PHARMACOLOGY AND TOXICOLOGY

Ultrastructural and Cytochemical Changes in the Respiratory Compartment of the Lungs in Rats after Combined Treatment with Fine Silicon Dioxide Powder and Uridine

N. P. Lebkova and V. I. Baranov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 6, pp. 633-637, June, 2004
Original article submitted October 14, 2003

Electron microscopy and cytochemical study of alveolar tissue of rat lungs were performed at the early stage after intratracheal treatment with fine silicon dioxide powder. The preparation was administered to animals receiving or not receiving intravenous injection of uridine. Dust particles permeated the cytoplasm, mitochondria, and nuclei of cells in the air-blood barrier of the alveoli. Uridine decreased the severity of dust-induced damage to cells and increased intracellular glycogen content.

Key Words: *silicon dioxide; lungs; uridine; electron microscopy*

Fine powder of silicon dioxide (particle size 10-100 Å, specific surface 100 m^2/g) is extensively used in various industrial processes. The effect of this powder on the lung tissue is poorly understood. Coarse-grained dust is less toxic than fine dust and can be removed from the respiratory tract with villi. A decrease in the size of dust particles is accompanied by an increase in the degree of their accumulation in the terminal compartment of the lungs. Small particles produce a strong toxic effect on the organism. Silicon dust is most toxic compared to other dusts [1].

The development of new effective approaches to the prevention and therapy of lung damage produced by ultradisperse dust inclusions requires a detailed study of intracellular structural and functional changes in pulmonary alveoli responsible for gas exchange between inspired and expired air.

Here we performed an electron microscopic and cytochemical study of uridine-produced changes in the submicroscopic structure and energy homeostasis in alveolar tissue of the lungs in rats at early stage after treatment with fine powder of silicon dioxide (SD).

Uridine serves as a stimulator of plastic and energy processes in cells. This substance is involved in the synthesis of RNA and glycogen (major intracellular carbohydrate substrate of energy) and increases the resistance of cells to hypoxia accompanying lung diseases [1,3]. Uridine and its phosphoric compounds have a positive effect during ischemic injury, myocardial infarction [3], atherosclerosis [5], and starvation [6].

MATERIALS AND METHODS

Experiments were performed on 15 outbred rats. The animals were randomly divided into 3 groups. Group 1 rats served as the control (n=4). Group 2 animals intratracheally received 0.5 ml physiological saline containing fine SD powder in a single dose of 5 mg (n=6). Group 3 animals (n=5) received intramuscular injections of uridine (water solution) in a daily dose of 50 mg/kg for 10 days; SD was administered on day 3 of treatment. Control and treated rats were kept in a vivarium under similar conditions.

Russian Medical Academy of Postgraduate Education, Moscow

0007-4888/04/1376-0556

The animals were anesthetized and killed after 10-day treatment with uridine (day 8 after SD administration). Small samples of alveolar tissue were taken from the upper region of the lungs, fixed in paraformaldehyde, postfixed in a buffered solution of OsO_4, dehydrated with alcohols in increasing concentrations and absolute acetone, and embedded into Epon-araldite mixture. Semithin sections for electron microscopy were stained with methylene blue (estimation of common characteristics and detection of lipids) and Schiff reagent (method of McManus for glycogen detection). For electron microscopy, ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-7A electron microscope.

RESULTS

No pathological changes were found in the lungs of control rats. We revealed no signs of atelectasis or emphysematous changes in lung tissue. Similar results were obtained during examination of semithin sections. Single lung macrophages with normal characteristics (coniophages) were present in the alveolar cavity. Many alveoli included aggregations of surfactant.

Electron microscopy of semithin sections revealed well-defined intercellular boundaries in the epithelial alveolar layer and endothelium of capillaries. Single erythrocytes and leukocytes were present in the capillary lumen. Dust particles were absent. Abnormal mitochondria were rarely found in type 2 large alveolar cells and other cells of the alveolar epithelium. Surfactant-producing osmiophilic lamellar bodies (OLB) contained a moderate amount of lamellar structures. Aggregations of glycogen granules were revealed in some cells around resorbed OLB.

Profound changes in the air-blood barrier of group 2 rats developed on day 8 after single intratracheal administration of SD. Macroscopic signs of emphysema and atelectasis of the lungs included swelling and formation of dense cyanotic focuses. Light microscopy revealed alveolar collapse. Dilation of alveoli was accompanied by an increase in the lumen volume and thinning of the wall. The alveoli contained a considerable number of coniophages and erythrocytes. Leukocytes and plasma cells were often seen. Ultrastructural changes included massive destruction of coniophages. It was accompanied by destruction of the cell membrane, mitochondria, and lysosomes and release of the intracellular detritus and absorbed quartz dust into the alveolar cavity. Considerable amounts of lipid inclusions, dust conglomerates, and diffuse polymorphous dust particles were present in macrophages and alveolar lumen. Most dust particles had small size. Sometimes we revealed large crystalline and bundle-like structures. Small dust particles permeated alveolar and endothelial cells and capillary lumen through the damaged plasmalemma. A considerable number of erythrocytes, lymphocytes, plasma cells, and neutrophils were present in capillaries. We revealed diapedesis of erythrocytes through the air-blood barrier.

Fragments of OLB were accumulated on the surface and in the lumen of alveoli, which reflects profound changes in the synthesis of surfactant [4].

Some alveolar cells were characterized by hypoplasia of OLB. Hyperplasia of OLB and accumulation of lipid drops were characteristic of other cells. Most OLB were completely exhausted. It was probably related to the inhibition of surfactant synthesis. Conti-

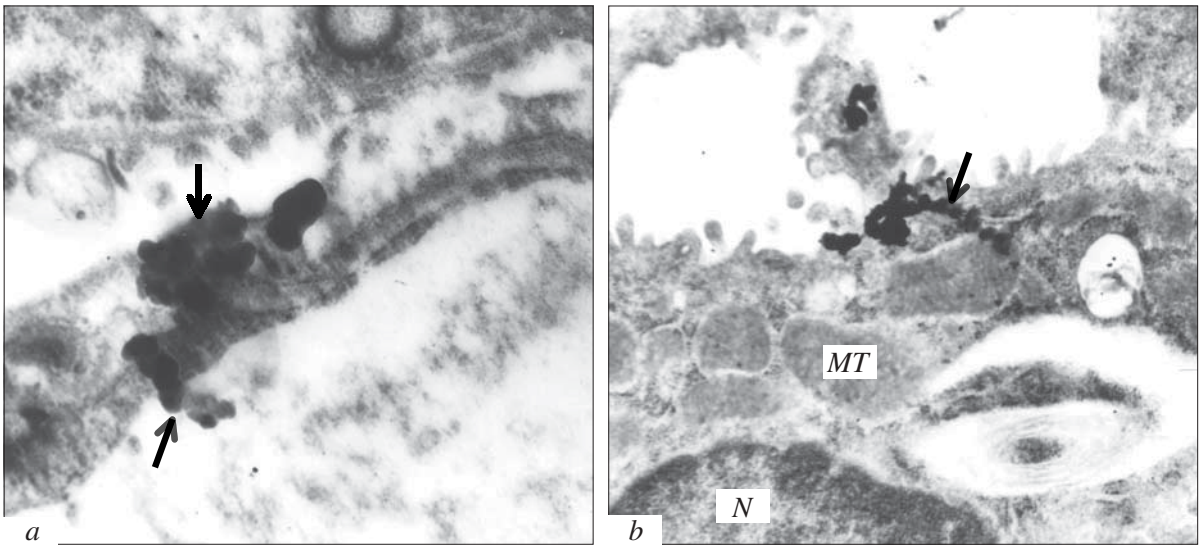

Fig. 1. Infiltration of alveolar tissue in the lungs with silicon dioxide. *a*) conglomerates of silicon particles (arrow) in the air-blood barrier (×31,200); *b*) permeation of silicon dioxide (arrow) into the alveolar cell (×32,500). *MT*: mitochondrion; *N*: nucleus.

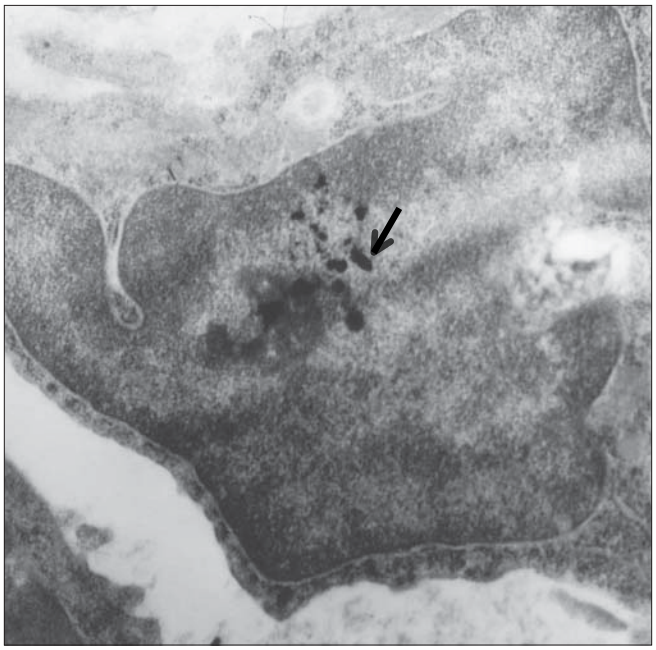

Fig. 2. Particles of silicon dioxide (arrow) in destructed nucleus of a large alveolar cell (×45,000).

nuous surfactant layer was absent on the alveolar epithelium. Components of surfactant in the alveoli were found more rarely than in control rats. Many mitochondria in alveolar cells and coniocytes had destructed membranes and cristae, were swollen, and included microgranular dust particles. Dust particles were also seen in the nuclei of alveolar cells (Figs. 1-3).

Under normal conditions, dust is eliminated from the alveoli with macrophages and surfactant produced by large alveolar cells [1.8]. Destructive changes in these cells reflect abnormal synthesis of surfactant and disturbances in evacuation of absorbed silicon particles from pulmonary alveoli. Silicon dust produces a strong damaging effect on coniophages. This is associated with the ability of dust particles to adsorb lipids and stimulate lipid peroxidation (LPO), since silicoses are accompanied by accumulation of lipids in the lungs [1,2]. Accumulation of lipid inclusions in coniophages of group 2 rats reflects activation of LPO.

Structural changes develop in the early period after accumulation of ultradisperse dust in pulmonary alveoli (day 8). These changes impair gas exchange in the air-blood barrier and promote the development of immune disorders. The number of bacteria in the alveoli and capillaries increased after inhalation of dust.

Light microscopy showed that in group 3 rats receiving uridine as a preventive and therapeutic drug (before and after administration of SD, respectively) atelectasis and emphysema of the lungs were less pronounced than in group 2 animals. Most alveoli were enlarged and did not contain erythrocytes and other formed elements of the blood. The count of destructed coniophages was low in alveoli. The amount of lipid inclusions in the cytoplasm of cells from these rats was lower than in animals not receiving uridine. We found glycogen inclusions that were absent in coniophages from group 2 rats. It was related to transforma-

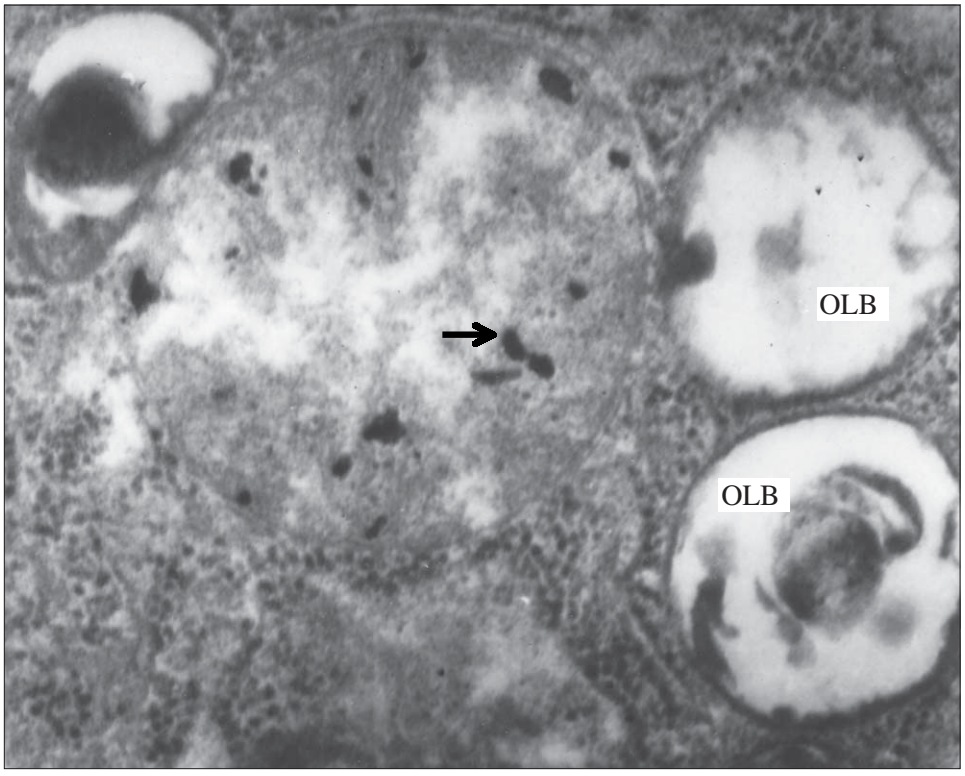

Fig. 3. Particles of silicon dioxide (arrow) in swollen mitochondrion with destructed cristae and exhausted osmiophilic lamellar bodies (OLB) in large alveolar cell (×87,000).

tion of intracellular lipids into glycogen. These changes were previously observed in cells of various tissues under extreme and pathological conditions. They serve as an adaptive reaction, which maintains intracellular energy homeostasis by activation of anaerobic glycolysis [6].

Similar changes in the amount of lipids and glycogen energy substrates (increase in glycogen content) were observed in type 2 large alveolar cells. They resulted not only from stimulation of glyco- and gluconeogenesis with the involvement of lipids for energy-related glycolysis, but also from high-intensity synthesis of the carbohydrate component in surfactant. The amount of this component in alveoli increased in uridine-receiving animals. In group 3 rats the number of OLB in type 2 large alveolar cells was higher than in group 2 animals. They underwent transition into lamellar concentric structures typical of surfactant and were accumulated in the hypophase and inner surface of the alveolar layer. Uridine considerably increased the amount of surfactant structures in the alveolar lumen. The structural relationship exists between intracellular lipids, glycogen, and surfactant [9,10]. However, the functional role of this relationship remains unclear.

Uridine is the major component of the key enzyme for carbohydrate synthesis. This compound stimulates the synthesis of glucose, glycogen, and other polysaccharides from fatty acids from in alveolar tissue during fat catabolism. Small amounts of carbohydrates can be formed from glycerol entering the composition of neutral lipids [6].

The mechanism of OLB formation in alveolar cells is poorly studied. It was hypothesized that various intracellular structures, including mitochondria, Golgi complex membranes, and pinocytotic vesicles, serve as a source of OLB. In our experiments uridine increased the number of microbodies (peroxisomes) in macrophages and type 2 large alveolar cells. Microbodies are the evolutionary precursor of mitochondria. Enzymes involved in the glyoxylate pathway and localized in these organelles convert fatty acids into carbohydrates [6,7].

Some authors believe that peroxisomes play a role in the synthesis of surfactant [7].

Peroxisomes transform excess intracellular lipids into carbohydrates. These changes are accompanied by inhibition of LPO, which reduces the toxic effect of reactive oxygen species on cells, tissues, and whole organism.

The amount of dust particles in alveolar and macrophageal cells of the air-blood barrier decreased in group 3 rats. These organelles rarely underwent destructive changes. The degree of intracellular edema was low. The amount of glycogen and the number of ribosomes in the cytoplasm increased. Positive ultrastructural intracellular changes in the terminal region of the lungs are probably related to the increase in functional activity of cell nuclei, since uridine is involved in the synthesis of nucleic acids.

Our results indicate that the ultradisperse powder of SD produce damage to alveolar tissue of the lungs, which is abolished by treatment with uridine. These findings are of considerable significance for the therapy and prevention of pneumoconioses caused by fine dust.

Study of the lungs showed that uridine holds much promise for the prevention of pathological changes produced by ultradisperse silicon dust.

REFERENCES

1. B. T. Velichkovskii, *Fibrogenic Dusts (Peculiarities of Structure and Mechanism for Biological Activity)* [in Russian], Gorky (1980).
2. B. T. Velichkovskii, *Vestn. Ros. Akad. Med. Nauk*, No. 6, 45-52 (2001).
3. V. V. Eliseev, N. R. Evdokimova, I. B. Krylova, *et al*., *Khim.-Farm. Zh*., No. 3, 270-273 (1989).
4. V. V. Erokhin, *Functional Morphology of the Respiratory Compartment in the Lungs* [in Russian], Moscow (1987).
5. N. P. Lebkova, *Vestn. Ros. Akad. Med. Nauk*, No. 9, 16-22 (2000).
6. N. P. Lebkova and O. A. Tugushi, *Problems of Lymphology and Quantitative Pathology* [in Russian], Moscow (1997), pp. 118-120.
7. A. M. Panchenko, A. M. Gerasimov, and V. D. Antonenkov, *Role of Peroxisomes in Cell Pathology* [in Russian], Moscow (1981).
8. L. K. Romanova, *Regulation of Reparative Processes* [in Russian], Moscow (1984).
9. O. S. Alwal, *J. Submicr. Cytol*., No. 2, 443-445 (1983).
10. E. Klika and L. Antalikova, *Folia Morphologia*, No. 2, 184-188 (1980).