CYTOPHARMACOLOGICAL EFFECT OF POLYVINYLPYRIDINE-N-OXIDE IN EXPERIMENTAL SILICOSIS

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The development of experimental silicosis during simultaneous administration of polyvinylpyridine-N-oxide was studied by scanning and transmission electron-microscopic and cytochemical methods. Most macrophages were found to preserve their active function and to phagocytose quartz and to remove it through the air passages. In the fibroblasts the liberation of proteins onto the cell surface was delayed and the intensity of formation of collagen fibers was reduced. Administration of the polymer prevented the development of fibrosis of the lungs.

KEY WORDS: *scanning electron microscopy; macrophages; fibroblasts; silicosis.*

With the introduction of the therapeutic and prophylactic use of polyvinylpyridine-N-oxide (PVNO), a polymer which is soluble in tissue fluid and which largely prevents autolysis of the macrophages during the cytotoxic action of quartz and reduces the intensity of the sclerotic changes in silicosis, it has become possible to carry out more purposive research in this field. However, the mechanism of action of PVNO still remains unexplained and, in particular, the site of its fixation is unknown [2-5].

In this investigation an attempt was made, by electron microscopy and quantitative cytochemistry, to demonstrate the mechanism of the antifibrogenic action of PVNO on the development and course of experimental silicosis.

EXPERIMENTAL METHOD

Experimental silicosis was induced in albino rats by a single intratracheal injection of 20 mg quartz (particle size up to 3 μ) in the form of a suspension in 0.6 ml physiological saline. Experiments were carried out on two groups of animals. The animals of group 1 received a subcutaneous injection of 1 ml of a 2% solution of PVNO (mol. wt. 10,000) simultaneously with the quartz, and thereafter weekly for 3 months. The animals of group 2 received quartz only and served as the control. Besides light microscopy, transmission and scanning electron microscopy also were used [1]. In the early stages of the experiment (4th, 7th, and 14th days) the macrophages in squash preparations of the lungs were studied for their phagocytic activity, their content of total proteins (per dry weight, by interference microscopy), their nucleic acid content (cytospectrophotometrically), and size, and the location and activity of acid phosphatase in the sections were determined.

EXPERIMENTAL RESULTS

During the first 24 h after injection of quartz the morphological picture of the lungs of the animals treated with PVNO and the controls was of the same type. Initially granulocytes (mainly neutrophils) predominated in the interstitial tissue, but under the influence of the toxic action of the quartz they disintegrated and were phagocytosed by macrophages. After 3 days there was a sharp rise in the number of macrophages conducting intensive phagocytosis of the quartz particles. Necrotic changes were found more frequently in the macro-

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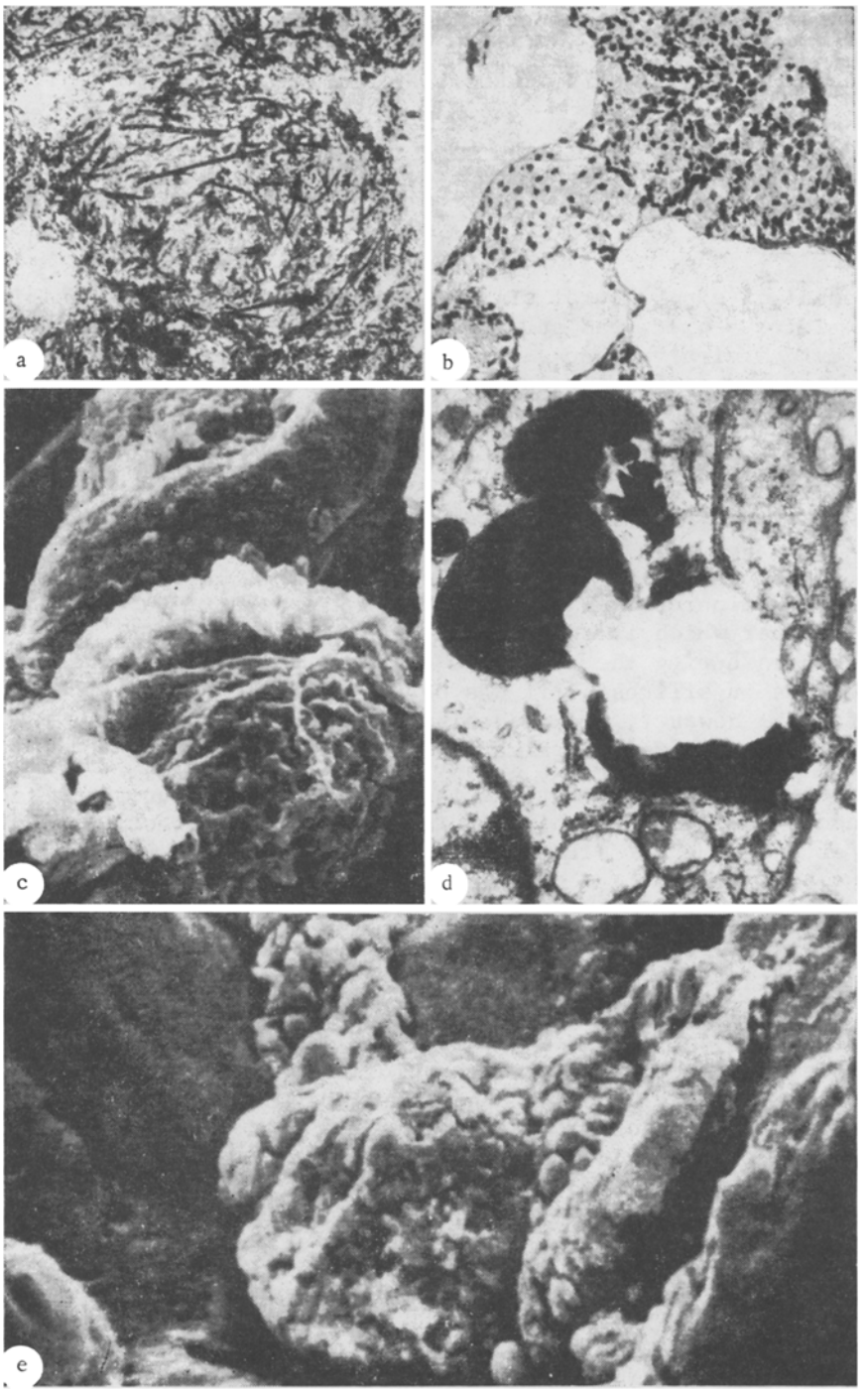

Fig. 1. Experimental silicosis of the lungs 6 months after injection of quartz particles: a) lungs of control animals: many collagen fibers (Van Gieson, 100×); b) lungs of treated animals: single collagen fibers (Van Gieson, 250×); c) fibroblast above, phagocytosing macrophage below (administration of PVNO, scan.; 5000×); d) integrity of ultrastructures of macrophage following PVNO administration (clear space shows where quartz was removed during cutting; 20,000×); 3) phagocytosing macrophage in alveolar cavity (scan.; 9000×).

phages of the control animals than of those receiving PVNO. Silicotic nodules consisting of groups of degeneratively changed macrophages, fibroblasts, and precollagen and collagen fibers were found in the lungs of the control animals 30 days after the injection of quartz. Meanwhile, in the treated animals, the degenerative changes in the macrophages were much

weaker and precollagen and collagen fibers were totally absent. The most marked antifibrogenic action of PVNO was discovered after 3-6 months or more. Many large silicotic nodules occupying wide areas and consisting of a mass of well-developed thick collagen fibers and fibroblasts were formed in the lungs of the control animals 6-12 months after injection of the quartz (Fig. 1a). The silicotic nodules in the lungs of the treated animals were small and they were formed by phagocytosing macrophages, single fibroblasts, and solitary thin precollagen and collagen fibers (Fig. 1b).

The most demonstrative difference in the response of the cells of the treated and control animals could be observed in the macrophages phagocytosing quartz particles. Initially the quartz particles appeared to stick to the cytoplasmic processes on the surface of the macrophage, but later they gradually sank into the depth of the cytolemma and penetrated inside the cell, the cytoplasmic processes joined together, and the particles of the mineral were surrounded by cytoplasm (Fig. 1c). As a result of counting the number of phagocytosing cells in squash preparations from the lungs it was found that the phagocytic activity of the macrophages of the treated animals was higher and the number of dying cells was smaller than in the untreated animals. To explain the results the level of biosynthesis was determined in the phagocytosing macrophages. The content of nucleic acids and total protein was found to be 30% higher in macrophages of animals receiving PVNO. The size of the macrophages of the treated animals increased within the same limits on account of an increase in the mass of the cytoplasm. Parallel with the quantitative cytochemical investigations an electron-microscopic study was made of the phagocytosing macrophages in the ultrathin sections in order to judge the degree of preservation of the organelles responsible for phagocytosis. Homogenization and clarification of the matrix of the mitochondria and lysosomes and disappearance of the organelle membranes took place in the macrophages of the control animals in zones of contact between quartz and organelles. Where several quartz particles underwent phagocytosis, the zone of proteolysis covered wide areas of the cytoplasm, including the nucleus also. The cell died.

In the experiments in which PVNO was given, the organelles in most of the macrophages containing quartz remained undamaged (Fig. 1d). The localization and high activity of acid phosphatase remained the same in the lysosomes, by contrast with the diffuse distribution of the enzyme in the cytoplasm of the macrophages of the control animals.

In the fibroblasts, dilatation of the tubules of the endoplasmic reticulum and filling of the cisterns with secretory proteins took place under the influence of the polymer, but the proteins were liberated slowly onto the surface of the cell. The study of fibroblasts in the scanning electron microscope during administration of PVNO showed that the cells had a nodular surface and an elongated, cylindrical shape (Fig. 1c). The residual macrophages of the treated animals, having phagocytosed the quartz, migrated along the interstitial tissue and were discharged into the lumen of the alveoli. Attached by their processes to the alveolar epithelium, they moved into the air passages and were removed from the lung (Fig. 1e).

The results described above thus show that after treatment with PVNO the ultrastructural organization of the macrophages is preserved and the polymer promotes intensive protein synthesis in them and active phagocytosis of the quartz particles, with their subsequent removal from the lungs through the air passages. The mechanism of the protective action of PVNO is evidently as follows: The polymer is fixed to the membranes of the organelles, shielding them from the cytotoxic action of the quartz. Secretion of proteins onto the surface of the cell is retarded in the fibroblasts and the intensity of formation of collagen fibers is weakened. The number of cells of the fibroblast series also decreases and this affects the formation of the total mass of collagen fibers in the regions of fibrosis.

LITERATURE CITED

1. V. D. Arutyunov, Yu. D. Batsura, and G. G. Kruglikov, Arkh. Patol., No. 9, 10 (1975).
2. B. A. Katsnel'son et al., in: Pathogenesis of the Pneumoconioses [in Russian], Sverdlovsk (1970), pp. 89-96.
3. I. M. Shnaidman, "Pathogenesis and simulation of the silicotic process," Author's Abstract of Doctoral Dissertation, Moscow (1974).
4. H. Antweiler, Beitr. Silikoseforsch., 5, 153 (1963).
5. I. Fetzer, Naturwissenschaften, 54, 518 (1967).