Therapeutic Efficiency of Early and Late Administration of Surfactant-BL during Bleomycin-Induced Damage to Rat Lungs

V. A. Volchkov, V. F. Dubrovskaya, O. V. Klestova, A. A. Val'kovich, V. A. Serzhanina, A. A. Seiliev, and O. A. Rosenberg

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Surfactant-BL was administered to rats via the inhalation route from day 1 or day 8 after intratracheal injection of bleomycin. Bronchoalveolar lavage and morphological characteristics of the lungs were compared. Administration of surfactant-BL at the early terms efficiently reduced the severity of bleomycin-induced alveolitis and atelectases.

Key Words: *alveolitis; bleomycin; rats; surfactant-BL*

Disturbances in the pulmonary surfactant (ST) system accompany many pulmonary diseases and are considered as important component of their pathogenesis [5]. However, ST preparations are now used only in the therapy of critical states [2,8].

Domestically produced preparation ST-BL obtained from cattle lungs [7] is successfully used for the treatment of respiratory distress syndrome in newborns [6] and adults [4]; it exhibits high therapeutic efficiency in a relatively low course dose [2]. At the same time, little is known about the possibility and degree of modifications in damaged lung tissue with ST-preparations administered at different stages of the pathological process. Bleomycin-induced alveolitis is a widely used model of pulmonary pathology. Our aim was to examine the therapeutic efficiency of the early and late administration of ST-BL preparation in this pathology.

MATERIALS AND METHODS

Experiments were carried out on the random-bred male albino rats (n=89) weighing 150-220 g. The rats under ether narcosis intratracheally received bleomycin (BM) dissolved in isotonic NaCl (10 mg/kg). Inhalation of ST-BL (15 mg/kg) were performed after BM injection on days 1, 3, and 5 in group 1 rats and on days 8, 10, and 12 in group 2 rats. Controls received BM alone.

Three or 10 days after the last administration of ST-BL, the rats were sacrificed under deep thiopental narcosis (25 mg/kg). Total lung sections (6-7 μ) were stained with hematoxylin and eosin according to Van Gieson method. Quantitative analysis of histological sections were made using an Avtandilov grid [1]. For each rat 4500-8000 points were processed.

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.

Architectonics and ultrastructure of the lung tissue were examined; the character and volume of lesions were assessed. We evaluated the percent of lung tissue occupied by atelectasis (absence of airiness due to cell obturation or alveolar collapse), alveolitis (partial decrease in alveolar volume), normal tissue (regions with normal or only slightly alternated tissue with available air).

Lavage samples were obtained by injection of sterile isotonic NaCl saline (3×4 ml) and collected

Central Research Radiology Institute, Ministry of Health of the Russian Federation, St. Petersburg. ***Address for correspondence:*** volchkov@mail.rcom.ru. V. A. Volchkov

into siliconized tubes; the concentrations of macrophages and leukocytes were determined.

The data were processed using Student's *t* test [3].

RESULTS

On day 1 after BM injections, numerous small alteration foci were observed in the lungs of control rats (lung airness was preserved). Changes in capillary ultrastructure attested to increased permeability and microcirculation disturbances in some loci of the lung tissue. Edema was observed in some regions of the interstitial tissue and in cells of the blood-gas barrier. Alveoli usually contained epithelial cells, leukocytes, and macrophages with signs of phagocytosis activation. In damaged regions, type II alveolitis (A-II) was accompanied by ultrastructural disturbances with partial or sometimes complete depletion of osmiophilic and lamellar bodies. The concentration of macrophages and leukocytes in the lavage fluid increased (Fig. 1). Therefore, in group 1 rats inhalations of ST-BL were started against the background of focal alveolitis during its edematous-destructive stage.

On day 8 after injection of BM, focal lesions were still observed in the lungs of control rats. In addition to interstitial and intracellular edema, infiltration of septa with inflammatory cells was revealed in thickened alveolar walls. The lumens of some alveoli contained the serous and hemorrhagic exudate, myelin membranes, damaged alveolocytes, blood cells, and phagocytising macrophages. Fibroblastic cells with signs of functional activation were observed in areas with partially or completely lost airiness. In addition to structural damages to cells of the alveolar epithelium and their depopulation, we observed hypertrophy of individual A-II cells with signs of alteration in the fine structure of lamellar bodies.

Lavage fluid contained primarily leukocytes, while the number of macrophages just slightly surpassed the control value.

Thus, in group II rats ST-therapy was initiated during the productive phase of the development of the focal alveolitis accompanied by signs of atelectasis and activation of fibroblast proliferation.

In group 1 rats examined on day 8 after BM injection (day 3 after termination of ST-therapy course), the sites with normal tissue structure occupied the same volume as in control rats (Table 1), while the percent of tissue involved in alveolitis was greater. In this case, ST-therapy significantly limited spreading of severe alveolitis and formation of airless areas in the lung tissue. Small tissue loci contained serous intraalveolar exudation, while in untreated rats we primarily observed hemorrhagic exudation. In addition, ST-producing A-II cells in group 1 rats demonstrated a lower degree of depopulation and better preservation of lamellar bodies. Atelectatic areas with fibroblast proliferation were more frequently seen in the lungs of untreated rats.

The number of leukocytes sharply increased in the lavage samples from rats of the control and the first experimental group. The percent of macrophages increased less dramatically, but in group 1 rats it significantly surpassed the control.

On day 15 after BM injection (day 10 after termination of ST therapy), the percent of air-filled tissue significantly increased due to shrinkage of atelectatic regions. The areas involved in alveolitis occupied approximately equal volume in the lungs of controls and group 1 rats. However, in group 1

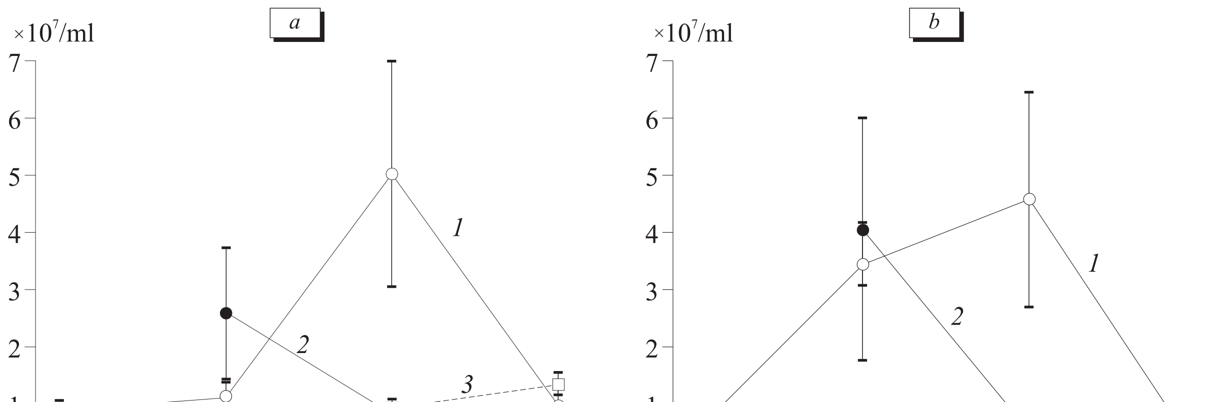

Fig. 1. Concentration of macrophages (*a*) and leukocytes (*b*) in lavage samples. *1*) control; *2*) group 1; *3*) group 2.

TABLE 1. Percentage of Lung Tissue with Various Structural Alterations (*M*±*m*)

Group	Days after BM injection	*n*	Normal	Alveolitis	Atelectasis
Control	8	6	18.70±0.23	14.80±0.23	66.50±0.43
	15	5	28.40±0.27	37.20±0.35	34.40±0.32
	22	6	27.80±0.27	55.20±0.38	17.00±0.23
Group 1	8	6	20.40±0.26	35.00±0.34	44.60±0.34
	15	5	46.0±0.3	41.40±0.34	12.60±0.21
Group 2	15	6	30.40±0.25	41.40±0.32	28.2±0.3
	22	6	48.00±0.26	26.80±0.28	25.20±0.27

Note. All differences from the control are significant at $^*p<0.001$.

rats structural disturbances in the blood-gas barrier became less severe and activation of the compensatory reparative processes was noted. In untreated rats, cells, detritus, and phagocytising macrophages were more frequently observed in the intraalveolar space.

In untreated rats, the number of leukocytes and macrophages in the lavage fluid attained the maximum during the observation period, while in group 1 rats, the number of these cells considerably decreased and did not differ from the corresponding indices of the day 1 after BM injection (Fig. 1).

In group 2 rats examined on day 15 after injection of BM (day 3 after termination of ST therapy) the percent of atelectatic tissue decreased, which was accompanied by an increase in the volume of normal lung tissue and tissue with sings of alveolitis (Table 1). At the same time, we observed more pronounced ultrastructural damage to cell elements in zones involved in alveolitis and the presence of alveolocytes, their fragments, and macrophages in the alveoli in untreated rats. The intensity of fibroblast proliferation in areas of atelectasis was similar to that in the lungs of control and group 2 rats. Cell concentration in lavage fluid was similar in groups 1 and 2 rats. In group 2 rats, the count of leukocytes and macrophages decreased to a level corresponding to day 1 after BM injection, while in untreated rats the number of these cells markedly increased.

On day 22 after BM injection (10 days after termination of ST-therapy), the percent of air-filled lung tissue in group 2 rats significantly increased due to decrease in the percent of areas involved in alveolitis. At the same time, the volume of atelectasis did not significantly change in comparison with previous term and significantly surpassed the control value. In foci of chronic inflammation, the alterations of elements of the blood-gas barrier in rats treated with ST-BL were less pronounced and were accompanied by structural normalization in most ST-producing cells.

Leukocyte count in lavage samples of both experimental groups did not differ from the control level. The percents of macrophages in treated and untreated rats were similar and corresponded to those observed on day 1 after BM injection.

Therefore, the therapeutic effect of ST-BL inhalations performed during the early stages of BM-provoked pulmonary pathology manifested at early terms. It efficiently moderated the severity of alveolitis and reduced the areas of atelectasis.

The therapeutic effect of ST-BL administrated during the productive phase of alveolitis accompanied by fibrous proliferation was slower, and it did not eliminate the causes leading to atelectasis in the lung tissue.

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