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ACTION OF DEXTRAN-MODIFIED HYALURONIDASE IN EXPERIMENTAL SILICOSIS

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Silicosis is an occupational disease that develops through exposure to industrial aerosols containing quartz. The treatment of silicosis is an important problem in occupational medicine. Theoretical, clinical, and experimental data [1-3,5] relating to the search for agents with which to treat silicosis show that as yet no sufficiently effective remedies free from side effects have been found. During the development of fibrosis of the lungs under the influence of dust, synthesis of collagen and proteoglycans and the formation of collagen fibrils are stimulated. Proteoglycans form intermolecular bonds in collagen, regulate fibrillogenesis, and give the collagen fibers their stability [8]. Hyaluronidase (EC 3.2.1.35) depolymerizes glycosaminoglycans [4], and this may lead to regression of fibrosis. Many years of experience of the use of native hyaluronidase in clinical practice has shown that the enzyme is effective only if applied locally [4]. On parenteral administration its activity is low, evidently because the body contains many inhibitors. Attempts have been made to increase the stability of the enzyme under physiological conditions by covalent binding to N-hydroxypolyethylenepiperidine [6]. However, because of the toxicity of the matrix, this preparation

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TABLE 1. Parameters of Fibrosis in Lungs after Administration of Stabilized Enzymes for 4 Months

Experimental conditions	Weight of lungs		Weight of LN, mg		HP content in lungs, mg	% of BC
	wet, g	dry, mg	wet	dry		
SiO_2	5,5±0,1	1669±190	870±63	113±11	18,1±0,2	530
SiO_2 + SH intraperitoneally	4,8±0,5	1107±35*	826±80	169±16	10,5±1,7	300
SiO_2 + SL intraperitoneally	4,7±0,1	854,2±16*	982±109	152±26	8,5±1,9	250
SiO_2 + SH by inhalation	4,6±0,3	943,8±24*	639±104	66±8	6,9±0,8	200
SiO_2 + SL by inhalation	7,3±0,6	899,2±166*	570±134	1±3	5,3±0,5	155
SiO_2 + dextran by inhalation	4,8±0,5	1128,5±154*	886±128	104±13	10,2±1,5	300
BC	2,1±0,1	363,2±36*	468±18	28±7	3,4±0,68	100

Legend. Asterisk indicates significance of differences between groups by Student's t test at the $P < 0.05$ level.

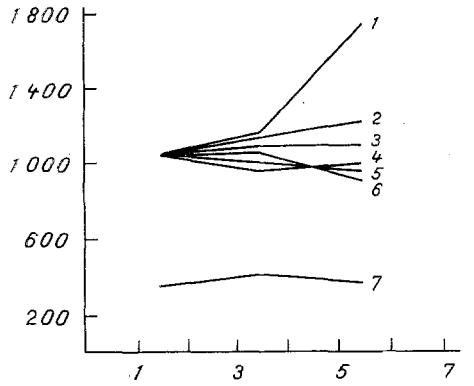

Fig. 1. Changes in dry weight of rat lungs during administration of stabilized enzymes and dextran to animals with silicosis. Abscissa, time (in months); ordinate, dry weight of lungs (in mg). 1) Untreated animals; 2) animals receiving dextran, 3) SH intraperitoneally; 4) SH by inhalation; 5) SL by inhalation; 6) SL intraperitoneally; 7) Biological control.

has not yet found an application. It seems more promising to use dextran for this purpose [9], more especially because other enzyme derivatives based on dextran have been used successfully in clinical practice for a long time [10].

The effect of hyaluronidase, stabilized by covalent addition to dextran, modified by partial oxidation, on the development of the pneumofibrosis in silicosis was the subject of the present investigation, for there are no data in the literature on the action of this compound in silicosis.

EXPERIMENTAL METHOD

Stabilized derivatives of hyaluronidase (from Reanal, Hungary) and of the pharmacopeial preparation lidase (USSR), with hyaluronidase action, were obtained by covalent interaction of the enzymes with aldehyde-dextran (mol. wt. 40 kilodaltons) [9] in 0.1 M phosphate buffer solution, pH 8.7, containing 0.15 M NaCl at 4°C for 24 h. After reduction of the derivatives with sodium borohydride, they were separated by ultrafiltration on an Amicon (USA) apparatus with XM-100 filter. The catalytic activity of the derivatives was monitored viscosimetrically [7]. Wistar albino rats initially weighing 180-200 g were used in the experiments. To create an experimental model of silicosis, 20 mg of highly dispersed (about 5 μ) SQ quartz dust (Czechoslovakia), suspended in 1 ml of 0.9% NaCl solution, was injected intratracheally into the animals. The development of fibrosis in the lungs was assessed by the usual parameters: the wet and dry weight of the lungs and lymph nodes (LN), their lipid and collagen protein (hydroxyproline) content [2]. The preparations of stabilized hyaluronidase and lidase (SH and SL, respectively) were dissolved in 0.9% NaCl solution and administered to the rats by inhalation in the form of an aerosol, produced by means of a modified Éidel'shtein's atomizer.

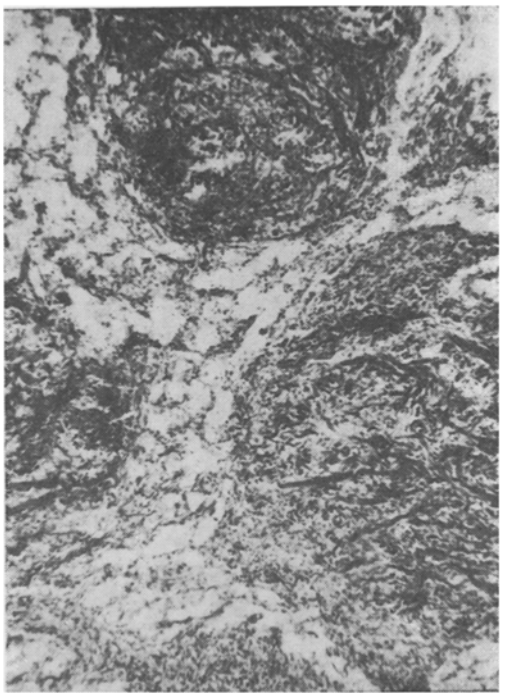

Fig. 2

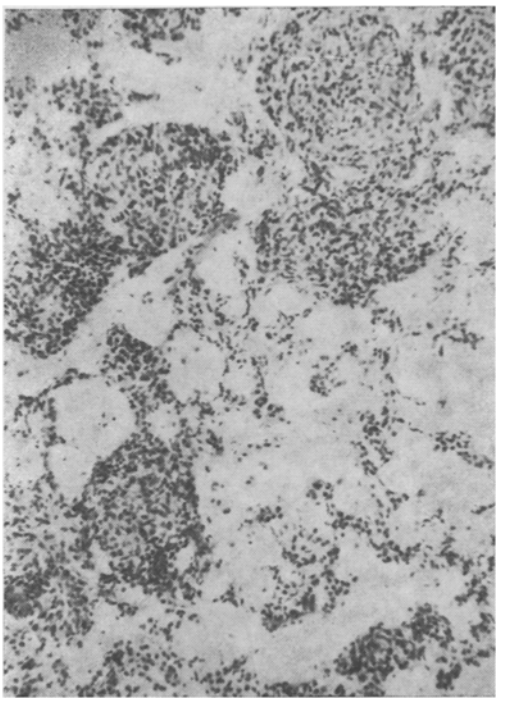

Fig. 3

Fig. 2. Large, confluent silicotic nodules with fibrocellular structure, accompanied by development of powerful bundles of collagen fibers in lungs of rats of the untreated group 5.5 months after administration of quartz dust. Here and in Fig. 3: stained by Van Gieson's method. 100×.

Fig. 3. Silicotic nodules at the stage of cellular structure with single collagen fibrils in lungs of rats treated by inhalation of SL for 4 months.

Air was pumped into the flask of the atomizer, with outlet tubes for five rats, by means of an inhaler, at the rate of 10 liters/min. Solutions of the stabilized enzymes were inhaled (2 conventional units of activity in each case) for 1 h three times a week for 4 months. The single dose of dextran was 24 mg per rat. In parallel experiments SH and SL were injected intraperitoneally into rats.

Morphological changes in the lungs were investigated by examination of sections stained with hematoxylin and eosin, and with picrofuchsine, by Van Gieson's method, for collagen proteins.

EXPERIMENTAL RESULTS

Toward the beginning of administration of the enzyme preparations (1.5 months after administration of SQ) marked fibrosis developed in the lungs: the weight of the lungs increased to 2.3 times that of the biological control (BC) and their hydroxyproline (HP) content reached 13.3 ± 1.2 mg (BS 3.3 ± 0.9 mg), which was three times higher than in the control. This is evidence that the treatment (administration of enzyme derivatives) was started when the fibrotic process had already commenced, as was confirmed by the results of the pathomorphological investigation.

After inhalation of dextran for 2 months a significant decrease was observed in the dry weight of the lungs compared with that in the other untreated animals, together with a marked decrease in the HP content. Administration of SH and SL also had an inhibitory action on the development of fibrosis and caused the HP content to fall by 2-2.5 times. After inhalation of 4 months these differences were even more marked (Table 1). In animals of the untreated group, however, the development of fibrosis in the lungs was intensified with respect to all parameters, the values of which were five times higher than BC. The corresponding data for animals receiving stabilized enzymes were virtually unchanged compared with the initial level of development of fibrosis. The results are thus clear evidence of marked inhibition of fibrosis in the lungs of animals of all groups which received SH, SL, and dextran (Fig. 1). The action of SL was observed to be most effective when given by inhalation, and this also was

confirmed histologically. In untreated animals, for instance, fibrosis was considerably intensified on account both of an increase in volume of the silicotic nodules and the development of powerful bundles of collagen fibers in them (Fig. 2). Only nodules with a predominantly fibrocellular structure were found in the lungs of all the animals receiving SL (Fig. 3).

Consequently, of the two preparations with stabilized hyaluronidase action (SH and SL), that stabilized by covalent addition of lidase to aldehyde-dextran (SL) proved to be the more effective.

The effectiveness of SL can be explained not only by its fibrolytic properties and its ability to depolymerize proteoglycans, but also by the antifibrotic action of dextran itself, which it exhibits when inhaled by animals in very small doses. The third component of the effectiveness of this derivative is the rational mode of its administration, by inhalation, which facilitates the fibrolytic action of the preparation when it enters the respiratory passages. All these factors contribute to the promising trends in the development of a course of treatment of silicosis by derivatives of stabilized enzymes.

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