STRUCTURAL CHANGES IN THE LUNGS AND PHOSPHOLIPIDS OF THE LUNG SURFACTANT IN RATS WITH EXPERIMENTAL BLEOMYCIN PNEUMOSCLEROSIS

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Acute toxic alveolitis (ATA), which may be caused by many different chemical agents or may develop as a result of allergic reactions, leads as a rule to pneumosclerosis and to other chronic nonspecific lung diseases [2, 8]. In the early stages of ATA, interstitial edema, lymphoid infiltration, and activation of macrophages are found. An important contribution to the development of the picture of ATA is made by the reaction of the type II alveolocytes, which most frequently takes the form of hypertrophy of these cells, and lipoproteinosis, namely an increase in the content of surfactant-like material in the alveoli [2, 7, 8]. An important role in maintenance of the normal function of the alveolar apparatus and of effective gas exchange is known to be played by constancy of the chemical composition and structure of the lung surfactant [2, 7]. Recently the possibility of development of corpuscular preparations for the treatment of lung diseases, on the basis of surfactant and (or) liposomes, has been discussed in the literature [10]. An important problem in the development of preparations of this kind is the choice of an adequate and reproducible model. Pneumosclerosis [1, 3] is a threatening and constant complication of chemotherapy and combination therapy of lung cancer by means of the antibiotic bleomycin (BM). Of a whole range of agents tested (paraquat, quartz sand, silica-gel) BM, when injected intratracheally, gives rise to a sufficiently adequate and highly reproducible clinical and morphological model of ATA and the subsequent pneumosclerosis [3, 6].

The aim of this investigation was to study structural changes in the lung tissue and phospholipids (PL) of the lung surfactant in bleomycin-induced ATA and pneumosclerosis, for comparison of the changes mentioned above has not been adequately investigated. It was hoped that the study described below would provide this information.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-200 g, bred at the Central Roentgeno-Radiological Research Institute, Ministry of Health of the USSR. BM in a dose of 10 mg/kg in 0.3 ml physiological saline was injected as a single dose through puncture of the tracheal wall under aseptic conditions and under ether anesthesia. At various times from 1 to 60 days after injection of BM, the rats were anesthetized (10-12 animals at each point), the trachea was exposed and ligated, after which the lungs were removed from the thorax. Animals of the control group underwent none of these procedures. Lungs for morphological examination were straightened out in a 10% solution of formalin and fixed for 5 days. Histological preparations were stained with hematoxylin-eosin and by Van Gieson's method. Activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and cytoplasmic and mitochondrial forms of glycerophosphate dehydrogenase (cGPDH and mGPDH respectively) was determined in the epithelial cells of the bronchi

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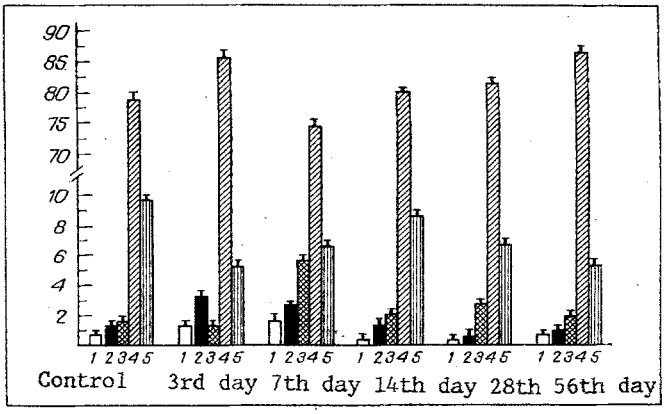

Fig. 1. Changes in phospholipid composition of lung surfactant in bleomycin pneumosclerosis: 1) lysophosphatidylcholine, 2) sphingomyelin, 3) phosphatidylserine, 4) phosphatidylcholine, 5) phosphatidylglycerol. Abscissa, time after injection of antibiotic (in days); ordinate, content of lipid phosphorus (in %).

and alveoli and the endothelium of the capillaries of the alveolar wall, and alkaline phosphatase (AlP) activity in the endothelial cells of the bronchial capillary were determined semiquantitatively in frozen sections through the lungs, 10 μ thick.

To study the phospholipids of the surfactant, washings were obtained by lavage of the lungs three times with buffered (5 mM Tris-HCl, pH 7.4) cold physiological saline in portions of 4 ml. The washings were pooled and centrifuged at 300*g* for 10 min at 4°C to remove cells and cell debris. PL were extracted with chloroform—methanol mixtures as in [5]. The extracts were dried on a rotary evaporator (RV0-64, Czechoslovakia) and redissolved in 1.5 ml chloroform. The content of lipid phosphorus was determined as in [11]. The composition of the PL fractions was analyzed by two-dimensional thin-layer chromatography on silica-gel with particle size of 15-25 μ, in chloroform—methanol μ NH_3 (65:25:5) and chloroform—acetone—methanol—acetic acid—water (30:40:10:10:5) systems [4]. The stain of PL was developed in iodine vapor, and zones of silica-gel corresponding to individual PL fractions were scraped off and their lipid phosphorus content was determined.

EXPERIMENTAL RESULTS

In histological specimens of the lungs 24 h after injection of BM, areas of atelectasis and dystelectasis alternating with areas of aeration were discovered. Hemorrhages into the lumen of the alveoli, edema of the alveolar septa, and their infiltration by leukocytes and macrophages, with contamination by a few eosinophils, and edema fluid in the lumen of the alveoli were observed. Disturbances of the lymph flow and hemodynamics were found. After 3-7 days the small foci of inflammation merged to form larger foci, the number of macrophages was increased, and desquamated epithelium began to appear in the lumen of the alveoli and the disturbances of the lymphatic flow and hemodynamics became more severe. The number of foci of cellular infiltration in the alveoli, the alveolar septa, and the lumen and wall of the bronchi gradually decreased toward the 14th day of observation. By the 30th day the signs of inflammation had subsided, but the alveolar septa remained thickened, with clearly distinguishable collagen fibers.

The results of the enzyme-histochemical analysis show that by the 3rd-7th day after injection of BM, LDH and SDH activity was increased in the epithelium of the bronchi and alveoli (with a maximum of LDH activity on the 30th day). cGPDH and mGPDH activity was increased by the 3rd day in the epithelium of the bronchi and alveoli, and by the 3rd-7th day an increase in LDH, cGPDH, mGPDH, and AlP activity was found in the endothelial cells of the capillaries. After the increase in enzyme activity in the epithelial and vascular components of the air-blood barrier, it gradually decreased toward the 30th and 60th days.

The total PL content increased during the development of bleomycin pneumosclerosis almost twofold, and by the end of the period of observation it amounted to 2.5 ± 0.23 mg PL per lung (1.3 ± 0.17 mg PL in the control).

Information on the time course of changes in composition of PL of the surfactant is given in Fig. 1, and shows that the reaction to injection of BM demonstrated considerable changes in fractional composition of PL. They consisted of an increase in the content of lysophosphatidylcholine, sphingomyelin, and phosphatidylserine toward the 3rd-7th days after exposure, followed by a return to the initial values by the 30th day.

The most marked changes were found in the phosphatidylglycerol content. They were wavelike in character. Initially (up to the 3rd day after injection of BM) a sharp decrease was observed in the phosphatidylglycerol concentration, followed by an increase until the 14th day, almost back to the original values, followed by another marked fall in the 4th-8th week of observation (Fig. 1). The content of the main fraction of PL, namely phosphatidylcholine, increased until the 4th week of development of the process and remained high until the 60th day. Analysis of the data showed that no change was found in the phosphatidylethanolamine, cardiolipin, and phosphatidic acid concentrations in PL of the surfactant (data not given).

The significant changes in the content and composition of PL of the surfactant correlate in time with changes in the morphological picture of ATA and changes in activity of the enzymes of energy metabolism studied (see above).

It must be pointed out that the increase in the relative and absolute content of lysophosphatidylcholine and sphingomyelin is characteristic of many destructive and degenerative processes, accompanied by proliferation of connective-tissue cells.

The changes discovered in the absolute and relative content of the individual PL fractions must evidently significantly disturb the surface-active properties of the surfactant, and are evidently reflected in function of the alveolar apparatus and in the exchange. Similar changes in the fractional composition of PL also were found in ATA of a different nature, and in particular, caused by introduction of silica-gel [9].

Thus characteristic combined morphological changes in the structure of the lung tissue and in the content and composition of PL of the surfactant take place during the development of acute toxic bleomycin-induced alveolitis and subsequent pneumosclerosis.

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