Intracellular Changes in Rat Hepatocytes after Intratracheal Administration of Highly Dispersed Silicon Dioxide and Uridine Effects on These Changes

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Rat hepatocytes were examined under electron microscope at early terms after intratracheal administration of highly dispersed silicon dioxide powder against the background of uridine treatment. Penetration of powder particles into hepatocyte cytoplasm, nuclei, mitochondria, and peroxisomes and development of bacteria in these cells were observed. Uridine reduced the destructive effect of powder on the organelles, increased glycogen content in hepatocytes, and inhibited the formation of capsulated bacterial forms in these cells.

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

Ultradispersed silicon dioxide (SD) powder with particles of different size (from 10 to 100 Å) and specific surface of up to 100 m^2/g, is now widely used in various industrial processes. These particles can be detected only under a high-resolution electron microscope. It is assumed that such fine powder, particularly silicon, is the most toxic for the body, because it can penetrate into cells [1,2, 11]. The mechanisms of damaging effects of highly dispersed SD particles on the subcellular organelles are little known. All available data of electron microscopic studies describe alveolar macrophages in silicosis. However, we experimentally detected penetration of SD nanoparticles not only in rat lung macrophages, but also in the alveolar epithelial and endothelial cells and microvessels [7]; the possibility of penetration of SD nanoparticles through vessels into cells of other organs thus cannot be excluded.

We examined rat hepatocytes under an electron microscope after intratracheal administration of SD. The choice of hepatic parenchymatous cells is explained by the fact that bacteria play an important role in the pathogenesis of liver diseases [4,11], and intracellular penetration of these bacteria with silicon powder seems to be quite possible. Uridine participating in the synthesis of RNA and glycogen, improving activity of mitochondria, and exhibiting antioxidant properties served as the stimulator of intracellular defense and compensatory processes after hepatocyte injury. The positive effect of uridine was detected in many pathological and critical conditions [3,6,8], including SD exposure of pulmonary alveolar cells [7].

MATERIALS AND METHODS

We examined the liver of animals, on which SD effects on the lungs were previously studied [7].

The study was carried out on 15 outbred rats divided into 3 groups (two experimental and one control). Animals of experimental group 1 (n=6) received a single intratracheal dose of ultradispersed SD powder (5 mg) in 0.5 ml saline. Group 2 (n=5) animals were daily intramuscularly injected with aqueous solution of uridine (50 mg/kg) for 10 days and after 2 days an SD dose. The control group consisted of 4 intact rats. The animals were kept

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under similar vivarium conditions. Fragments of the liver were collected from the right lobe of narcotized animals on day 8 after SD administration. The specimens were fixed in paraformaldehyde buffer solution, postfixed in osmium tetroxide buffer solution, dehydrated in ascending alcohols and absolute acetone, and embedded in epon and araldite mixture. Semithin and thin sections were made on an LKB-4800 ultratome. Semithin sections were stained with methylene blue (for review and detection of fat), by Schiff's reagent after Mac-Manus (for detection of glycogen), and examined under an light microscope. Ultrathin sections were contrasted with uranylacetate and lead citrate and examined under a JEM-7A electron microscope.

RESULTS

In group 1 animals nanoparticles from the sinusoids and intracellular spaces penetrated into hepatocytes (Fig. 1, *a*) and accumulated in the cytoplasm, mitochondria, peroxisomes, and nuclei of these cells. Mitochondria into which SD penetrated were swollen, their membranes and cristae were damaged or almost completely destroyed (Fig. 1, *b*, *d*). Increased condensation of heterochromatin and a drop in the content of euchromatin were seen in hepatocyte nuclei with accumulation of SD (Fig. 1, *c*). Many peroxisomes with damaged membrane contained electron-dense structures looking like SD particles or very small spheroid bacteria (Fig. 1, *d*).

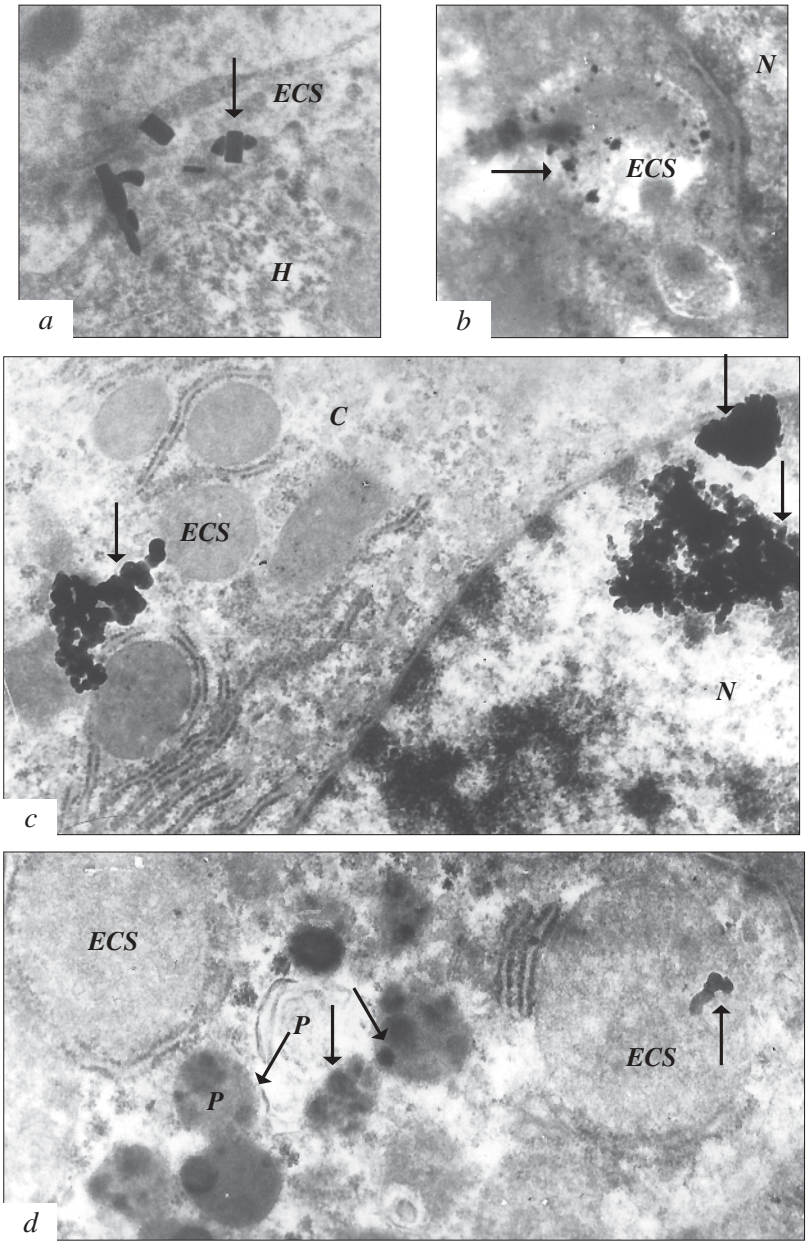

Fig. 1. Location of ultradispersed silicon dioxide (SD) in hepatocytes (arrows). *a*) penetration of SD particles into hepatocyte, ×15,000; *b*) SD particles in destroyed mitochondrion, ×25,000; *c*) accumulation of SD particles in the cytoplasm and nucleus, ×30600; *d*) SD particles in peroxisomes, ×30,600. H: hepatocyte; MT: mitochondrion; ECS: extracellular space; P: peroxisome; C: cytoplasm; N: nucleus.

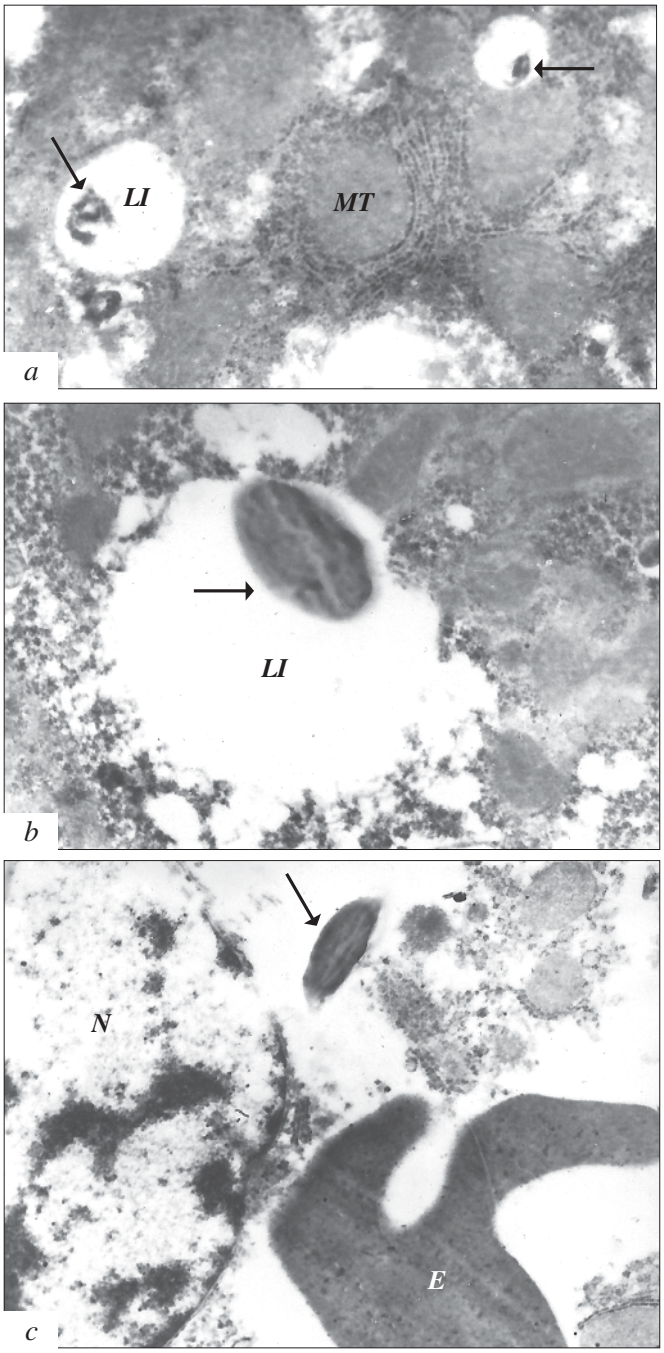

Fig. 2. Location of bacteria in hepatocytes (arrows). *a*) forming capsule-free bacteria in lipid incorporations of cytoplasm, ×25,500; *b*) formed capsulated bacterium in lipid inclusion of the cytoplasm, ×8250; *c*) hepatocyte with damaged nucleus, capsulated bacterium, and erythrocytes penetrating from capillary, ×7500. LI: lipid incorporations; MT: mitochondrion; E: erythrocyte; N: nucleus.

Chain-like structures located in the cytoplasm (Fig. 1, *c*) also greatly resembled bacteria. However, bacteria at different stages of their development (from small spheroid and twisted capsule-free to large capsulated forms) were most often seen in lipid inclusions in the cytoplasm (Fig. 2, *a*, *b*) and inside damaged hepatocytes (Fig. 2, *c*).

The destructive effect of quartz dust predominantly on the pulmonary macrophagal mitochondria, accumulation of lipids in these cells, presence and probable multiplication of phagocytosed bacteria, development of pulmonary tuberculosis and other infectious diseases in occupational silicosis are persuasively proven [4,11].

The destructive effect of SD on macrophages in silicosis is attributed to the capacity of silicon dust to adsorb lipids on its surface and cause their peroxidation [1,2,4,11]. The same mechanisms can account for destructive changes in the plasma membrane, nuclear, mitochondrial, peroxisomal, and other hepatocyte membranes, containing phospholipids, and for the appreciable increase in the content of intracellular fat.

Numerous microorganisms are adsorbed on the dust inhaled with air [10]. Ultramicroscopic nanobacteria, which are considered to be very early developmental forms of larger bacteria, can be present among them. They were detected in sea water, oceanic precipitations, in some soils and rocks. Nanobacteria are very closely connected to silicon [9]. These bacteria can be present in highly dispersed SD powder, used in our experiments, and penetrated together with it through the vascular system from the trachea or lungs into the liver.

Combined mechanical (SD particles), hypoxic (decreased energy production in damaged mitochondria), and toxic (LPO with participation of SD and bacteria) exposure can cause hepatocyte death, leading to the release and persistence of capsulated virulent intracellular bacteria in the circulation.

The main defense and compensatory mechanism in intracellular infection is enhanced regeneration of organelles determined by regulatory and protein-producing function of intact or little damaged nuclei.

Destructive changes in hepatocytes were less pronounced in group 2 animals. The content of euchromatin in their nuclei increased, the nucleoli were larger and closer to the karyolemma, the number of nuclear pores, through which ribosomes penetrated from the nucleus into the cytoplasm, was greater. The endoplasmic reticulum was presented mainly by the granular form. The majority of mitochondria had normal structure. There were virtually no lipid inclusions in the hepatocyte cytoplasm in this group of animals, while the content of glycogen inclusions increased significantly. Peroxisomes filled with glycogen were seen more often than in group 1. We consider that this was due to uridine-induced activation of the glyoxylate cycle enzymes and catalase in peroxisomes (these enzymes promoting glyconeogenesis from fatty acids) [6]. This

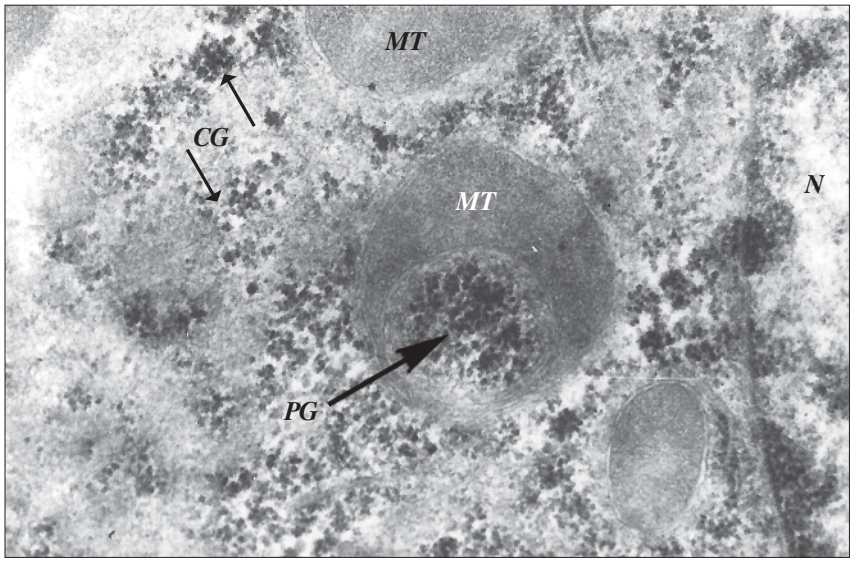

Fig. 3. Association of a mitochondrion with glycogen-containing peroxisome, ×38,250. MT: mitochondrion; PG: peroxisome with glycogen (arrow); CG: cytoplasmic glycogen; N: nucleus.

was paralleled by intensification of the energy-producing function of mitochondria due to intensification of glycolysis under conditions of hypoxia developing in the cells after penetration of SD. At the ultrastructural level this process manifested by close contacts of the mitochondria with glycogen-containing peroxisomes (Fig. 3).

The majority of bacteria in hepatocytes of animals treated with uridine before SD inhalation had no lipid capsules; this seemed to be due to predominant utilization of fatty acids for glycogen synthesis, but not for capsule formation. This sign can indicate that bacterial toxicity associated with lipid components of their capsules decreases in this case [10]. Structures similar to dust particles were rarely seen in the hepatocytes of control rats, but their nature and origin were not clear.

Hence, our experiments demonstrated the destructive effect of intratracheally administered SD powder on hepatocyte ultrastructure and the possibility of reducing this effect with uridine.

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