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ANTIFIBROUS ACTION OF ALDEHYDE-DEXTRAN MODIFIED SUPEROXIDE DISMUTASE IN EXPERIMENTAL SILICOSIS

A. V. Maksimenko, E. L. Grigor'eva, L. M. Bezrukavnikova, A. D. Petrov, E. G. Tishchenko, O. G. Arkhipova, and V. P. Torchilin

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Applications of modified superoxide dismutase (SOD) are promising in connection with the campaign against lung diseases [3]. For instance, the covalent SOD — Ficoll conjugate possessed prolonged activity in the blood stream. In experimental pulmonary microembolism the permeability of the blood vessels of the lungs for proteins is increased and migration of neutrophils to the endothelium is observed, greatly increasing the risk of involvement of the vascular wall. Prolonged administration of native SOD reduces the pathological increase in permeability of the vessels and demonstrates a protective effect of the preparation. Injection of Ficoll in such a situation reduces adhesion of neutrophils to the endothelium. The above factors mean that the SOD — Ficoll covalent conjugate can be regarded as a promising agent for the treatment of lung diseases that depend on the behavior of neutrophils. In this case both enzyme and carrier (Ficoll) can exhibit a protective effect (preventing accumulation of superoxide radicals and reducing adhesion of neutrophils to the endothelium on account of inhibition of intravascular coagulation respectively) [3]. The useful antifibrotic action of another carrier used to stabilize enzymes, namely dextran, has been described also in lung diseases [4].

The aim of this investigation was to study the action of a preparation of aldehyde-dextran modified SOD (the SOD — AD conjugate) in rats with experimental silicosis. Silicosis is an occupational lung disease which develops as a result of exposure to industrial aerosols containing quartz. Modified enzymes have proved themselves to be effective

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Institute of Work Hygiene and Occupational Diseases, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 265-267, September, 1991. Original article submitted November 10, 1990.

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TABLE 1. Distribution of ^{125}I-Labeled SOD Preparations among Organs (in % of injected dose) after Injection into CBWA Mice (M ± m)

Organ	Preparation	Time after injection of preparation					
		5 min	30 min	1 h	4 h	24 h	48 h
Blood	Native SOD	38,7±5,1	13,5±1,2	11,9±1,4	6,9±2,1	0,3±0,2	0,2±0,2
	SOD-AD	53,3±4,4	28,1±4,5*	20,8±0,9*	9,5±1,2	0,7±0,3	0,3±0,1
Liver	Native SOD	11,5±1,7	4,8±2,8	3,1±0,2	2,1±0,6	0,4±0,05	0,2±0,009
	SOD-AD	16,8±0,9*	16,2±1,0*	15,1±0,4*	13,5±0,4*	11,1±0,4*	9,8±1,7*
Kidneys	Native SOD	30,2±1,6	24,2±5,9	17,1±2,2	4,3±1,0	0,4±0,08	0,2±0,09
	SOD-AD	10,6±1,5*	11,1±0,7	10,6±0,2*	8,3±0,9*	5,2±1,3*	3,8±1,0*
Spleen	Native SOD	0,6±0,1	0,2±0,05	0,2±0,09	0,2±0,2	0,1±0,04	0,008±0,04
	SOD-AD	0,5±0,1	0,7±0,1*	0,7±0,1*	0,6±0,1	0,5±0,07*	0,5±0,2
Lungs	Native SOD	1,9±0,9	1,0±0,4	0,6±0,1	0,5±0,2	0,05±0,04	0,03±0,01
	SOD-AD	1,6±1,2	1,1±0,4	0,8±0,2	0,6±0,2	0,07±0,02	0,09±0,1
Heart	Native SOD	0,7±0,2	0,3±0,08	0,2±0,08	0,1±0,05	0,1±0,04	0,08±0,01
	SOD-AD	0,7±0,2	0,5±0,1	0,4±0,05	0,3±0,1	0,06±0,02	0,08±0,02
Total	Native SOD	83,6	44,0	33,1	14,1	1,15	0,6
	SOD-AD	83,5	57,7	48,4	32,8	17,6	14,6

Legend. Asterisk indicates values for which $p < 0.05$ compared with values for native SOD.

potential remedies for the treatment of silicoses (expanding the arsenal of these preparations currently available for pulmonology).

EXPERIMENTAL METHOD

The SOD — AD preparation was obtained as described previously [1].

Pharmacokinetic Study. Retention of ^{125}I-labeled enzyme preparations [2] in the body and their distribution among the organs of inbred male CBWA mice (weight 30 ± 3 g) were determined by the following method. The labeled enzyme preparation (100 μl) was injected into the caudal vein of the mouse. After definite time intervals the animals were decapitated and blood collected and the organs removed, washed with physiological saline, and gently dried. The radioactivity of the samples was then determined on a "Compugamma" gamma counter (LKB, Sweden). Each value is the mean of six experimental determinations. Statistical analysis of the data was carried out by Student's t test ($p < 0.05$).

Comparative Biomedical Study of the Action of Enzyme Preparations in Experimental Silicosis. The investigation was conducted on male Wistar rats. In response to injection of quartz dust into their lungs, silicosis resembling human silicosis in its morphology developed rapidly. Experimental silicosis was produced by a single intratracheal injection of 20 mg of quartz dust with particle size of under 5 μ. The respirable dust fractions were isolated by sedimentation in water. Dust particles entering the lungs caused the development of fibrosis, which results in an increase in their mass, and in particular, an increase in their content of connective-tissue proteins (measured as hydroxyproline). The course of fibrosis was therefore monitored by measuring the wet weight of the lungs and lymph nodes, the dry weight of the lungs and lymph nodes, and the content of hydroxyproline in dry tissue [4], and calculating the relative weights of the lungs and lymph nodes (i.e., their weight per 100 g body weight).

The animals began their inhalations 3 weeks after receiving the injection of dust. SOD in native or modified form was administered in a dose of 200 U per animal. The inhalations continued for 2 months, 3 times a week. Each inhalation lasted 1 h. The parameters given above were determined when administration of the preparations ended.

EXPERIMENTAL RESULTS

Pharmacokinetic Investigation. No significant differences in the accumulation of native or modified SOD were observed in the spleen, lungs, and heart of the mice after intravenous injection (Table 1).

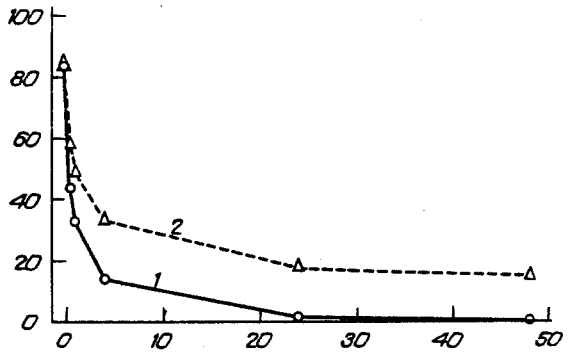

Fig. 1. Radioactivity of samples after intravenous injection of preparations of native (1) and aldehyde-dextran-modified (2) SOD into mice (for all organs tested). Abscissa, time (in h); ordinate, radioactivity (in % of injected dose).

TABLE 2. Parameters of Fibrosis in Rat Lungs after Inhalation of Native and Modified SOD (M ± m)

Parameter	Control	Silicosis (untreated)	Native SOD	SOD-AD conjugate
Body weight, g	423±16	461±59 (100)	475±21 (103,2)	445±15 (96,5)
Dry weight of lungs, g	0,5147±0,1686	1,1449±0,0468 (100)	1,1678±0,0513 (102,4)	0,9386±0,0801* (82,0)
Relative dry weight of lungs, g/100 g	0,1237±0,0429	0,2718±0,297 (100)	0,2696±0,231 (99,2)	0,2119±0,0169* (78,0)
Dry weight of lymph nodes, g	0,0233±0,0019	0,0939±0,0191 (100)	0,1113±0,0203 (118,5)	0,1261±0,0187 (134,3)
Relative dry weight of lymph nodes, g/100 g	0,0055±0,0004	0,0207±0,0034 (100)	0,0233±0,0035 (112,4)	0,0283±0,0036* (136,6)
Hydroxyproline content in lungs, mg	5,6055±0,2795	12,0191±1,2843 (100)	12,6802±1,2371 (105,5)	9,9103±0,6800* (82,5)
Hydroxyproline content in lymph nodes, mg	0,0323±0,0029	1,1479±0,1186 (100)	1,1628±0,01342 (101,3)	1,6521±0,2593* (143,9)

Legend. Asterisk indicates values for which $p < 0.05$ compared with group of rats with untreated silicosis. Change (in % of value for group of rats with untreated silicosis) shown in parentheses.

More native than modified SOD was found in the kidneys, evidence of definite resistance of the SOD-AD conjugate to proteolysis. As a result of modification, the period of stay of SOD-AD in the blood stream and liver was prolonged in character (the relative half-life of the modified and native forms of the enzymes was 1.4 and 10 respectively). On the whole, modification of SOD increases the duration of retention of the preparation of the body (Fig. 1). These results encourage the hope that schemes of administration of modified SOD can be simpler than those for native SOD.

The similar although no level of accumulation of the preparations in the lungs (Table 1) indicates that another method of administration of this preparation than intravenously (for example, by inhalation or intraperitoneally) may be preferable for the treatment of experimental lung diseases.

The Antifibrotic Action of SOD Preparations. To study the effect of different forms of SOD on the development of fibrosis in the rats' lungs, the preparations were inhaled. The results of determination of the relative weights of the lungs and lymph nodes are given in Table 2. No significant differences were found between the relative weights of the liver, kidneys, spleen, and heart of the animals of all treated groups compared with rats with experimental silicosis. The relative weights of the lungs of rats receiving the SOD-AD conjugate were significantly lower than values of this parameter for animals with silicosis. Treatment with modified SOD also significantly reduced the hydroxyproline concentration compared

with the control. However, the relative weights of the dry lymph nodes were significantly higher than in the group of animals with silicosis. Under these conditions native SOD had no inhibitory action in general on the development of fibrosis in the lungs in experimental silicosis, and the action of dextran was less perceptible [4].

Thus thanks to modification of SOD, the SOD-AD conjugate has a marked antifibrotic action, reducing the relative weight of the lungs by 18-22%. The search for optimal doses and schedules of administration of this preparation and its use as a component of systems of regulated drug release [5] will probably enhance its therapeutic effect.

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