ALUMINIUM ADMINISTRATION ON ACETYLCHOLINESTRASE ACTIVITY OF DIFFERENT REGIONS OF RAT BRAIN

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SUMMARY: The present investigation was undertaken to study short and long term effects of aluminium on acetylcholinestrase activity of different areas of rat brain.

Intraperitoneal administration of 2 mg/kg aluminium as $AlCl_3$ daily for 15 to 60 days reduced acetylcholinestrase activity of cerebellum controls from 16.3 ± 0.7 to 14.2 ± 0.9, mid-brain from 15.8 ± 0.7 to 12.3 ± 0.7 and brain cortex from 8.7 ± 0.7 to 1.0 ± 0.4 μ mole enzyme/mg protein/min respectively ($p < 0.05$). Aluminium (10 mg/kg) administration daily for 15 and 30 days reduced acetylcholinestrase activity of cerebellum controls from 16.2 ± 0.8 to 12.1 ± 1.0, mid-brain from 15.8 ± 0.4 to 9.6 ± 0.5 and brain cortex from 8.4 ± 0.6 to 5.4 ± 0.4 μ mole enzyme/mg protein/min respectively ($p < 0.05$). Serum aluminium of aluminium treated animals was elevated significantly ($p < 0.05$). The neurotoxicity of aluminium has been considered in the discussion.

Key Words: Aluminium, acetylcholinestrase, neurological disorders.

INTRODUCTION

Aluminium is the third most abundant element in the earth's crust of which it comprises 8%. It is a chemically-reactive metal and does not occur naturally in the elemental form, it is found in compounds such as silicates and oxidase (1). We are exposed to aluminium through the use of deodorants, in cooking pots, in water where it is used as a flocculent and in drugs (2). Aluminium industry is another place where people come into contact with aluminium (3).

The recognition of aluminium as a toxic substance in patients with chronic renal failure followed the work of Alfrey *et. al.* (4) who showed that aluminium could readily cross dialysis membranes and lead to hyperaluminemia in patients on regular hemodialysis. Dialysis osteodystrophy (5), hypochromic microcytic anemia (6) and neurological disorders are the most common disturbances encountered in (aluminium toxicity). Recently there have been a number of reports which suggest a possible role for aluminium in the pathogenesis of Alzheimer's disease (7) and dialysis encephalopathy (8). Although aluminium is an element without a known function in brain metabolism, it has been found in both senile plaques (9) and neurofibrillary tangles (10) the two major pathological markers for Alzheimer's disease.

The exact mechanism by which aluminium interferes with brain function is still a matter of speculation. Therefore, the present project was established to study the effect of aluminium toxicity on acetylcholinestrase activity of the cerebellum, mid-brain and brain cortex.

MATERIALS AND METHODS

All chemicals were of reagent grade and obtained from Sigma Chemical Company (Germany). Deionized water was used throughout this study. Laboratory glassware was soaked

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Table 1: Aluminium determination in rat serum.

Al Dose (mg/kg)	Days of injections	Serum aluminium (ug/l)	
		Control	Treatment
2	15	1.1 ± 0.05	22.5* ± 3.0
2	30	1.5 ± 0.07	37.5* ± 6.6
2	60	1.2 ± 0.06	54.1* ± 6.3
5	15	1.5 ± 0.05	36.7* ± 2.8
5	30	1.4 ± 0.04	86.0* ± 5.2
10	15	1.0 ± 0.05	84.0* ± 7.3
10	30	1.5 ± 0.07	159.1* ± 10.1
20	1	1.5 ± 0.07	12.1* ± 32

*Rats were injected with varying amounts of aluminium daily for 1 to 60 days. Each figure is Mean ± SE of four different animals. * $p \leq 0.05$.*

overnight in 10% HNO_3 and washed three times with distelled water with aluminium concentration less than 1 μg/l.

Animals and treatment

Male Wistar rats were kept in the faculty animal house at standard conditions and fed on basal diet and water until their weights reached between 200-250 grams. Animals were injected i.p. with 0.2 ml saline containing varying amonts of aluminium as $AlCl_3$ for different periods of times. Controls were injected only with 0.2 ml of saline.

Animals were killed by decapitation between 8-9 AM and blood samples were collected carefully in acid pre-washed test tubes.

Sera were separated from red blood cells by centrifugation and used for aluminium determination.

Brain was carefully removed. Cerebellum, mid-brain and brain cortex were dissected and used for protein and acetylcholinestrase (ACE) determination. Aluminium analysis in sera was determined by the method of Moshtaghie *et. al.* (11) using a Perkin-Elmer (Model, HGA-600) Flameless atomic absorption spectrophotometer with aluminium hollow Cathode lamp operating at 25 mA. The wavelenght used was 309.3 nm with spectral band width of 0.7 nm. Argon gas was used as an inert gas. Acetylcholinestrase activity of dissected areas was determined using acetylcholine chloride as the enzyme substrate as reported elsewhere (12).

Protein concentration was determined by the method of Lowry *et. al.* (13). Student's t-test was used to show statistical differences between aluminium treated and control animals and calculated at $p < 0.05$.

RESULTS

Aluminium concentrations in the sera of rats treated with aluminium (2-20 mg/kg) and also in controls were first determined. Data presented in Table 1 show that administration of aluminium for different periods of times lead to the significant elevation of serum aluminium ($p < 0.05$). The elevation of serum aluminium was not only dependent on the amount of injected aluminium, but also related to the duration of injection (Table 1).

The effect of varying amounts of aluminium on different regions of rat brain acetylcholinestrase activity was studied next (Table 2). Acute aluminium (20 mg/kg) administration did not change the enzyme activity significantly ($p < 0.05$).

Daily administration of aluminium (2 mg/kg) for 15, 30 and 60 days reduced the acetylcholinestrase activity of cerebellum compared to controls from 16.2 ± 0.8 to 15.6 ± 0.7 in 15 days; from 16.3 ± 0.7 to 14.1 ± 1.4 in 30 days and from 16.5 ± 0.6 to 12.9 ± 0.7 mmole enzyme/mg protein/min in 60 days of aluminium treatment (Table 3). Administration of aluminium (5 mg/kg) daily for 15 and/or 60 days reduced the activity of acetylcholinestrase of cerebellum from 16.1 ± 0.8 in controls to 14.9 ± 0.5 in aluminium treated rats after 15 days and, from 16.1 ± 0.8 to 12.3 ± 1.9 in 30 days of aluminium administration (Table 4). At the same time the mid-brain enzyme activity was reduced from 16.1 ± 0.5 to 12.5 ± 0.5 for 15 days and from 15.6 ± 0.3 to 8.6 ± 0.9 for 30 days respectively. When brain cortex enzyme activity of these groups was measured, it was found that 15 days of aluminium administration (5

Table 2: Effect of a single injection of aluminium on acetylcholinestrase activity of different regions of rat brain.

Treatment	Acetylcholinesterase activity (μ mole enzyme/mg protein/min)		
	Cerebellum	Mid-brain	Brain-cortex
Control	16.49 ± 0.5	15.7 ± 0.6	8.4 ± 0.8
Al-treated	16.1* ± 0.5	15.1* ± 0.7	8.1* ± 0.4

*Rats were injected daily with aluminium (20 mg/kg) as $AlCl_3$. After 24h animals were killed and acetylcholinestrase activity was determined in different regions of brain. Data are presented as Mean ± SE of four separate experiments. * $p \leq 0.05$.*

Table 3: Effect of 2 mg/kg of aluminium on acetylcholinestrase activity of different regions of rat brain.

Treatment	Acetylcholinesterase activity (μ mole enzyme/mg protein/min)		
	15 days	30 days	60 days
Control Cerebellum	16.2 ± 0.8 15.6 ± 0.7	16.3 ± 0.7 14.1 ± 1.4	16.5 ± 0.6 12.9 ± 0.7*
Control Mid-brain	16.1 ± 0.5 14.6 ± 0.6	15.6 ± 0.3 12.9 ± 0.7*	15.8 ± 0.6 9.4 ± 0.9*
Control Brain-cortex	8.4 ± 0.6 8.1 ± 0.16	8.5 ± 0.6 7.1 ± 0.5	9.4 ± 0.9 5.8 ± 0.5*

*Rats were administered daily with aluminium (2 mg/kg) for 15, 30 and 60 days. Animals were killed at the end of the experimental time. Blood samples were collected and treated as mentioned in the legend for Table 2. Data are presented as the Mean ± SE of four separate series of experiments. * $p \leq 0.05$.*

mg/kg) changed the activity from 8.4 ± 0.6 in untreated controls to 1.6 ± 0.3 in aluminium treated and from 8.5 ± 0.6 of controls to 5.1 ± 0.3 μmole enzyme/mg protein/min in 30 days following daily aluminium administration. Data are presented in Table 4.

Increasing amounts of aluminium (10 mg/kg) was then injected to animals and enzyme activity was measured in cerebellum, mid-brain and brain cortex areas of rat brain following 15 and 30 days of aluminium treatment. It was found that cerebellum enzyme activity in controls was reduced from 16.1 ± 0.8 to 12.5 ± 1.2 in 15 days and from 16.3 ± 0.7 to 11.7 ± 0.9 mmole enzyme/mg protein/min in 30 days respectively. Mid-brain enzyme activity was reduced from 16.1 ± 0.5 of controls to 12.1 ± 0.6, of aluminium treated animals in 15 days and from 15.6 ± 0.3 to 7.1 ± 0.4 enzyme units in 30 days of aluminium injection.

Lastly, when brain-cortex enzyme activity was measured it was found that 10 mg/kg aluminium administration to rat changed the activity of cholinestrase of brain-cortex from 8.4 ± 0.6 to 6.6 ± 0.8 in aluminium treated in 15 days and from 8.5 ± 0.6 to 4.1 ± 0.3 μmole enzyme/mg protein/min in 30 days respectively (Table 5).

DISCUSSION

Following the first reports that neurotoxic concentrations of aluminium were presented in the cerebral cortex of patients with Alzheimer's disease (7) a controversy arose concerning the significance and universal reproducibility of these findings. Data which have been presented in this paper tried to elucidate the relationship between aluminium toxicity and brain dysfunction in relation to the appearance of neurological disorders in hemodialyzed patients with aluminium overload. Data presented in Table1 show that treatment of rats with increasing amounts of aluminium over different periods of times lead to the elevation of serum aluminium. The levels of aluminium thus achieved are related to the duration of treatment and to the amounts of aluminium administration.

There is some experimental evidence for alternation by polyvalent cations in general and aluminium ions in particular of the synthetic and metabolic enzymes of cholinergic neurotransmission.

Altered levels of choline acetylase and acetylcholinestrase have been reported in brain tissue of rabbits with neurofibrillary degeneration induced by intracisternal injection of aluminium salt (14). Determination of acetylcholinestrase activity of different regions of rat brain treated with aluminium in the present investigation lead to the significant reduction of the enzyme activity (Tables 3-5). These results are in agreement with the earlier reports

Table 4: Effect of 5 mg/kg aluminium on acetylcholinestrase activity of different regions of rat brain.

Treatment	Acetylcholinesterase activity (μ mole enzyme/mg protein/min)	
	15 days	30 days
Control Cerebellum	16.1 ± 0.8 14.9 ± 0.5	16.1 ± 0.6 12.3 ± 1.9
Control Mid-brain	16.1 ± 0.5 12.5 ± 0.5*	15.6 ± 0.3 8.6 ± 0.9*
Control Brain-cortex	8.4 ± 0.6 7.6 ± 0.3	8.5 ± 0.6 5.1 ± 0.3*

*Rats were injected with aluminium (5 mg/kg) daily for 15 and/or 30 days. Animals were killed at the end of the experimental time and blood samples were collected and treated as mentioned in the legend for Table 2. Data are presented as the Mean ± SE of four separate experiments. * $p \leq 0.05$.*

Table 5: Effect of 10 mg/kg aluminium on acetylcholinestrase activity of different regions of rat brain.

Treatment	Acetylcholinesterase activity (μ mole enzyme/mg protein/min)	
	15 days	30 days
Control Cerebellum	16.1 ± 0.8 12.5 ± 1.2	16.3 ± 0.7 11.7 ± 0.9*
Control Mid-brain	16.1 ± 0.5 12.1 ± 0.6*	15.6 ± 0.3 7.1 ± 0.4*
Control Brain-cortex	8.4 ± 0.6 6.6 ± 0.8	8.5 ± 0.6 4.1 ± 0.3*

*Rats were injected with aluminium (10 mg/kg) daily for 15 and/or 30 days. Animals were killed at the end of the experimental time. Blood samples were collected and treated as mentioned in the legend for Table 2. Data are expressed as the Mean ± SE of four separate experiments. * $p \leq 0.05$.*

(15) carried out with rabbit as an experimental animal model for neurotoxic effect of aluminium.

The exact mechanism by which acetylcholinestrase activity was reduced following aluminium administration is still not sufficiently elucidated. Aluminium may interfere with either synthesis of acetylcholinestrase or inhibits choline uptake by synaptosomes. Acetylcholine has been implicated in human memory and cognitive functioning and dramatic decreases in choline acetyltransferase activity in the cortex and hippocampus of Alzheimer's disease patients have been reported (16). The higher reduction in acetylcholinestrase activity of mid-brain may suggest that this part of brain is much more susceptible to aluminium intoxification. We believe that more investigations still should be done to study the exact mechanism that aluminium causes toxicity in each individual part of brain and interferes with biochemical events.

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