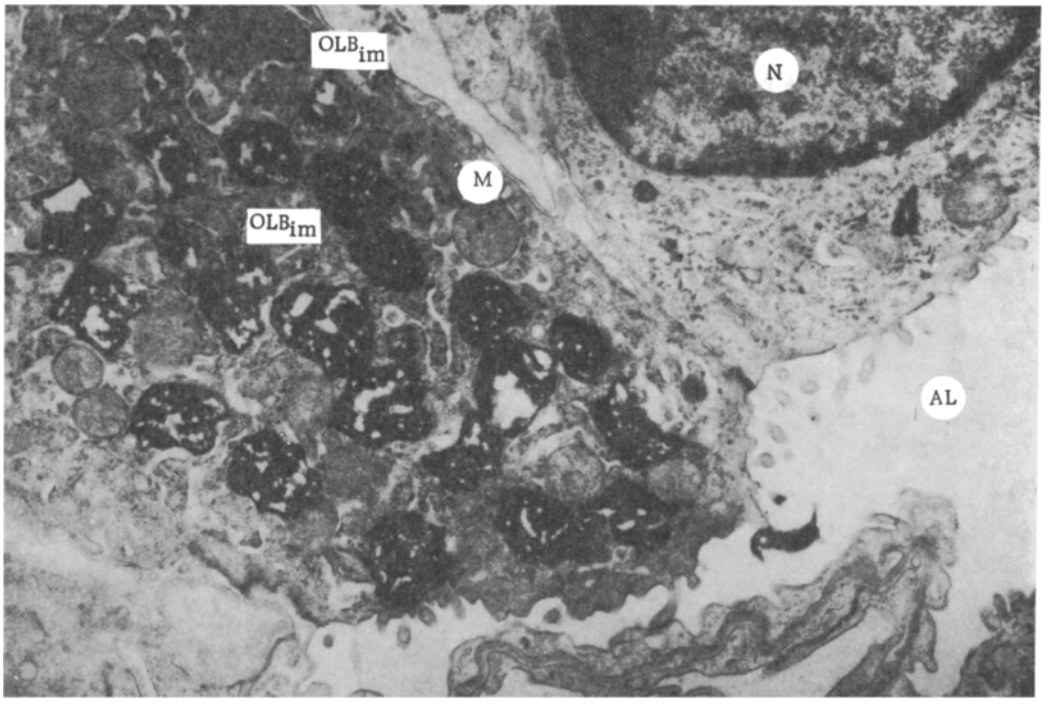

Fig. 2. "Dark" AII with well developed ultrastructure, almost completely filled with OLB_m and OLB_{im}. AL) Alveolar lumen. Three months of USI with isoniazid. 13,680×.

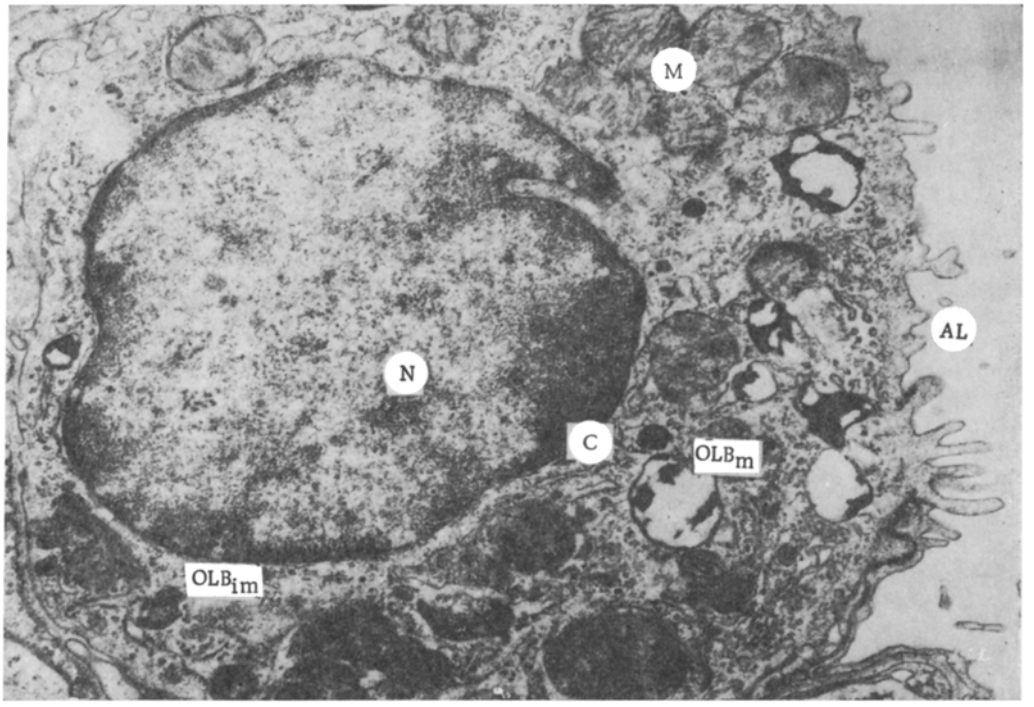

Fig. 3. AII with well developed ultrastructure, containing a considerable number of OLB_m and OLB_{im}. Fourteen days after the end of USI with the chemical preparations. B, 600×.

aerosol therapy (primary reduction). Toward the 15th day some recovery of surface activity took place (Table 1). Starting with the 16th inhalation there was a gradual decline of surface activity, which continued for 3 months of inhalations. By the 90th day the index of activity had fallen to 0.57 ± 0.01. Seven days after the end of the inhalations an increase in surface activity of the lung surfactants was observed. The index of activity reached a value of 0.72-0.07, whereas 14 days after the end of the inhalations the surface activity of the surfactants was almost completely restored and IS reached the value of 0.35 ± 0.06.

In the group of animals that inhaled isoniazid, a lowering of the surface activity of the surfactant happened directly after the first inhalation. IS decreased to 0.85 ± 0.08. The decrease in the surface activity of the surfactant in this case is less than during application of streptomycin, except that during inhalation by animals of isoniazid the surface activity of the surfactant remained constant for two months inhalation, and only after the 60th inhalation was further decrease in surface activity observed. On the 90th day of inhalation the surface activity decreased and IS reached the value 0.76 ± 0.04. After cessation of inhalation a stop in surface activity of the surfactant was noted. After 7 days IS rose to 0.87 ± 0.06, after 14 days it rose to 0.99 ± 0.05. The above data were obtained by intravenous introduction of isoniazid into dogs infected with tuberculosis [1]. In the earlier period under the influence of medication, surface activity of the lung surfactant stopped, and after four to six weeks its depression was observed at the center of the injury as well as in the parts of the lung far from the center.

An electron-microscopic study of the lungs of these same rats, having been treated with ultrasonic inhalations (USI) of streptomycin or isoniazid, showed that most AII were cells with moderate or poor development of the cytoplasmic reticulum of the lamellar complex, and with a small number of osmiophilic lamellar bodies (OLB). The mitochondria have a translucent matrix (Fig. 1). This is evidence of depression of intracellular surfactant synthesis in the animals. Disintegrating type AII cells were found more frequently in the experimental rats of this group than in the control. Usually these were large alveolocytes with enlarged mitochondria, deprived of cristae, with a well-marked endoplasmic reticulum, with a nucleus deprived of chromatin, and with a double set of intracellular structures and secretory granules.

Meanwhile, in some cases we observed AII cells almost completely packed with mature and young OLB. These cells have a well developed ultrastructure, a dark cytoplasmic matrix (Fig. 2). and they resembled "dark" AII with increased functional potential. Their appearance was evidently connected with compensatory secretion of surfactant in regions where AII activity was reduced because of microcirculatory disturbances in the alveolar walls.

Appreciable changes were observed in the ultrastructure of AII 7-14 days after the end of inhalations in animals receiving the tuberculostatics for a long time. Considerable accumulations of mitochondria with well-developed cristae appeared in the cell cytoplasm (Fig. 3). Tubules of cisterns (C) were in close contact with them. The number of cisterns and of osmiophilic bodies was appreciably increased. Besides mature OLB, these cells contained numerous young secretory granules. Activation of synthetic and secretory processes was evidently observed in AII in the animals of this group, possibly due to cessation of the toxic action of the chemotherapeutic agents.

During long-term aerosol therapy with antituberculosis drugs, it is thus necessary to monitor the state of the SSL: If surface activity of the surfactants falls by more than 30-40%, administration of the chemotherapeutic agents by USI must be interrupted or special measures used to correct the lung surfactants.

LITERATURE CITED

1. Yu. Ya. Agapov, I. M. Bondarev, and V. P. Novoselova, Patol. Fiziol., No. 4, 65 (1976).
2. V. A. Berezovskii and V. Yu. Gorchakov, Surface-Active Substances of the Lungs [in Russian], Kiev (1982).
3. A. L. Brant, V. A. Rudenko, and L. I. Medvedev, Abstracts of Proceedings of the 3rd All-Union Conference on Aerosols [in Russian], Vol. 2, Moscow (1977), pp. 71-73.
4. V. Yu. Gorchakov, "Changes in surface-active properties of lung surfactant in hypoxia and hyperoxia," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Kiev (1979).
5. V. Yu. Gorchakov, New Problems and Methods in Modern Medicine [in Russian], Kiev (1982), pp. 84-86.
6. E. N. Nesterov, G. N. Panevskaya, and G. V. Kobozev, Arkh. Patol., No. 3, 54 (1978).
7. N. S. Pilipchuk and R. G. Protsyuk, The Use of Aerosol Therapy in Combined Treatment of Polmonary Tuberculosis, Technical Recommendations [in Russian], Kiev (1983).
8. R. G. Protsyuk, Current Problems in Physiopulmonology [in Russian], Kiev (183), pp. 74-75.
9. L. K. Romanova, A. A. Zhavoronkov, B. L. Lempert, et al., Fiziol. Cheloveka, 3, No. 6, 1006 (1977).
10. S. I. Eidel'shtein, Principles of Aerosol Therapy [in Russian], Moscow (1967),pp. 209-215.

11. J. A. Clements, R. P. Jonson, and L. Y. Gribet, J. Appl. Physiol., 16, 444 (1961).
12. W. Heubner, Z. Ges. Exp. Med., 19, 264 (1920).
13. R. E. Pattle, Nature, 175, 1125 (1955).
14. E. R. Weibel and J. Gil, Resp. Physiol., 4, 42 (1968.