EFFECT OF THE CHELATING AGENT $Na_2MgEDTA$ ON COLLAGEN FORMATION AND SOME ASPECTS OF LIPID METABOLISM IN EXPERIMENTAL SILICOSIS

L. G. Babushkina and B. A. Katsnel'son

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Injection of $Na_2MgEDTA$ into rats receiving metacrystobalite dust by intratracheal insufflation intensified the increase in the phospholipid and cholesterol content of the lungs characteristic of silicosis. Meanwhile, the development of silicotic sclerosis was retarded. The chelating agent had no significant effect on the hydroxyproline and lipid content of healthy lungs.

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A number of chelating agents have an inhibitory action on physiological collagen formation [9]. According to data in the literature the chelating agent $Na_2MgEDTA$ [1, 2] lowers the content of cholesterol and phospholipids in the aortic wall of rabbits with experimental atherosclerosis. According to one hypothesis [10, 11], chronic pathological accumulation of lipids in lung tissue leads to the development of silicotic pneumofibrosis. In earlier investigations [1-6] the authors showed that lipids accumulate in the lungs in silicosis.

The object of the present investigation was to determine whether the chelating agent $Na_2MgEDTA$ inhibits the accumulation of lipids in the lungs under normal conditions and in silicosis and also whether it inhibits the development of silicotic fibrosis.

EXPERIMENTAL METHOD

Silicosis was induced in male rats by intratracheal injection of 50 mg of highly fibrogenic metacrystobalite dust [6]. The rats received a subcutaneous injection of $Na_2MgEDTA$ solution in bidistilled water in a daily dose of 100 mg/kg for 3 months. The rats were sacrificed in groups (A, receiving metacrystobalite; B, receiving metacrystobalite and treated with chelating agent; C, intact rats; D, receiving the chelating agent only) of 7-12 animals by decapitation 1 and 3 months after the beginning of the experiment. The body weight, the weight of the lungs and tracheobronchial lymph glands, the content of total hydroxyproline [8], total lipids [4], and dust [14], and the phospholipid phosphorus (by Briggs's method) and cholesterol (by Levchenko's method [7] as modified by ourselves [1]) were determined in the lungs and the lymph glands. The dry tissue of the lymph glands was analyzed as mixed samples from several rats, so that the mean values presented below are given without statistical analysis.

EXPERIMENTAL RESULTS AND DISCUSSION

It is clear from Table 1 that after 1 month the metacrystobalite caused a marked increase in the fresh and dry weight of the lungs and lymph glands and also a marked increase in their content of hydroxyproline, which continued to increase after 3 months. At this time the lipid content in the lungs containing dust was increased almost 6 times, and at the same time the content of phospholipids and cholesterol also showed a significant increase. After 1 month the lymph glands contained 0.369 mg phospholipid phosphorus and 0.110 mg cholesterol (per rat of group A) compared with 0.129 and 0.039 mg in the control (group C), while subsequently these two indices still continued to rise. However, the increase in phospholipids in the lungs of the rats receiving dust at these times was smaller than the increase in total lipids. (The analogous index for cholesterol after 1 month was the same in groups A and B, and the difference after 3 months was not significant.) These findings, as we have previously pointed out [1-3], conflict with views [10, 12] that the phospholipids are the principal fractions accumulating in the lungs in silicosis.

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TABLE 1. Changes in Weight and Content of Some Components in the Lungs of Rats Receiving Metacrystobalite Dust and (or) a Course of Subcutaneous Injections of $Na_2MgEDTA$ (mean value)

Index	1 Month						3 Months					
	group A	group B	P	group C	group D	P	group A	group B	P	group C	group D	P
Wt, of lungs (in mg):												
fresh	4146	4683	> 0.05	1194	1035	> 0.5	5319	4770	> 0.1	1339	1417	> 0.1
dry	867.5	1022	< 0.01	226	224	> 0.5	1074	983	> 0.1	271	269	> 0.5
Content in lungs:												
hydroxyproline (in μg)	9940	10,260	> 0.5	2490	2110	≈0.1	13,850	11,970	< 0.1	2810	2960	> 0.1
lipids (in mg)	171.2	223.9	< 0.05	28.4	26.2	> 0.5	175.2	169.3	> 0.1	32.5	29.4	> 0.1
dust (in mg)	40.6	40.7	—	—	—	—	31.9	31.5	—	—	—	—
phospholipids (in mg)	12.8	19.2	≈0.01	3.9	3.9	> 0.5	11.7	16.5	< 0.05	4.8	4.1	< 0.1
cholesterol (in mg)	15.7	22.6	≈0.01	2.4	3.1	> 0.1	18.6	22.0	0.1	2.9	3.5	> 0.05
per mg lipids												
phospholipids (in μg)	74.9	85.8	> 0.1	136.2	149.0	< 0.5	66.9	97.5	< 0.05	147.7	138.5	> 0.5
cholesterol (in μg)	91.5	101.0	> 0.5	90.0	117.8	> 0.1	106.0	129.7	< 0.1	87.7	119.2	< 0.01

In the healthy animals administration of the chelating agent (groups D and C) did not affect the indices of lipid metabolism and merely a slight tendency toward an increase in the cholesterol content was observed. The effect of dust on these indices of lipid metabolism was similar in character in animals receiving Na_2-MgEDTA (groups B and D).

Comparison of the results for groups A and B shows that through the action of the chelating agent the content of phospholipids and cholesterol in the lungs of the rats receiving dust was higher at both times than in those receiving dust alone, but the total lipid content was higher only after 1 month. The ratio of both fractions to the total lipids was also a little higher.

Turning to the effect of the chelating agent on the fibrogenic action of the dust, it was found that the hydroxyproline content was lower in group B than in group A only after 3 months. Although this decrease was not significant ($P > 0.05$), whereas during the previous 2 months the hydroxyproline content in group A rose by 3910 μg ($P < 0.02$), in group B it rose by only 1710 μg, which was not significant ($P = 0.1$). There was thus some delay, very slight in magnitude yet determinable, in the development of silicotic fibrosis. Further evidence of this was given by the hydroxyproline content in the lymph glands of the animals: in groups A and B 72.6 and 58.1 μg respectively after 3 months.

It is interesting to note that if the antisilicotic action of the chelating agent is judged from the content of total lipids, it is extremely slight at this period. Evidently, the increase in the phospholipid and cholesterol content produced by the chelating agent partially compensated for the smaller increase in the content of neutral fat. Since delay of fibrogenesis took place despite this increase, two hypotheses can be put forward to explain this discrepancy. Either, as we have already postulated [1-3], it is the neutral fat accumulating in the dust-laden lungs and not the phospholipids which plays the role of "fibrogenic factor," attributed to the phospholipids [10, 12], in silicosis, or the chelating agent partially blocks one of the links in collagen synthesis in response to a particular fibrogenic stimulus.

It should also be mentioned that, compared with the literature citations mentioned at the beginning of this paper [13], the results are evidence of certain differences in the mechanisms of accumulation of lipids in the lungs during silicosis and in the intima of the aorta during atherosclerosis, for in the latter case the same chelating agent in the same dose causes a decrease, and not an increase, in the accumulation of phospholipids and cholesterol.

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