POSSIBLE WAYS OF INFLUENCING THE MECHANISMS OF SCLEROSIS IN EXPERIMENTAL SILICOSIS

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Cytochemical study (acid phosphatase, succinate dehydrogenase, NAD- and NADP-diaphorase) of peritoneal coniophages showed inhibition of the cytotoxic action of quartz by polyvinylpyridine-N-oxide, hydrocortisone, and chlorpromazine. Morphological and biochemical investigations showed that these compounds delayed the development of experimental silicosis in albino rats. By contrast, trypsin, which accelerates death of the coniophages, intensified collagen formation in the lungs of animals exposed to the action of dust.

A special place in the pathogenesis of silicosis has recently been ascribed to the macrophages [11, 12], which are generally considered to secrete fibrogenic substances under the influence of quartz. On the basis of investigation [4, 5, 7-9] the following hypothesis was put forward to explain the development of the most important components of the pathogenesis of silicosis. The first component – the phagocytosed quartz – causes damage to the mitochondrial membranes and disturbance of the energy metabolism and biosynthetic activity of the coniophage, followed by disintegration of the phagolysosome membranes and uncontrolled liberation of hydrolases into the cytoplasm. The second component is that as a result of autolysis of the coniophage and resulting changes in phospholipase activity lysolecithin, a substance reducing the ability of cells to absorb oxygen, and perhaps other fibrogenic factors also are secreted into the surrounding medium. The third component is that fibroblasts, which are highly competitive under hypoxic conditions, activate their primary function of collagen formation. The increased synthetic activity of the fibroblast takes place against a background of its sharply reduced energy metabolism, with the result that atypical mucopolysaccharides and collagen are produced.

Accordingly, the possible ways of deliberately influencing the development of the pathological process may be: reducing the surface activity of quartz in various ways, administration of stabilizers of mitochondrial and lysosomal membranes, removal of disintegration products of the coniophages either by specific hydrolysis or by the formation of inert complexes, the administration of substances stimulating oxido-reduction, and the specific inhibition of the synthetic function of the fibroblasts.

This paper describes results showing that the development of pneumosclerosis in experimental silicosis can be inhibited and intensified by the stabilization and labilization of the mitochondrial and phagolysosomal membranes of the coniophage.

EXPERIMENTAL METHOD

Two test objects were used: peritoneal macrophages and the lungs of rats with experimental silicosis. In the experiments of series I the cytotoxic effect of quartz dust was studied during the action of the substances indicated below. Impression films were made 24, 48, and 72 h after injection of 100 mg crystalline dust containing 92.8% silica into the peritoneal cavity of control and experimental animals. The cytochemi-

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cal reactions for succinate dehydrogenase, NAD- and NADP-diaphorase, and acid phosphatase [2-4, 13] were used as indicators of the state of the mitochondrial and lysosomal membranes. Each group of experimental animals additionally received one of four substances by intraperitoneal injection throughout the experiment: hydrocortisone (total dose per animal 60 mg), chlorpromazine (1-4 mg), polyvinylpyridine-N-oxide with molecular weight 80,000 (10-30 mg), and trypsin (2 mg 6 h before taking the films).

The object of the experiments of series II was the morphological (sections stained with hematoxylin-eosin, picrofuchsin, impregnation by Gomori's method) and biochemical (determination of collagen by the method of Slutskii and Sheleketina [6]) study of sclerosis in the lungs of rats with experimental silicosis under the influence of the preparations mentioned. The experiments with hydrocortisone were carried out on 60 rats (experimental and control) in which silicosis was induced by intratracheal injection of 50 mg of the above-mentioned dust. Three months later 30 animals received 2.5 mg hydrocortisone daily by gastric tube for one month. The experimental and control animals were sacrificed at the same time four months from the beginning of the experiment.

Chlorpromazine was injected in a dose of 2 mg three times a week during the last (fourth) month of the experiment into 15 rats with experimental silicosis; the same number of animals formed the control group. The effect of trypsin (this part of the work was done jointly with M. A. Son) on collagen formation in the lungs was studied in 181 rats at various times (from one to five months) after injection of the dust. Trypsin was injected intratracheally in a dose of 0.2 mg once every four days.

EXPERIMENTAL RESULTS

After 24 h the peritoneal exudate of the control animals contained coniophages and polymorphs actively engaged in phagocytosis of dust. The mainly diffuse character of the deposition of the reaction product in the cells in tests for oxido-reductases was evidence of a marked injury to the mitochondrial membranes by quartz even in the early stage of dust phagocytosis. In a few cells diffuse deposits of diformazan were less well defined. At the same period in animals receiving hydrocortisone, chlorpromazine and, in particular, polyvinylpyridine-N-oxide, the largest number of phagocytic elements consisted of those in which the mainly granular deposits of diformazan were combined with a less marked diffuse distribution of the dye.

After additional treatment with trypsin the diffuse deposits of diformazan in the coniophages were intensified.

In the reaction for acid phosphatase in the control animals, both uniform punctate deposits of granules of lead sulphide in the lysosomes and larger deposits of the reaction product, confluent in some places, were observed in the phagolysosomes. In the three groups of experimental rats the deposits of lead sulphide were mainly discrete in character.

Trypsin induced an increase in the diffusion of lead sulphide in the cytoplasm of the coniophages.

With an increase in the duration of exposure to dust (48-72 h), the diffuse deposition of reaction products in the tests for the mitochondrial and lysosomal enzymes increased in intensity and became complete in an increasingly large number of macrophages; some of the cells were destroyed. Destroyed cells were found more often after the treatment with trypsin but, conversely, less often after treatment with hydrocortisone, polyvinylpyridine-N-oxide, and chlorpromazine. When 10,000 coniophages were counted in the impression films from the control and experimental animals at the third period of sacrifice a statistically significant ($P < 0.001$) decrease was found under the influence of polyvinylpyridine-N-oxide, hydrocortisone, and chlorpromazine in the number of cells with evidence of marked or complete diffusion of the histochemical reaction product in the test for acid phosphatase (40 ± 1.4, 43 ± 1.1, $46 \pm 1.8\%$ compared with $54 \pm 2.2\%$ in the control), and an increase in the number of these cells to $66 \pm 1.4\%$ was found after treatment with trypsin.

Morphological investigation of the lungs of rats with experimental silicosis receiving hydrocortisone showed inhibition of sclerosis in the lungs, as reflected by some decrease in the number and in the degree of maturity of the silicotic nodules. These results are in agreement with those of the biochemical rats. For instance, the collagen content in the lungs of the control animals was 60.32 mg, or 21.26% of the dry weight of the organ, whereas in the experimental animals the figures were 48.88 mg or 16.5%. The difference between these indices in the two cases is statistically significant. Results of a study of the antisilicotic action of polyvinylpyridine-N-oxide published previously [1] show regression of the pathological process after intratracheal injection or inhalation of the dust by the animals which received subcutaneous injections of the compound.

Morphological investigation of the lungs of a rat with experimental silicosis and with silicosis treated with trypsin 1, 3, and 5 months after injection of the dust, however, showed an increase in collagen formation in the lungs. This was reflected by an increase in the number of small nodules in the lungs after administration of trypsin in the early periods of dust administration and the frequent confluence of larger silicotic nodules with the formation of continuous fields of airless silicotic connective tissue in certain areas five months after administration of the dust. Biochemical investigation showed that in the animals receiving trypsin 1.5 months after the beginning of the experiment there was a statistically significant ($P < 0.001$) increase in the relative (13.3% compared with 11.9% in the control) and a small but not significant increase in the absolute (34.87 mg compared with 33.28 mg in the control; $P > 0.5$) content of collagen in the lungs. Administration of trypsin when the pathological process was well established (in the last, fifth month after administration of dust) also showed a statistically significant increase in the content of collagen proteins: relative to 14.58% (12.85% in the control) and absolute to 85.26 mg (75.11 mg in the control).

The results of the morphological, cytochemical, and biochemical tests thus indicate that the substances used, depending on their stabilizing or labilizing action on the membranous structures of the coniophage, can inhibit or accelerate the development of pneumoconiosis. It should be mentioned in this connection that the slowing of development of experimental silicosis described previously [14] in rats under the influence of chloroquine phosphate can be explained in the light of investigations [10] showing the stabilizing effect of chloroquine on the phagolysosomal membranes of the hepatocytes.

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