THE MECHANISM OF ALUMINIUM IN THE PREVENTION OF PNEUMOCONIOSIS*

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Most experiments with animals, and particularly those with rabbits and guinea-pigs, have shown that aluminium and alumina are not fibrogenic[1-3] when administered in relatively small quantities. King *et al.*,[4] on the other hand, obtained massive fibrosis in the lungs of rats with large amounts of intratracheally injected alumina. With metallic aluminium powder, alone and mixed with a little quartz, a severe fibrosis was produced. Engelbrecht *et al.*[5] demonstrated that the conflicting reports concerning the effects of alumina were due to a species difference in the fibrogenic reaction of the tissues to intratracheally injected dusts.

From human experience in industry, it is claimed that administration of small amounts of aluminium has a beneficial influence on established silicosis as well as a retarding effect on its development.[6-8] However, Goralewski[9] observed that subjects exposed to high concentrations of aluminium developed a diffuse fibrosis with a tendency to spontaneous pneumothorax.

In recent *in vitro* experiments[10] it was found that high concentrations of aluminium chloride inhibited cytochrome c oxidase activity in homogenates and isolated enzyme systems, while in low concentrations a stimulating effect was observed. This finding might explain the specific mechanism of aluminium in the prevention of silicosis, as well as some of the conflicting reports in the literature.

Experiments were therefore designed to investigate the *in vivo* effects of large amounts of aluminium as well as the influence of small amounts in combination with other fibrogenic dusts such as quartz and asbestos.

MATERIALS AND METHODS

Albino rats weighing 140 ± 10 G were used. Six groups of 24 animals each were injected intratracheally with suspensions of carbon, quartz, asbestos, aluminium, and dust mixtures of asbestos + aluminium and quartz + aluminium, respectively.

The following materials were used:

(*i*) Quartz dusts (Dowson and Dobson) very finely powdered and specially treated to remove impurities.

(*ii*) Carbon, pure activated (Merck).

(*iii*) Asbestos (crocidolite) obtained from the Pneumoconiosis Research Unit, Johannesburg, milled until 85% of the particles were smaller than 5μ.

(*iv*) Aluminium (McIntyre Research Foundation) consisting of 15% aluminium and 85% aluminium oxide. This dust has been used in Canada for silicosis therapy.

Suspensions of quartz, carbon, asbestos and aluminium, and of dust admixtures containing 10 parts quartz + 1 part aluminium and 10 parts asbestos + 1 part aluminium, were respectively prepared in isotonic saline (50 mg./ml. for single dusts and 50 mg. + 5 mg./ml. for dust admixtures). All the suspensions were sterilized and administered via intratracheal injection (50 mg. dust/rat) according to the technique of Ross *et al.*[11]

The experiment lasted 140 days. Two animals from each group were killed by decapitation at intervals of 10, 20, 30, 40, 60, 80, 100 and 140 days. Special care was taken to prevent blood entering the lungs.

The lungs of 2 rats from each group were dissected and carefully weighed. De-ionized water was added in a ratio of 9·5 ml. to 0·5 G of tissue to obtain a 5% homogenate. The tissue was homogenized in a Waring Blendor for 6 × 20 seconds and the temperature was kept at ±4°C by cooling on ice.

Determinations

Dry weight determinations were done on each homogenate. Duplicate samples of 5 ml. of a 5% homogenate were pipetted accurately in preweighed evaporation dishes and dried in an oven at 105°C for 24 hours to constant weight.

Cytochrome c oxidase activity of each homogenate was determined simultaneously and in duplicate in a Warburg apparatus at 38°C as previously described.[12]

At set intervals one animal from each group was anaesthetized with ether before killing. Their tracheas were exposed and 10 ml. of 15% formol-saline was injected into the lungs. The lungs were dissected and preserved in the same fixation fluid. Blocks of lung tissue were selected from the left and right lung of each animal in a sagittal plane near the hilum, embedded in wax and sectioned serially. Two sections from each lung were stained with haematoxylin and eosin and another was impregnated with silver.[13]

The grading of the amount and maturity of the fibrosis produced in the lung at set intervals was done according to the method of Ross *et al.*[11]

RESULTS AND DISCUSSION

In this comparative investigation different chemical and histological parameters were investigated to assess the effects, if any, of small concentrations of aluminium powder on the fibrogenicity of quartz and asbestos dusts in lung tissue after intratracheal administration. Carbon dust, which is relatively inert, was used as an experimental control in addition to normal control animals. The following parameters were investigated: wet weight, dry weight, cytochrome c oxidase activity, grade of fibrosis, amount of fibrosis and total fibrosis.

Wet Weight

After the intratracheal injection of suspensions of single dusts and of dust admixtures, a marked increase in wet lung weight was observed (Table I).

The most pronounced increase occurred over the first 10 days after injection. In the case of quartz dust a progressive increase in weight was maintained over the whole experimental period. It appeared that the initial increase in weight is not a reflection of the fibrogenicity of a

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specific dust, although quartz is by far the most fibrogenic dust (see later).

TABLE I. AVERAGE WET WEIGHT OF RAT LUNGS IN G AT SET INTERVALS AFTER INTRATRACHEAL ADMINISTRATION OF CARBON, QUARTZ, QUARTZ + ALUMINIUM, ASBESTOS, ASBESTOS + ALUMINIUM AND ALUMINIUM DUSTS (50 MG./ML./RAT FOR SINGLE DUSTS AND 50 MG. + 5 MG./ML./RAT FOR DUST ADMIXTURES)

Days	Control	Carbon	Quartz	Quartz + aluminium	Asbestos	Asbestos + aluminium	Aluminium
10	0·84	1·59	1·83	1·43	1·75	1·77	1·72
20	0·81	1·58	2·20	1·55	1·34	1·55	1·52
30	0·84	1·54	2·27	1·90	1·80	1·12	1·89
40	0·91	1·71	2·18	1·67	1·76	1·67	1·79
60	1·05	1·62	2·37	1·48	1·40	1·43	1·80
80	0·91	1·97	3·07	2·27	1·29	1·48	1·61
100	1·10	1·52	3·42	1·85	1·35	1·68	1·89
140	0·83	1·54	4·42	2·01	1·39	1·82	1·48
Av.	0·91	1·63	2·72	1·77	1·51	1·57	1·71

A small amount of aluminium dust (5 mg.), simultaneously administered with quartz, significantly retarded the weight increase due to quartz. Addition of the same amount of aluminium to asbestos dust did not have a similar effect. A slight increase in weight was found instead. Aluminium dust, in a massive dose (50 mg.), itself produced a weight increase of the lungs, comparable to the effect of any of the other dusts used, except silica.

Dry Weight

The average percentage increase in dry weight of lung tissue after the intratracheal injection of suspensions of dust and dust admixtures is given in Table II. Compared with the control value, the findings indicate a slight increase in percentage dry weight of lung tissue for all dusts, except in the case of asbestos.

The increase in percentage dry weight might be due to dust retained in the lung, as well as to the deposition of collagen which is more compact in nature than normal lung tissue. Although the total wet weights of lungs injected with quartz + aluminium and quartz, respectively, were markedly different, the percentage dry weight remained practically the same.

The relatively low percentage of dry weight of lung tissue after the injection of asbestos dust could be interpreted to reflect a state of oedema in the lungs of this group. A small amount of aluminium in combination with asbestos increased the average percentage of the dry weight to the same order as that of carbon and aluminium, respectively.

TABLE II. PERCENTAGE DRY WEIGHT OF LUNG TISSUE AT SET INTERVALS AFTER INTRATRACHEAL INJECTION OF DUST SUSPENSIONS (50 MG./RAT FOR SINGLE DUSTS AND 50 MG. + 5 MG./RAT FOR DUST ADMIXTURES)

Days	Control	Carbon	Quartz	Quartz + aluminium	Asbestos	Asbestos + aluminium	Aluminium
10	20·2	21·0	21·2	21·8	19·8	20·4	20·6
20	20·4	22·8	22·8	23·4	21·4	22·2	20·4
30	19·6	21·6	22·0	23·2	18·8	21·2	22·0
40	20·2	21·2	22·6	23·2	19·6	21·2	21·4
60	19·8	21·2	22·6	23·8	18·2	21·4	22·2
80	20·4	22·2	24·1	23·6	19·6	22·4	21·6
100	20·4	20·8	24·4	22·2	18·0	19·2	22·6
140	20·4	20·6	23·8	22·6	20·6	21·0	18·8
Av.	20·1	21·4	23·0	23·0	19·6	21·2	21·2

Cytochrome c Oxidase

The cytochrome c oxidase activity of lung tissue from the different groups was determined in homogenates and expressed in terms of μlitres O_2/mg. wet lung tissue/hour (Table III).

The values obtained demonstrate clearly that all the dusts and dust admixtures, except quartz, enhanced the activity of this enzyme. Quartz dust gave a marked inhibition compared with the normal and experimental control. A small amount of aluminium in combination with quartz caused a reversion of the effect of quartz alone on the cytochrome c oxidase activity. So pronounced was this stimulatory effect that values even higher than those for carbon were obtained. However, a small amount of aluminium had no definite effect in combination with asbestos dust. In a massive dose (50 mg.) aluminium alone produced an even bigger increase in the cytochrome c oxidase activity of lung tissue than either carbon or asbestos dust.

Grade, Amount and Total Fibrosis

The grade, amount and total fibrosis produced by various dusts and admixtures over a period of 140 days after intratracheal injection were histologically assessed (Table IV).

TABLE III. CYTOCHROME C OXIDASE ACTIVITY OF LUNG TISSUE AFTER INTRATRACHEAL ADMINISTRATION OF SUSPENSIONS OF SINGLE DUSTS AND DUST ADMIXTURES (μLITRES O_2/MG./HOUR)

Days	Control	Carbon	% difference from control	Quartz	% difference from control	% difference from carbon	Quartz + Al	% difference from control	% difference from carbon	Asbestos	% difference from control	% difference from carbon	Asbestos + Al	% difference from control	% difference from carbon	Aluminium	% difference from control	% difference from carbon
10	11·50	13·39	+16·43	10·46	− 9·04	−21·88	11·28	− 1·91	−15·76	11·53	+ 0·26	−13·89	12·98	+12·87	− 3·06	14·61	+27·04	+ 9·11
20	10·23	11·67	+14·08	11·39	+11·34	− 2·40	11·28	+10·26	− 3·34	10·72	+ 4·79	− 8·14	9·73	− 4·89	−16·62	13·94	+36·27	+19·45
30	10·38	12·41	+19·56	10·18	− 1·93	−17·97	12·70	+22·35	+ 2·34	10·45	+ 0·67	−15·79	10·87	+ 4·72	−12·41	12·75	+22·83	+ 2·74
40	11·07	14·03	+26·74	10·87	− 1·81	−22·52	12·98	+17·25	− 7·48	14·35	+29·63	+ 2·28	14·39	+29·99	+ 2·57	12·77	+15·36	− 8·98
60	12·08	13·63	+12·83	12·61	+ 4·39	− 7·48	13·40	+10·93	− 1·69	12·20	+ 0·99	−10·49	13·68	+13·25	+ 0·37	14·08	+16·56	+ 3·30
80	11·09	12·81	+15·51	10·45	− 5·77	−18·42	12·84	+15·78	+ 0·23	13·02	+17·40	+ 1·64	14·11	+27·23	+10·15	14·36	+29·49	+12·10
100	12·08	13·05	+ 8·03	8·34	−30·96	−36·09	13·28	+ 9·93	+ 1·76	12·33	+ 2·07	− 5·52	13·12	+ 8·61	+ 0·54	14·89	+23·26	+14·10
140	11·68	11·81	+ 1·11	10·32	−11·64	−12·62	16·36	+40·07	+38·53	13·88	+18·84	+17·53	13·22	+13·18	+11·94	13·28	+13·70	+12·45
Av.	11·26	12·85	+14·29	10·58	− 5·68	−17·42	13·02	+15·58	+ 1·82	12·31	+ 9·33	− 4·05	12·76	+13·12	− 0·82	13·84	+23·06	+ 8·03

TABLE IV. GRADE (G), AMOUNT (A) AND TOTAL FIBROSIS (T) PRODUCED AT SET INTERVALS OVER A PERIOD OF 140 DAYS AFTER THE INTRATRACHEAL INJECTION OF DUSTS AND DUST ADMIXTURES

Days	Carbon G × A = T	Quartz G × A = T	Quartz + Al G × A = T	Asbestos G × A = T	Asbestos + Al G × A = T	Aluminium G × A = T
10	1·0 × 1·5 = 1·50	1·5 × 1·5 = 2·25	1·0 × 1·5 = 1·50	1·5 × 1·5 = 2·25	1·5 × 3·0 = 4·50	1·5 × 2·5 = 3·75
20	1·5 × 1·0 = 1·50	2·0 × 3·5 = 7·00	1·0 × 2·5 = 2·50	1·5 × 1·0 = 1·50	1·0 × 3·5 = 3·50	1·0 × 2·0 = 2·00
30	1·5 × 1·5 = 2·25	1·5 × 3·0 = 4·50	1·0 × 3·0 = 3·00	1·5 × 1·5 = 2·25	1·5 × 3·0 = 4·50	1·5 × 2·5 = 3·75
40	1·5 × 1·5 = 2·25	2·0 × 2·0 = 4·00	1·0 × 3·5 = 3·50	1·5 × 2·0 = 3·00	1·0 × 2·5 = 2·50	1·5 × 2·5 = 3·75
60	1·5 × 2·0 = 3·00	2·0 × 3·0 = 6·00	1·0 × 2·0 = 2·00	1·5 × 2·5 = 3·75	1·0 × 2·0 = 2·00	1·5 × 2·5 = 3·75
80	1·0 × 1·5 = 1·50	2·0 × 3·0 = 6·00	1·0 × 2·5 = 2·50	1·5 × 3·0 = 4·50	1·5 × 1·5 = 2·25	1·0 × 3·5 = 3·50
100	1·5 × 2·0 = 3·00	3·0 × 3·0 = 9·00	1·5 × 3·5 = 5·25	1·5 × 2·0 = 3·00	1·5 × 2·0 = 3·00	1·0 × 2·5 = 2·50
140	1·5 × 1·5 = 2·25	3·0 × 4·0 = 12·00	1·5 × 4·5 = 6·75	1·0 × 3·0 = 3·00	1·0 × 4·0 = 4·00	1·5 × 3·0 = 4·50
Av.	2·16	6·34	3·38	2·91	3·28	2·61

The findings indicate that all dusts and dust admixtures used were slightly fibrogenic on intratracheal administration. Quartz dust was particularly outstanding in this respect, producing an average total fibrosis of 6·34, nearly twice as high as any other dust during the experimental period.

A small amount of aluminium in combination with quartz greatly reduced the fibrogenicity of the latter dust, especially as far as the grade of fibrosis was concerned. A similar amount of aluminium, administered simultaneously with asbestos, did not have any retarding effect on the fibrogenic response of this dust. Instead it had a cumulative effect. Although a small amount of aluminium suppressed the fibrogenicity of quartz dust and aggravated the fibrous response to asbestos, by itself it produced fibrous changes in lung tissue when administered in a relatively high dose.

The mechanism of aluminium in retarding the fibrogenic response of lung tissue to quartz is still uncertain. Denny *et al.*[1] suggested that aluminium might prevent the quartz particles from dissolving. Emmens and Fries[14] regarded the capacity of aluminium to flocculate colloidal silicic acid as the basis of its protective action. Engelbrecht and Burger[15] demonstrated that aluminium chloride (4×10^{-3}M) was essential for the optimal activity of cytochrome c oxidase activity *in vitro*. When colloidal silicic acid (1·67 mM) was added to such a system, containing no aluminium, a significant inhibition was obtained. In the presence of aluminium a marked stimulatory effect was observed. These results indicate that aluminium is somehow involved in the protection of this enzyme system against the toxic effects of silicic acid.

In the present *in vivo* experiments aluminium in combination with quartz enhanced the cytochrome c oxidase activity of lung tissue to such an extent that values much higher than those for normal lungs and lungs injected with quartz only were obtained. At the same time, the increased cytochrome c oxidase activity of the tissues was accompanied by a marked reduction in the grade of, and the total, fibrosis. If the reduction in the fibrous response is associated with the increase in cytochrome c oxidase activity, this finding could be interpreted as indicating that aluminium alleviates the hypoxic state of the cells induced by quartz.

Aluminium has no such function in combination with asbestos dust. It appears, therefore, that the mechanism whereby asbestos causes lung fibrosis is different from that of quartz. Asbestos is a silicate and it is known that silicates are less fibrogenic than free silica. Aluminium could perhaps combine with silicic acid in the lung to form a practically insoluble aluminium silicate which would produce only a limited fibrous response.

Aluminium dust in a relatively large dose (50 mg.) produces marked fibrosis in the lungs of rats. At the same time, the cytochrome c oxidase activity of the tissues is accelerated. Other dusts, inert and slightly fibrogenic, such as carborundum, carbon, asbestos, etc., have similar effects.

The increase in cytochrome c oxidase activity of lung tissue after intratracheal injection of dust is most probably due to an accumulation and concentration of mononuclear and phagocytic cells in the lung which have a higher metabolic rate than lung cells. The toxicity of a specific dust would determine the degree to which these cells as well as lung cells are damaged and thus also the metabolic rate or cytochrome c oxidase activity. It has been demonstrated that aluminium in a relatively high concentration (4×10^{-3}M) inhibits the cytochrome c oxidase activity.[10] However, it is doubtful whether this concentration of aluminium, after intratracheal injection, will ever be reached in lung tissue. Although partial cell damage may occur by inhibition of the cytochrome c oxidase activity, the net effect of a slightly fibrogenic dust such as aluminium will still be an increase in the total metabolic rate, as observed in the present experiments.

The practical implementation of aluminium therapy for the prevention of pneumoconiosis in general is not supported by the present results. In cases of uncomplicated silicosis it might be useful in retarding the development of nodular silicosis. In silicatosis and especially asbestosis it might be very dangerous, producing lung damage and fibrosis greater than that caused by any single dust.

SUMMARY

Six separate groups of young albino rats were intratracheally injected with suspensions of carbon, quartz, asbestos and aluminium dusts and with dust admixtures of 50 mg. quartz + 5 mg. aluminium and 50 mg. asbestos + 5 mg. aluminium, respectively. Animals from each group were killed at set intervals over a period of 140 days. The following parameters were carefully investigated: Wet weight and percentage dry weight of lungs; cytochrome c oxidase activity/unit wet weight of lung tissue; and the grade, amount and total fibrosis.

Of all dusts investigated, quartz dust caused the biggest increase in wet and dry lung weight as well as the highest grade of fibrosis and total fibrosis. However, it caused a marked inhibition of the cytochrome c oxidase activity compared with other dusts. A small amount of aluminium, administered simultaneously with quartz, retarded the increase in lung wet weight as well as the grade and total fibrosis produced by quartz dust alone. The cytochrome c oxidase activity was markedly increased in the lung tissue of these animals. Contrary to the effects of aluminium in combination with quartz, it caused a slight increase in lung wet weight, in total fibrosis and in the activity of cytochrome c oxidase when simultaneously administered with asbestos dust. Aluminium in a dose of 50 mg. produced an increase in lung wet weight, in total fibrosis and in the activity of cytochrome c oxidase of lung tissue comparable to the effects of carbon and asbestos dusts.

The differential effect of aluminium on the influence of quartz and asbestos dusts in lung tissue is discussed. A possible mechanism for aluminium in the prevention of silicosis is suggested. Aluminium therapy in cases of silicatosis may be dangerous.

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