PHARMACOLOGY AND TOXICOLOGY

Effectiveness of Correction of Bleomycin-Induced Lung Injury by Early and Late Administration of Surfactant-BL

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Rats were exposed to inhalation of surfactant-BL starting from the first or eighth day after intratracheal administration of bleomycin. At the early stages, the preparation effectively attenuated damage to ultrastructural components of the lung tissue and reduced the severity and extent of subsequent pulmonary pathology.

Key Words: *rats; bleomycin; surfactant-BL; lung; ultrastructure*

Surfactant-BL (SBL), a Russian-made preparation isolated from cattle lungs, is used for the treatment of respiratory distress syndrome in children and adults [4] and in the complex therapy of pulmonary tuberculosis [5].

Previous studies have shown the therapeutic benefit of SBL inhalations in reducing the severity of bleomycin-induced alveolitis and atelectasis [2]. However, changes at the cellular level determining therapeutic effects of SBL on damaged lung remain unknown.

Here we compared the effects of SBL inhalations during early and delayed bleomycin-induced alveolitis on the dynamics of damage to various structural components in the lung.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats weighing 150-220 g. The rats under ether narcosis intratracheally received bleomycin dissolved in isotonic NaCl (10 mg/kg). Inhalations of SBL (15 mg/kg body weight) were performed on days 1, 3, and 5 after bleomycin administration in group 1 rats and on days 8, 10, and 12 in group 2 rats.

Three or ten days after SBL inhalation, the rats were sacrificed under thiopental narcosis (25 mg/kg). Lung tissue samples were fixed in 3% glutaraldehyde in cacodylate buffer (pH 7.4) and embedded in araldite. Ultrathin sections were examined under a JEM-1200 electron microscope. At each time point, samples of experimental and control lung tissue from three animals of total area 5500-6500 μ^2 were studied.

We evaluated the percentage of capillaries, dense and loose collagen fibrils, and the volume of alveoli filled with exudate and cell detritus. The number of type II alveolocytes and leukocytes per unit area of lung tissue and the number of macrophages and dead cells per unit volume of alveoles were determined. Only sections containing cell nuclei were counted. The thickness of 500-600 collagen fibrils in each of the comparison groups was measured. Quantitative analysis of TEM images (×3000) was performed using

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Image-Pro Plus 2.0 (Media Cybernetics) software and an Avtandilov grid.

Significance of differences between mean values of sample fractions was assessed using Student's *t* test [1].

RESULTS

One day after bleomycin administration, the percentage of dilated capillaries of interalveolar septa was 30% of the lung tissue (Table 1). Edema and destruction of the endothelium were associated with increased permeability of their walls, the presence of fluid in septal interstitium and exudate in the alveoli. Dead alveolar epithelial cells and detritus were found (Table 2). The content inflammatory cells in the lung tissue increased, which was accompanied by an increase in the number of fibroblasts with cytoplasmic processes protruding into intraalveolar exudate containing fibrin clots and appearance of collagen fibrils (carnification). The numbers of type II alveolocytes and osmiophilic lamellar bodies in them markedly decreased.

Thus, the onset of SBL inhalations in group 1 rats coincided with the development of edematous alterative phase of alveolitis.

By day 8 after bleomycin administration (day 3 after SBL treatment), the volume of capillary network in the alveolar septa of control animals decreased by more than 2 times (Table 1). Lung tissue damage increased with increasing cell infiltration. Serosanguineous exudate occupied about 50% alveolar volume, the relative content of cell detritus, dead cells, and collagen fibrils significantly increased (Table 2).

In SBL-treated animals, the decrease in the volume of intraalveolar exudate and cell detritus and the number of dead cells (Tables 1, 2) was accompanied by recovery of some structural components. This primarily concerned the number and ultrastructure of surfactant-producing cells. At that stage, carnification proceeded more rapidly in controls, causing more severe pulmonary disease. Some differences in the fibrillar architectonics of accumulated fibrous tissue were also noted between the two groups. The volumes of collagen bundles with densely packed fibrils were the same but SBL-treated animals showed significantly increased percentage of thin loosely arranged fibrils. It is believed that such fibrils are forming the bundles of collagen fibrils and can be involved in metabolism [3]. Average fibrillar thickness in SBL-treated rat lungs was 30% lower than in controls.

By the 15th day of the experiment we observed alleviation of lung tissue damage in all experimental animals (Tables 1, 2). However, the degree of compensatory and restorative mechanisms was different. In control rats, the percentage of capillary network remained unchanged in comparison with the previous term, whereas in SBL-treated animals it exceeded the previous values.

In controls, serous exudate was detected in the alveoli; in groups 1 and 2, it was not found. In the alveoli of SBL-treated rats the amount of cell detritus and number of dead cells decreased nearly 5-fold. Interstitial edema and leukocyte infiltration of lung were less pronounced. Type II alveolocytes in these animals were more abundant. The percentage of loosely arranged collagen fibrils was higher than in control rats. The fibrils were thicker than at the preceding time point, although their thickness was still 12% less than in non-treated animals.

In group 2 rats receiving SBL during productive phase of inflammation individual differences in ultrastructural abnormalities of alveoli were revealed.

By the 15th day (day 3 after the end of SBL treatment), compensatory and restorative mechanisms were

TABLE 1. Changes in the Percentage of Structural Components of Alveolar Walls ($M \pm m$)

Group	Time after bleomycin administration, days	Capillaries	Dense collagen fibrils	Loose collagen fibrils	Leukocytes	Type II alveolocytes
Control	1	30.0±3.87	3.0±0.7	0.80±0.42	1.20±0.28	1.40±0.38
	8	10.40±1.82	3.40±0.92	0.50±0.18	1.70±0.43	0.60±0.22
	15	10.40±1.92	7.10±0.98	1.20±0.21	1.50±0.33	1.70±0.17
	22	14.00±2.53	8.60±1.95	1.50±0.45	1.60±0.31	0.70±0.28
1st	8	10.40±2.19***	3.40±0.49	3.20±0.39***	0.70±0.21*	2.00±0.35**
	15	17.80±2.28*	5.00±0.63	2.70±0.38***	0.50±0.13**	2.30±0.19*
2nd	15	18.50±2.44*	5.90±0.96	1.00±0.19	0.70±0.18*	2.30±0.21*
	22	11.00±2.03	8.30±0.71	1.20±0.18	1.00±0.22	1.80±0.27**

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with controls at the same time point.

TABLE 2. Changes in the Percentage of Exudate and Structural Components of the Alveoli (*M*±*m*)

Group	Time after bleomycin administration, days	Exudate	Fibrils	Detritus	Dead cells	Macrophages
Control	1	29.50±6.42	0.90±0.38	2.10±0.36	0.40±0.16	2.60±0.46
	8	49.20±8.06	5.90±1.19	3.30±0.58	2.90±1.12	2.90±0.42
	15	10.90±3.67	0.10±0.09	5.20±0.96	1.70±0.31	1.6±0.4
	22	41.80±6.63	3.70±1.03	4.50±0.81	0.80±0.24	2.00±0.41
1st	8	19.40±5.81**	1.10±0.38***	1.60±0.37*	0.20±0.12*	1.50±0.34*
	15	0.00±0.00**	0.00±0.00	1.10±0.28***	0.30±0.12***	1.00±0.25
2nd	15	0.00±0.00**	0.00±0.00	4.40±1.06	0.00±0.00***	1.30±0.32
	22	7.90±2.47***	0.00±0.00***	5.00±0.93	0.70±0.21	1.40±0.26

seen in the lungs of all the experimental animals, but were more pronounced in SBL-treated rats. The decrease in leukocyte infiltration of the alveolar walls was associated with an increase in the number of alveolar type II cells. In SBL-treated rats, exudate and dead cells were completely absent, although the content of cell detritus and mature macrophages was low in the majority of alveoli in examined rats (Tables 1, 2). In group 2, similarly to group 1, the percentage of capillaries was higher than in controls.

By day 22 after bleomycin administration, aggravation of chronic alveolitis was observed; abnormalities in the architectonics of alveolar zones prevailed over restoration in all the animals. In controls, carnification loci appeared in enlarged exudate volume (Table 2). In groups 1 and 2, only traces of intra-alveolar exudate without signs of organization were found. Differences in the number and ultrastructure of type II alveolocytes persisted, which attested to better functional capacities of surfactant-producing cells in SBL-treated rats. Cell composition, infiltration area, and percentage of dense and loosely arranged bundles of collagen fibrils in alveolar walls did not differ significantly in compared groups.

Thus, electron microscopy showed that exudative inflammation accompanied by injury and depopulation of cells in the alveolar walls underlies the bleomycin-induced alveolitis. SBL inhalations, especially during the early stage, reduced the severity of damage to various ultrastructures and contributed to more rapid and complete recovery of cells, especially surfactant-producing type II alveolocytes. SBL therapy led to resorption of carnification loci. The decrease in the thickness and more loose arrangement of collagen fibrils in the lungs of SBL-treated rats can reflect attenuation and delay of pneumosclerosis in bleomycin-induced alveolitis under the effect of SBL therapy.

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