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Effect of Chronic Aluminum Exposure on the Levels of Conjugated Dienes and Enzymatic Antioxidants in Hippocampus and Whole Brain of Rat

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The reported association between elevated tissue levels of aluminum (Al) and certain human neurological disorders have evoked increasing attention on the neurotoxic effects of aluminum. High levels of Al have been reported in hippocampal neurons comprising neurofibrillary tangles in senile dementia of Alzheimer's type, amyotropic lateral sclerosis and Parkinsonian dementia of Guam (ATSDR 1992). Aluminum is considered to be the causal factor for a high incidence of dialysis encephalopathy (Alfred et al. 1976). It has been shown that the incidence of Alzheimer's disease was higher in places with a high Al content in drinking water compared to low level areas (Martyn et al. 1989). Varied uses of Al in pharmaceutical preparations, foods, water purification and many house-hold items have increased the risk of its exposure to general population (ATSDR 1992). The exposure may be as high as 500 mg/kg/day in children with uremia who are treated with Al containing phosphate binding gels (Andreoli 1984). Aluminum ingestion in humans and experimental animals have been reported to produce behavioural dysfunctions (Bowdler et al. 1979; Lal et al. 1993). The mechanism of Al neurotoxicity is not understood at present. Attempts made in this direction have reported its interaction with blood-brain barrier function, decreased membrane fluidity, glutathione depletion and increased brain lipid peroxidation (ATSDR 1992; Lal et al. 1993; Fulton and Jeffery, 1994). These studies indicate the possibility that oxidative stress may be one of the possible mechanisms of Al-induced neurotoxicity.

Since Al has been reported to be in high concentrations in hippocampal neurons in certain neurological diseases and there is wealth of evidence implicating hippocampal impairment and memory dysfunction, we attempted to investigate the effect of chronic Al intoxication on

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the status of enzymatic antioxidants and the extent of peroxidative damage in hippocampus and whole brain of rat.

MATERIALS AND METHODS

Nitroblue tetrazolium, thiobarbituric acid, reduced glutathione, oxidized glutathione and β-nicotinamide adenine dinucleotide reduced form were purchased from Sigma Chemical company, St. Louis, MO, USA. tert-Butylhydroperoxide of purum grade was purchased from Fluka Chemie AG, Buchs, Switzerland. Pyrogallol was obtained from J.T. Baker Chemical Company, Phillipsburg, NJ, USA. All other reagents used in this study were of analytical grade.

Eighty adult male albino rats of Drucrey strain weighing 185 ± 10 g were obtained from central animal facility of the Industrial Toxicology Research Centre, Lucknow. Rats were housed in stainless steel cages under standard animal house condition with a light/dark cycle of 12 hrs each, and were given pellet diet (Lipton India Limited) ad libitum. They were divided into two groups of forty animals each. One group of the animals was exposed orally to $AlCl_3$(0.5 mg Al/ml in drinking water) for a period of 12 months. Another group having equal number of animals was kept under identical conditions and was given tap water (0.04 µg Al/ml), served as control. Body weight and the water consumption were recorded for all the rats every morning during the course of the experiment.

Sixty rats, thirty each from control and Al-treated groups, were selected randomly and sacrificed by decapitation for the present study. Rest of the animals were used for some other investigations. Brains were dissected out quickly, rinsed in cold isotonic saline. Hippocampus region was separated immediately from forty brains, twenty from each group. The hippocampus regions of two rats were pooled to make one sample. Five samples of hippocampus and whole brain each from control and Al-treated groups were homogenized (10%, w/v) in 50 mM phosphate buffer, pH 7.4 containing 1 mM EDTA for the biochemical analyses. Remaining five samples of hippocampus and whole brain from each group were used for Al estimation using DC Plasma Emission Spectrophotometer following acid digestion (Berman 1980).

Conjugated dienes were measured according to the method of Recknagel and Glende (1984) and were expressed as nmoles/g tissue using a value of 2.52×10^4 as molar extinction coefficient of conjugated dienes at 234 nm. Catalase [Hydrogen peroxide: hydrogen peroxide oxidoreductase] was assayed by measuring the decomposition of hydrogen peroxide at 240 nm (Aebi, 1983). The enzme activity was expressed as umoles of hydrogen peroxide decomposed/min/mg protein using its extinction coefficient (0.041/mmole/Cm).Glutathione peroxidase [NAD(P)H: oxidized glutathione oxidoreductase] was assayed in presence of tert-butylhydroperoxide as substrate (Rotruck et al. 1973) and was expressed as umoles of GSH oxidized/min/mg protein. The activity of superoxide dismutase [Superoxide:superoxide oxidoreductase] was determined using pyrogallol and nitroblue tetrazolium (Shukla 1987). It was expressed as units/min/mg protein. One unit is defined as the amount of enzyme required for the 50% inhibition in the pyrogallol autooxidation. The protein content was measured by the Folin-phenol method as described earlier (Shukla et al. 1984) using bovine serum albumin as standard.

The significance of the difference between control and Al-exposed groups was evaluated by Students 't' test. p values <0.05 were considered to be significant.

RESULTS AND DISCUSSION

The results of this study showed that an oral exposure of rats to Al for 12 months did not produce any appre-

Table 1. Effects of aluminum exposure for 12 months on body and brain weights, protein and aluminum contents of hippocampus and whole brain of rats.

Parameter	n	Control	Al-exposed
Weight (g)			
Body	30	430±12.2	376±8.9**
Whole Brain	10	2.079±0.04	2.207±0.08
Hippocampus	20	0.131±0.005	0.143±0.004
Protein (mg/g)			
Whole Brain	5	80.3±2.12	84.3±2.94
Hippocampus	5	74.3±3.15	72.4±3.01
Aluminum (µg/g)			
Whole Brain	5	3.25±0.44	6.36±0.53*
Hippocampus	5	4.25±0.57	9.06±0.67**

Data represent mean±SE of n samples. Hippocampus from two rats were pooled to make one sample. Statistical analysis was done by Students t-test and $p<0.05$ were considered to be significant, *$p< 0.01$;**$p<0.001$. $AlCl_3$ (0.5 mg Al/ml) was given in drinking water, ad libitum.

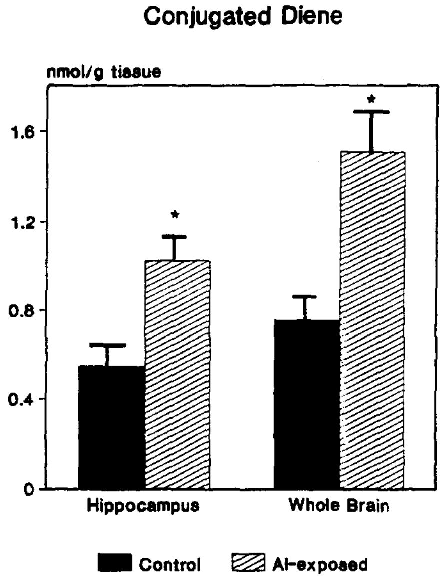

Figure 1. Levels of conjugated diene following chronic Al exposure. Each column is mean of 5 samples with vertical bar representing SE mean. *p< 0.001

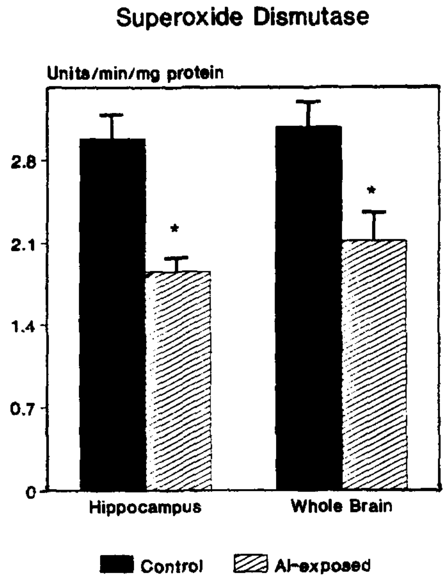

Figure 2. Activity of superoxide dismutase following chronic Al exposure. Each Column is mean of 5 samples with vertical bar showing SE mean. *p< 0.001

ciable effect on the food and water intake compared to control animals. The average intake of water in both groups was found to be 27±4 ml/rat/day. The body weight of Al-exposed rats were comparable to the control animals up to two months after the treatment (Table 1). However, a significant decrease in body weight was noticed at later periods of time and at the termination of the experiment the body weight of the treated animals was found to be decreased by 13 percent. The weight of whole brain and hippocampus region and their protein contents measured at end of the experiment was found to be comparable in both the groups. Aluminum exposure of rats for 12 months increased the levels of Al significantly in whole brain and hippocampus regions by 96% ($p<0.01$) and 113% ($p<0.001$), respectively, compared to controls. The magnitude of increases were higher in comparison to those observed after six months of identical Al exposure (Lal et al. 1993).

This study showed that chronic exposure of rats to Al increased the levels of conjugated dienes (Fig. 1) in hippocampus region (87%, $p<0.001$) and whole brain (100%, $p<0.001$) compared to respective controls. The

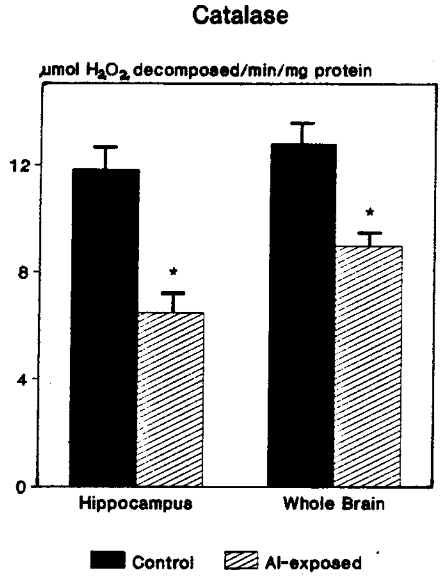

Figure 3. Catalase activity following chronic Al exposure. Each column is mean of 5 samples with vertical bar representing SE mean.
*p< 0.001

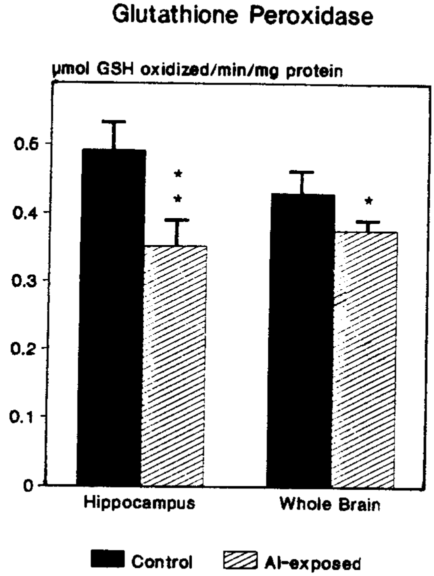

Figure 4. Glutathione peroxidase activity following chronic Al exposure. Each vertical column is mean of 5 samples with vertical bar representing SE mean.
*p< 0.01, **p< 0.001

levels of conjugated dienes are taken as an index of lipid peroxidation in the tissue which is related to cellular damage (Niki and Nakano, 1990). Although there are certain reports which show that Al has a potential to peroxidize lipids in the brain and other tissues (ATSDR 1992; Lal et al. 1993), there is very little information about the possible interaction of Al with the status of antioxidative enzymes. The activity of superoxide dismutase, in the present study, was found to be reduced by 38% ($p<0.001$) in hippocampus region and by 31% ($p<0.001$) in whole brain of Al-exposed rats compared to controls (Fig. 2). Superoxide dismutase is an important antioxidative enzyme as it scavenges superoxide anion free radical which if not removed efficiently from biological system, may generate an array of other free radicals and reactive oxygen species. The mechanism of Al-induced inhibition of this enzyme is not known at present, however, there is one report that Al in concentrations similar to that found in serum of uraemic patients was inhibitory to superoxide dismutase activity in concentration dependent manner in vitro (Shainkeim-Kestenbaum et al. 1989).

The activity of glutathione peroxidase, an enzyme that catalyzes the removal of highly oxidizing lipid hydroperoxides and hydrogen peroxide was found to be decreased (Fig. 3) in both hippocampus region (29%, $p<0.01$) and whole brain (13%, $p<0.05$). The activity of catalase was also significantly lowered in hippocampus (45%, $p<0.001$) and whole brain (30%, $p<0.001$) (Fig. 4). It may further decrease the removal of hydrogen peroxide from these tissues.

The susceptibility of an organ to oxidative damage is determined by the overall balance between prooxidant and antioxidant factors at cellular level. Brain is highly susceptible to oxidative damage as it has higher concentrations of easily peroxidizable substance polyunsaturated fatty acid and high oxygen tension. However, a concomitant presence of a high concentration of certain antioxidant enzymes help to maintain a low steady state levels of oxidizing species and avoids unwanted oxidative damage to the tissue (Yusa et al. 1984). It appears from the data that decreased levels of these vital enzymatic antioxidants in the brain of Al-treated rats may have some role in the oxidative damage following Al exposure. Golub et al. (1992) reported that feeding mice on dietary aluminum lactate (1 mg Al/g diet) for a comparatively shorter period of 90 days did not produce any change in the lipid peroxidation and the levels of antioxidative enzymes, namely superoxide dismutase, glutathione peroxidase and glutathione reductase. It appears that an unaltered strong tissue antioxidant defense in above study following this magnitude of dietary Al exposure may be responsible for saving the tissue from peroxidative damage.

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