Effects of chronic Aluminum administration on brain and liver enzymes activities and essential elements concentration in Rats: protective role of riboflavin and vitamin A

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ABSTRACT

Aluminum (Al) has been implicated in the pathogenesis of several clinical disorders, such as dialysis dementia, a neurological disorder that can develop in patients on renal dialysis. Therefore, the present experiment was carried out to determine the effectiveness of riboflavin and vitamin A in alleviating the effects of Aluminum chloride on brain and liver functions and contents of Cu, Zn and Fe, lipid peroxidation and antioxidant enzyme activities in Sprague Dawley rats. Four groups of rats were used. Al was given in a dose of 20 mg /kg body wt/day to the first group for 12 weeks, whereas the second and the third groups were given a combination of Al and riboflavin or vitamin A. The fourth group was taken as a control. Experimental data showed significantly decreased Fe concentration in hepatic and cerebral homogenates in Al and Al plus Vitamin A treated groups; whereas Al plus riboflavin treated group showed no significant decrease. Non significant changes in the concentration of Cu and Zn in Al plus vitamin A and Al plus riboflavin treated groups, while Al treated group showed significant increase in Zn concentration. The activities of the antioxidant enzymes were significantly reduced in Al treated group, while Al plus vitamin A or riboflavin treated groups showed improvement in the activities of these enzymes. Brain function enzymes showed significant decrease, except CPK, which showed significant increase in Al treated group, whereas combination of Al plus vitamin A or riboflavin showed no significant changes in most of serum hepatic and cerebral function enzymes. The present results indicate that exposure to aluminum resulted in significant changes in the hepatic and cerebral content of Fe, Zn and Cu; also the activities of the antioxidant and serum function enzymes were changed. Combination of Al plus riboflavin or vitamin A showed improvement in hepatic and cerebral functions, possibly due to decreased Al absorption from intestine.

Key words: aluminum toxicity, brain function enzymes, Fe, Cu and Zn

INTRODUCTION

Aluminum (Al) is the most widely distributed metal in the environment and is extensively used in modern daily life. Aluminum enters into the body from the environment and from diet and medication such as antacids, buffered aspirins, and antidiarrheal products. However, there is no known physiological role for aluminum within the body and hence this metal may produce adverse physiological effects ***(Turgut, et al. (2004)***.
Al sulfate is extensively added as a coagulant agent during the purification process of drinking water in order to folliculate the organic matter and so that clarify the water.

Elevated levels of Al in brain and bone have been associated with serious neurological diseases and osteodystrophic lesions in hemodialysed patients suffering from chronic renal failure due to use of dialysis solutions containing high levels of aluminum ***(Cannata et al. 1998)***

Al has been also proposed as a potential risk factor in the etiology of certain neurological disorders such as Alzheimer's disease, amyotrophic lateral sclerosis and Parkinsonism dementia in subjects with normal renal function. Al accumulates in the brain of those individuals ***(Lovell et al. 1993)***.

Oral exposure to Al may result in accumulation of Al in hippocampus of brain and thus affect some essential elements (Zn, Fe, Cu and Ca) contents in the hippocampus at different degrees ***(Yang et al. 2002)***

High accumulation of aluminum in hippocampus, which could disturb the normal distribution of iron and zinc, decrease the activities of antioxidant enzymes and increase the level of lipid peroxidation, which may leads to the neurotoxicity of aluminum ***{Domingo, et l. (1993); Jia et al. (2001)}.***

Considering the widespread presence of aluminum in the environment and the susceptibility of the brain and liver to peroxidative damage; the aim of this study was to investigate the effects of chronic Al exposure either alone or in combination with riboflavin or vitamin A on brain and liver contents of Cu, Zn and Fe, lipid peroxidation and antioxidant enzymes activities in rats. In addition serum hepatic and brain function enzymes were assayed.

MATERIAL AND METHODS

Animals:

This study was performed on forty adult male albino Sprague Dawley rats weighing between 150-180 g. The Animals were placed in stainless steel cages and maintained on a 12 hours light-dark cycle and 27ºC ± 1ºC room temperature and hygienic conditions. Rats were observed for 12 days prior to experimentation and water was offered *ad libitum*. Rats were divided into four equal groups each one consists of ten rats. The first group served as control, and fed well-balanced laboratory rat diet. The second group received aluminum chloride Al Cl_3 (Sigma) orally at a dose of 20 mg Al/kg body weight/day dissolved in bi-distilled water for 12 weeks. The third group takes the same aluminum dose together with diet enriched with vitamin A (30 IU /g) as retinyl acetate (five times the content of vitamin A in the control group) according to ***(Sato and Lieber 1981)***. The fourth group received the same aluminum dose with riboflavin in a dose of 15 mg/kg body weight orally ***(Satanovskaya and Sadovnik 1990)***. After the end of experimental period the rats were fasted overnight with free access for water and then scarified under diethyl ether anesthesia.

Biochemical assays:

The blood samples were collected by heart puncture before the animals were sacrificed by cervical dislocation. Sera were separated and used freshly for determinations of the activities of Gamma glutamyl transferase *(*GGT*) {EC 2.3.2.2}* (***Persijn and Van 1976)***, Alanine amino transferase *(*ALT*) {EC 2.6.1.2}*; Asparate amino transferase *(*AST*) {EC 2.6.1.1}*; alkaline phosphatase *(*ALP*) {EC 3.1.3.1.}* ***(Belifield and Goldburg 1971),*** Monoaminooxidase (MAO*) {EC1.4.3.4}* ***(Mc Eween 1969)*** Acetylcholinestrse (AChE) {EC 3.1.1.7} ***(Den Blaauwen et al 1983),*** 5'-Nucleotidase (5'-NT) ***(Bertrand and Buret 1982) and*** Creatine phosphokinase (CPK) {EC 2.7.3.2} ***(Rosano et l 1976)***

Preparation of homogenates:

Liver and brain tissue samples were quickly dissected, rinsed in ice-cold saline to clear them of blood. About one gram of the liver and brain were homogenated immediately with 9.00 ml potassium phosphates buffer solution pH 7.40, then briefly solicited and centrifuged at 3000 rpm for 15 min. the supernatant was separated and used freshly for biochemical assays. Lipid

peroxides was ascertained by measuring malondialdehyde (MDA) by thiobarbituric acid method ***(Uchiyama and Mihar 1976)***

Fe, Zn and Cu were determined in homogenates using Pirkin Elmer Model 23000 Atomic Absorption Spectrophotometer according to ***Watanabe (1996).***

The activities of GSH-Px *(EC1.11.1.9)* ***(Chiu et al. 1976);*** GR-ase *(EC1.6.4.2)* (***Bergmayer 1983);*** t-SOD *(EC 1.15.1.1)* ***(Misra and Fridovich 1972)*** and Catalase *(EC 1.11.1.6)* (***Sinha 1972)*** were assayed***.***

Statistical analysis:

Data were expressed as mean ± SE. Differences between groups were examined for statistical significance using the Student's *t*- test as explained by ***Petrie and Waston (1999).***

RESULTS

1- Changes in trace elements:

Figure (1) shows the effect of Al on trace elements concentrations in brain and liver tissues either alone or in combination with vitamin A or riboflavin. Administration of Al for 12 weeks resulted in significant ($p<0.05$) decrease of iron concentration in both liver and brain tissues; whereas Al plus vitamin A showed significant ($P< 0.05$) decrease of iron in liver tissue and non significant decrease in brain tissue. Combination of Al and riboflavin resulted in non significant decrease of iron concentration in both liver and brain tissues.

Zn concentration showed significant increase in liver tissues of all treated groups; whereas in the brain showed significant increase in Al treated group and non significant increase in Al plus vitamin A or riboflavin groups.

Non significant decrease in the concentration of Cu in liver and brain tissues of Al treated groups. While Al plus vitamin A or riboflavin treated groups showed non significant increase in Cu concentration in liver tissue and non significant decrease in brain tissue.

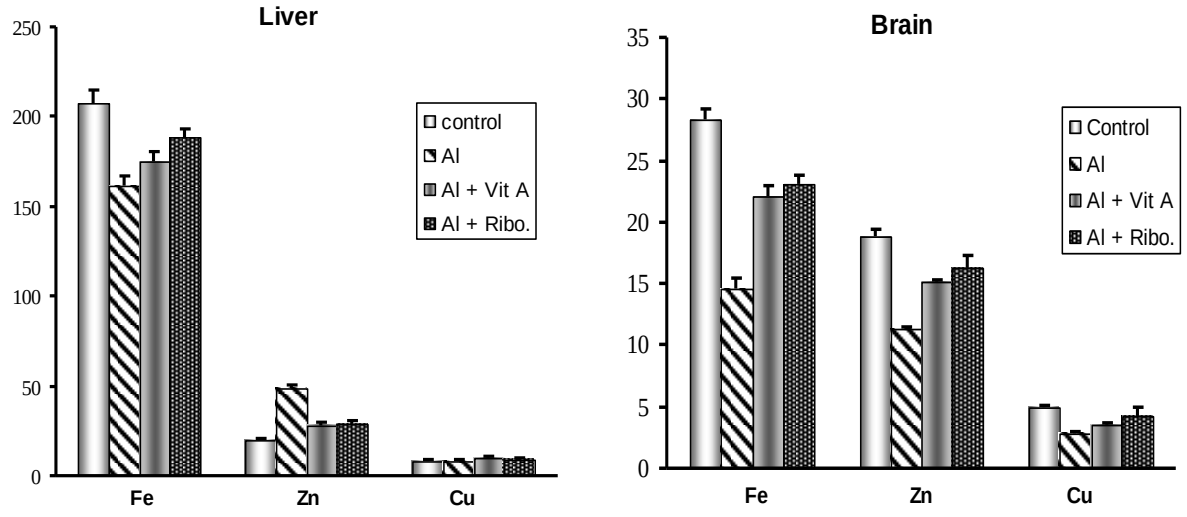

FIG. 1: changes in Fe, Zn and Cu in liver and brain tissues. Results are shown as mean ± SE $N = 10$.

2- Changes in Lipid peroxides and Glutathione redox cycle:

Significant increase ($p<0.05$) in lipid peroxides in the brain tissue of Al and Al plus vitamin A treated groups was recorded. In the liver tissue there was high significant ($p< 0.01$) in lipid peroxide concentration in Al treated group; while in Al plus vitamin A or riboflavin there was non significant decrease as shown in table (1).

Al treated group showed significant decrease ($p<0.05$) in the activity of GSH-px, catalase, and t-SOD and non significant decrease in the activity of GR-ase in the brain tissue. While Al plus vitamin A or riboflavin showed non significant decrease in the activity of the enzymes in the brain tissue.

In the liver tissue, Al treated group showed significant increase ($P<0.05$) in the activity of GSH-px and non significant increase in the activity of GR-ase; but showed significant decrease in the activity of catalase and non significant decrease in the activity of t-SOD. Non significant changes in the activity of redox cycle enzymes in Al plus vitamin A or riboflavin.

Table 1: Changes of hepatic and brain MDA, GSH-Px, CAT, t-SOD and GSH-RD in control and experimental groups

	Liver				**Brain**			
	Control	**Al**	**Al + Vit A**	**AL + Ribo.**	**Control**	**Al**	**Al + Vit A**	**Al + Rib**
MDA *(nmol/mg protein)*	1.50 ± 0.11	5.40 ± 0.60**	0.59 ± 0.04*	0.91 ± 0.09	0.40 ± 0.17	1.11 ± 0.30*	0.98 ± 0.04	1.02 ± 0.06
GSH-Px *(U/g protein)*	445.00 ± 17.10	506.90 ± 20.56*	493.33 ± 11.20	479.11 ± 13.10	16.30 ± 0.50	11.25 ± 0.60*	15.10 ± 1.65	13.33 ± 0.93
Catalase *(U/g protein)*	29.80 ± 1.06	15.40 ± 1.30*	19.31 ± 0.99	21.20 ± 1.10	3.10 ± 0.10	1.50 ± 0.04*	2.01 ± 0.13	3.20 ± 0.17
t-SOD *(U/g protein)*	8.80 ± 0.32	7.40 ± 0.41	8.90 ± 0.67	7.80 ± 0.35	6.70 ± 0.50	2.80 ± 0.40*	4.49 ± 0.09	6.22 ± 0.23
GR-ase *(U/g protein)*	49.11 ± 1.70	52.80 ± 1.09	44.56 ± 0.99	41.22 ± 1.00	18.40 ± 0.80	16.90 ± 0.7**0**	17.00 ± 0.47	18.88 ± 0.55

Results are shown as mean ± SE *N* = 10.

P values: significant *<0.05, high significant **<0.01 and very high significant ***<0.001.

3- Changes in serum hepatic and brain functions enzymes:

The results of the changes in the serum parameters including, hepatic and brain function enzymes are showed in Table (1). Non significant decrease in the activities of MAO, AChE, and 5'-NT in all treated groups was found, while CPK showed increased activities in all treated groups.

Al treated group showed significant increase in the activities of ALT, AST, GGT and ALP. While Al plus vitamin A or riboflavin treated groups showed significant increase in the activity of ALT and AST, and non significant increase in the activities of GGT and ALP.

Table 2: Changes of some hepatic and brain functions enzymes in control and experimental groups

	Liver function enzymes					**Brain functions enzymes**			
	control	**Al**	**Al + Vit A**	**AL + Ribo.**		**Control**	**Al**	**Al + Vit A**	**Al + Ribo**
ALT *(U/L)*	44.80 ± 1.72	136.90 ± 3.60**	116.00 ± 2.33**	100.70 ± 1.98*	***MAO*** *U/ml*	23.00 ± 0.80	9.00 ± 0.33	12.99 ± 0.67	18.88 ± 0.75
AST *(U/L)*	151.50 ± 2.11	197.50 ± 3.90*	177.90 ± 3.09*	182.00 ± 3.33*	***AChE*** *U/ml*	6.40 ± 0.88	2.22 ± 0.34	4.99 ± 0.56	4.89 ± 0.50
GGT *(U/L)*	1.70 ± 0.07	6.33 ± 0.17**	1.46 ± 0.08	1.99 ± 0.23	***5'-NT*** *U/ml*	4.99 ± 0.67	2.23 ± 0.22	3.61 ± 0.31	3.54 ± 0.55
ALP *(U/L)*	18.30 ± 0.93	54.00 ±1.30*	25.44 ± 1.25	27.70 ± 1.30	***CPK*** *U/l*	21.00 ± 0.56	33.00 ± 2.00	28.00 ±1.99	30.89 ± 2.01

Results are shown as mean ± SE *N* = 10.

P values: significant *<0.05, high significant **<0.01 and very high significant ***<0.001.

DISCUSSION

The role of heavy metals in the pathogenesis of neurodegenerative disease is currently receiving considerable attention. Aluminum is present in many manufactured foods and medicines and is added to drinking water for purification purposes. It has been proposed that aluminum is a contributing factor to several neurodegenerative disorders such as Alzheimer's disease. However, this remains controversial primarily due to the unusual properties of aluminum and a lack of information on its cellular sites of action (***Levesque, et al. 2002***). Presence of aluminum in dialysis fluids has been shown to be an etiological factor contributing to several neurological disorders known as dialysis dementia***. (Nayak and Chatterjee 2003)***

Fe and Al are carried mainly by transferrin and chelated by the same compounds (desferrioxamine) and both may compete for absorption and cellular uptake. The decrease in liver and brain Fe in the present study (Fig 1) may be attributed to the competition of Al with Fe to be transported from the intestine via transferrin. This result agrees with the data obtained by (***Deloncle et al. 2001***) who demonstrated that the radio-labeled iron absorption was significantly reduced in rats with Al intoxication and also in intestinal cells previously incubated with Al. also Al affect Iron homeostasis by interfering with iron regulatory proteins ***(Ward, et al. 2001)***

The Zn and Cu contents in brain homogenate decreased significantly in aluminum treated group, this result agrees with (***Yang et al 2002***) who recorded decreased Fe, Cu and Zn content in hippocampus of brain oral exposure to Al. Accumulation of aluminum and accompany with reduction of iron, copper and zinc in brain might interpret the neurotoxicity of aluminum.

Combination of vitamin A or riboflavin resulted in non significant decrease in Fe, Zn and Cu.

In liver homogenate, significant increase in Zn and non significant decreased in Cu concentrations were recorded.

Peroxidation, proteins and nucleic acids, has been related to a number of pathophysiological and Neurological situations of brain. Aluminum salts has been shown to accelerate the iron induced peroxidation of brain phospholipid liposomes ***(Oteiza 1993)***. The

end products of lipid peroxidation are often highly cytotoxic. The brain is the organ most susceptible to peroxidative damage ***(Julka, and Gil, 1996)***.

The increase in lipid peroxides recorded in this study (table1) is most related to the ability of Al to augment lipid peroxidation by enhancing the production of reactive oxygen intermediates. This can be confirmed by the reported data of ***Moyer (1999)*** who suggested that the diseases of the central nervous system associated with the presence of aluminum had free radical mediated oxidative reaction as causative mechanism.

Free radicals are in fact potent deleterious agents causing cell death or other forms of irreversible damage, e.g., free radicals appear to modify DNA base pairs every day. Neurons appear to be particularly susceptible to attack by free radicals for the following reasons: *1*) their glutathione content, an important natural antioxidant, is low; *2*) their membranes contain a high proportion of polyunsaturated fatty acids; and *3*) brain metabolism requires substantial quantities of oxygen ***(Christen et al. 2000)***.

The mechanism of Al prooxidant action may be produced through its interaction with the membranes, subtle changes in the rearrangement of lipids which could attack and facilitate the propagation of lipid. Peroxidation leads to loss of membrane integrity, decrease its fluidity, disrupt the functioning membrane bound enzymes receptors and ion channels, which leads finally to cell death. ***(Fraga et al.1999)*.**

The effect of metals on free radical reaction is usually ascribed to their ability to participate in redox reactions in which they donate or accept a single electron. Aluminum due to its electronic configuration, does not participate in redox reactions, consequently, its effect is probably due to a direct interaction with cell components, rather than to reactions with oxidative reactive species. ***(Timbrell, 2002).***

In the present study, the decreased activities of t-SOD, GSH-Px, GR-ase and catalase in brain homogenate from rats exposed to aluminum compared to control group, can be attributed to a direct interaction of aluminum with free radical scavenging enzymes. Therefore it accentuates the oxidative insult to tissues. These enzymes are first line of defense against the oxygen free radical and the decrease in their activities may contribute to the oxidative stress to the brain tissues***. (Swain, and Chainy, 1998).***

Al plus vitamin A or riboflavin treated groups showed non significant changes in the activities of antioxidant enzymes in brain tissue compared to control group.

Liver homogenate showed increased activity of GSH-px and GR-ase; while the activities of catalase and t-SOD were decreased. This result has partial agreement with ***Abubakar et al (2003)*** who recorded that hepatic catalase and reduced glutathione levels were both reduced in animals treated with aluminum

Decreased activities of serum brain function enzymes including MAO, AChE, and *5′-N* were found in Al treated group. This result agrees with previous data recorded by ***Zatta et al. (1998)*** **and** ***Dave et al (2002).*** Cholinesterases are a large family of enzymatic proteins widely distributed throughout both neuronal and non-neuronal tissues. Al may interfere with various biochemical processes including acetylcholine metabolism, and can thus act as a possible etiopathogenic cofactor. Monoamine-oxidase catalyses the oxidative deamination of various primary amines such as norepinephrine, serotonin, dopamine and others. Altogether these findings indicate that long-term Al feeding results in inhibition of AChE, and decreased activity of MAO, which could represent the mode of action through which Al may contribute to pathological processes in Al-induced neurotoxicity.
Administration of Al plus vitamin A or riboflavin showed improvement in the activities of brain function enzymes compared to Al treated group.

Serum liver function enzymes including AST, ALT, GGT and ALP showed increased activities after long term administration of Al. these results are in accordance with ***Wilhelm et al (1996)*** who found an increased release of the enzymes AST and ALT into the hepatic perfusate due to high dose of Al.

Taken together, the present findings documented that the brain is particularly susceptible to aluminum toxic effects. Al exposure significantly enhanced neuronal lipoperoxidative damage; decrease Fe, Cu, and Zn content, with concomitant alterations in the antioxidant defense status that may be responsible for a consistent rise in the cell load of oxidative stress. This may contribute, as an aggravating factor, to the development of neurodegenerative events. Combination of Vitamin A or riboflavin with Al decreased its toxic effect and improved brain and liver function possibly due to decreased absorption of aluminum.

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