Deposition of aluminium in tissues of rabbits exposed to inhalation of low concentrations of Al_2O_3 dust

H B Röllin, P Theodorou, T A Kilroe-Smith

Abstract

With strong evidence coming to light for the toxicity of aluminium (Al), especially to the brain, investigation into the effects of inhalation of low concentrations of Al dust in animal models has become important. This study follows up previous observations on the effects of Al on the concentrations of essential metals in serum of workers exposed to inhalation of low concentrations of Al dust, with a study of the concentrations of Al in tissues of rabbits exposed to Al dust at one twentieth of the threshold limit value (TLV). Even at this low concentration, the amount of Al in the brains of these animals was nearly two and a half times as high as that of the control animals. The concentrations in other tissues were similar to normal. At the same time, the concentrations of Al in the serum was only slightly raised, indicating that this variable is a poor marker for the effects of Al on the body. It is suggested that an extensive study is needed to determine a more correct TLV and health based permissible concentration for occupational exposure to Al.

New evidence of a link between Al and neurotoxic effects in occupationally exposed subjects emerged at the second international conference on Alzheimer's disease and related disorders, held in July 1990 in Toronto, Canada. Sandra Rifat of the University of Toronto studied a group of retired Canadian mine workers who had been exposed during their working life to inhalation of a finely ground Al powder, thought to be a prophylactic measure against silicosis. She found that those miners had suffered a degree of mental impairment.[1] She also found a clear dose–response effect. Although Al can be absorbed through the respiratory organs, little research has been done on its toxic effects in workers exposed in industry, although such exposure is common. Hošovski *et al* studied the mental abilities of workers exposed to high concentrations of Al dust in an Al foundry and found changes in their psychomotor and intellectual abilities.[2] In a previous study of foundry workers we established that very small changes in serum Al concentrations (although statistically significant) are accompanied by conclusive changes in the concentrations of two essential metals—namely, copper and zinc.[3] The most interesting observation was that these changes were seen at an atmospheric Al dust concentration well below 1 mg/m^3 as measured at those foundries. We concluded that the effect on the essential metals was possibly due to the displacement of these metals from the tissues by Al. To study this further, we needed to establish if a deposition of Al in body organs occurred as a result of exposure to low concentrations of Al dust similar to the concentration that is present in these foundries. We therefore designed an experiment that enabled us to monitor changes in the concentrations of Al in the serum of rabbits exposed to low concentrations of Al dust (0·56 (SD 0·17) mg Al/m^3) over a period of five months. After completion of the dust exposure period selected tissues were removed and analysed for Al content. We chose rabbits because it was found by Yokel[4] that aluminium has the ability to produce behavioural defects in rabbits and persistently raised tissue concentrations of Al that mimic the pattern in man.

Materials and Methods

Sixteen young adult female specific pathogen free New Zealand white rabbits were used. The animals were divided without conscious bias into groups of eight controls and eight exposed. Each was housed in a separate cage for the duration of the experiment and given a standard laboratory diet and tap water. The group of eight exposed rabbits was dusted in the dusting chamber with Al_2O_3 at a concentration of

Department of Biochemistry, National Centre for Occupational Health, PO Box 4788, Johannesburg, 2000, South Africa
H B Röllin, P Theodorou, T A Kilroe-Smith

0·56 (SD 0·17) mg Al/m³ for five months, eight hours a day, five days a week. Care was taken to dispose of food leftovers at the end of each dusting day, and food containers were rinsed. New food was then supplied to prevent accumulation of Al in the food. Every two weeks 2 ml of blood were withdrawn from an ear vein of each rabbit. Precautions were taken to avoid contamination during collection. Becton-Dickinson syringes were used to sample the blood, which was then transferred into Sterilin tubes (Sterilab Services catalog No 144 AS). These were shown to be Al free. After clotting, the blood samples were centrifuged at 3000 rpm. The resulting serum was transferred into other Sterilin tubes and immediately frozen and stored at −20 °C until analysed.

On the day of completion of the experiment, the animals were killed with pentobarbital sodium at a dose of 200 mg/kg body weight and exhaustively perfused through the left ventricle with 0·9% aqueous NaCl. The NaCl solution had been previously tested and found to be Al free. The brain, lung, liver, heart, kidney, and sternum were removed. They were rinsed with purified water, blotted on a clean tissue, and dried in an oven at 80 °C for 24 hours. The dry tissues were powdered in an agate mortar, defatted with chloroform methanol mixture (2:1), and dried again at 80 °C for 12 hours. The dried defatted tissues were again powdered and stored in Sterilin tubes at −20 °C until analysed. Serum and representative tissue samples were analysed for content of Al using a Varian Model AA-975 atomic absorption spectrophotometer equipped with a GTA-95 furnace and autosampler as previously described.[5] All samples were analysed using the method of addition to avoid matrix interferences. Tissues were dry ashed in platinum crucibles to eliminate contamination. To test the method for serum a certified standard of Al in serum was also measured (Seronorm trace elements from Nycomed AS, Norway). A study of the recovery of Al from various tissues has been reported previously.[5] The statistical significance of differences in Al concentration between exposed and control groups with respect to serum and the tissues was determined by Student's *t* test.

Results

The figure shows the change in Al concentrations in serum with time of exposure. It may be represented by the equation: Al concentration = 0·00906 × days of exposure + 2·15 with a regression coefficient of r = 0·47.

A cyclic change in the concentration of Al apparently occurs with an average period of about 53 days, but with a steady slow rise in the serum concentration.

The table shows the mean Al concentrations in different tissues on completion of exposure to dust as compared with the controls. Each tissue, whether for the standard graph or for the individual Al determinations, was weighed in duplicate (±20 mg dry tissue) and each of the duplicate samples was analysed in triplicate.

The lung has the highest concentration of Al. This is to be expected if clearance from the lung is slower than the deposition by inhalation, resulting in accumulation.

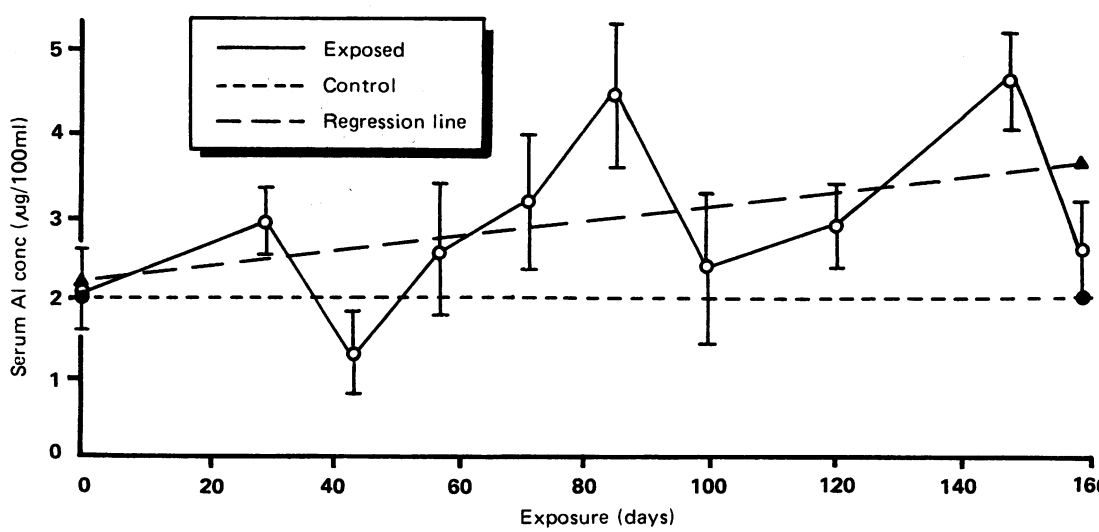

Figure Changes in serum Al concentrations in rabbits with time of exposure.

Table Comparison of Al concentrations in controls and dusted rabbits

Tissue	*Controls (n = 8) Al conc**	*Exposed (n = 8) Al conc**	*% Of control*	*p Value†*
Brain	4·1 (2·9)	10·1 (4·1)	247	<0·005
Lung	1·7 (1·3)	270 (149)	15 800	<0·001
Liver	ND	1·2 (1·0)	—	—
Kidney	3·0 (2·0)	4·9 (3·7)	165	NS
Heart	10·7 (2·2)	7·5 (2·7)	70	<0·005
Bone	18·2 (5·0)	22·2 (4·1)	122	<0·02

*Mean μg Al/g dry tissue (SD).
†p = Probability for a two tailed Student's *t* test.
ND = Not detectable.

The sternum showed a minor increase in concentration (22%), whereas the brain showed a highly significant increase (147%). Heart muscle showed a 30% decrease.

Discussion

The appreciable accumulation of Al in the body tissues of rabbits exposed to very low concentrations of Al_2O_3 dust (0·56 (SD 0·17) mg Al/m^3) over a period of only five months is surprising. That serum Al concentrations only increased slightly confirms the limited binding capacity of plasma for Al as reported by Kavalchik *et al*,[6] and suggests that concentrations of Al in serum are not a good reflection of body burden. Excess of Al appears to be translocated to other tissue stores. Our results show that rabbit organs have the ability to store Al. The high concentration of Al in lung tissue confirms the very slow rate of uptake of Al_2O_3 in vivo. Nevertheless, we found a big increase in the Al concentrations in the brain ($2p < 0·005$), which is particularly disturbing in the light of the neurotoxic effects of this element. Also, one cannot overlook the fact that the Al concentrations in the serum of exposed animals were lower than those presumably associated with encephalopathy in patients receiving dialysis.[7] Moreover, the cyclic variation in serum Al concentration appears to disqualify it as a reliable marker for body burden of Al. A reliable marker for body burden of Al must still be found.

We suggest that extensive studies need to be carried out on the effect of inhalation of Al in industrial workers in an attempt to reach a correct estimate of the proper TLV and health based permissible concentration for occupational exposure; this appears to need a downward revision in view of our findings on exposures to concentrations one twentieth of the present TLV.[8]

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Requests for reprints to: H B Röllin, Department of Biochemistry, National Centre for Occupational Health, PO Box 4788, Johannesburg 2000, South Africa.

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