Impact of Aluminum on Protein Malnourished Rat Brain

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Citation

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Abstract

Available reports suggesting that aluminum and protein deficiency both are causing deficits in the neurobehavioral development learning. It has been aimed to study the impact of protein malnutrition on the general responses of brain to aluminum exposure. Male albino rats of Wistar strain weighing 80-100g were injected intraperitoneally, at a dose of 4.2 mg / Kg body weight (15 % of LD50) / day for 4 weeks while they were maintained on either 18% protein diet or 6% protein diet. Rat brain aluminum content was significantly increased in the aluminum-exposed rats of the normal protein diet group but this increase was insignificant in the low protein diet group. The changes in brain weight, acid and alkaline phosphoesterases, aspartate and alanine aminotransferases, reduced glutathione, thiobarbituric acid reactive substances, DNA, RNA and protein contents suggested a nonspecific impact of dietary protein status on the response of brain to aluminum exposure.

Name of the department and Institution where the work was done:

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INTRODUCTION

A largely neglected issue of immense societal and public health importance is the issue of brain development and learning in Third World children ([1]). The dependence of brain development on good nutrition has been recognized for decades. In India protein energy malnutrition has been identified as a major health and nutrition problem ([2]). Reports are available showing deficits in the neurobehavioral development as an impact of protein deficiency ([3,4,5]). On the other hand, aluminum has been recognized as a potent toxin causing impairment in the brain development and learning ([6]). There are several reports indicating the worsening of toxic impacts of metals by protein malnutrition specifically in case of brain ([7,8]). In this context, the present investigation is aimed to study the impact of protein malnutrition on the general responses of brain to aluminum exposure.

MATERIALS AND METHODS

ANIMALS AND DIETS

Male albino rats of Wistar strain weighing 80-100g have been used for the present study. The animals were maintained with normal (18%) protein diet for 7 days and then the animals were divided into four groups (containing six rats in each group) of equal average body weight. The animals of half of the groups were continued with the diet containing 18% protein (casein) while those of the remaining groups were maintained on the diet containing 6% protein as described earlier ([7,8]).

ALUMINUM EXPOSURE

Aluminum chloride ($AlCl_3$. $6H_2O$) was administered to the animals of one of the groups from each dietary regimen, intraperitoneally, at a dose of 4.2 mg / Kg body weight (15 % of LD_{50}) / day for 4 weeks.

After the period of treatment was over, the animals were sacrificed by cervical dislocation and the whole brain was removed, washed with ice-cold saline, blotted dry and immediately transferred to the ice chamber.

ESTIMATION OF ALUMINUM

The wet weight of each brain was noted followed by digestion with acid mixture containing nitric acid, sulfuric acid and perchloric acid in the ratio of 6 : 1 : 1, over a regulated heater. After the digestion, the acid mixture was evaporated with occasional addition of triple distilled water and the colourless-odourless solution was used for the estimation of aluminum content by Atomic Absorption Spectrometer (Model – Spectra BQ20, Varian, Australia).

ASSAY OF PHOSPHATASES

Broad-spectrum phosphomonoesterases (acid and alkaline) were measured using p-nitrophenyl phosphate (PNPP) as substrate according to the method described by Dasgupta and Ghosh ([9]). The brain regions were homogenized in ice-cold 0.25 M sucrose solution. The 2% (w/v) homogenates were used as source of the phosphatase enzymes.

ASSAY OF TRANSAMINASES

For assay of transaminase activities, the brains were homogenized in ice-cold 0.1M phosphate buffer (pH 7.5) containing 0.9% NaCl. Transaminase activities of brains were assayed following the methods as described by Lal et al ([10]).

ASSAY OF REDUCED GLUTATHIONE

The brains were homogenized in ice-cold 0.1M phosphate buffer (pH 7.4) and then the reduced glutathione content of brains were assayed by using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as described by Rathore et al ([11]).

ASSAY OF LIPID PEROXIDATION

Brain homogenates (ice-cold 0.25M tris buffer, pH 7.4) were used to measure the content of thiobarbituric acid reactive substances by the method as described elsewhere ([11]).

ASSAY OF NUCLEIC ACIDS

Extraction and estimation of DNA and RNA content of brains were carried out according to the method described by Nayak and Chatterjee ([8])

ESTIMATION OF PROTEIN

The protein content of each brain region was measured by the Folin-Ciocalteau reagent following the modified method of Lowry ([12]).

STATISTICAL ANALYSIS

Student's ‘t'-test was used to determine the significance of differences, if any, between the means, and probability levels of 5% or less were considered significant ([13]).

RESULTS

Gain in body weight during the course of aluminum exposure of animals fed on an adequate protein diet or an inadequate diet is presented in figure 1. Slightly higher gain in body weight was observed in the aluminum-treated rats maintained on adequate protein diet. But this increase was not statistically significant. However, any difference in body weight gain was not observed in between the aluminum-treated and control animals which were maintained with low protein diet.

Figure 1

Figure 1: Comparison of changes in body weight of different groups of rats during the period of treatement.

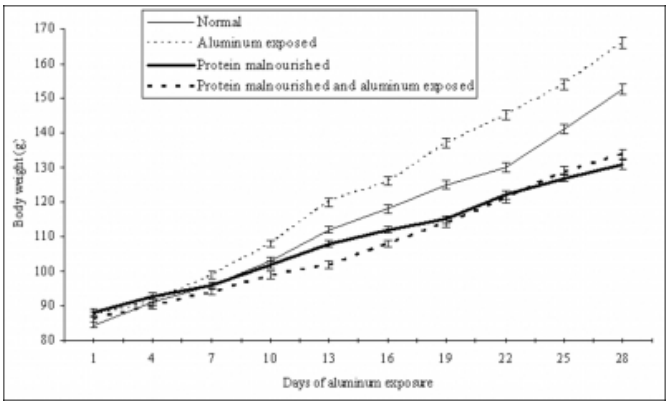


Figure 2

Table 1: Impact of aluminum on general metabolic enzymes of brain in normal and protein malnourished rats.

Parameters	Normal		Protein malnourished	
	Control	Aluminum exposed	Control	Aluminum exposed
Acid phosphatase (Units / 100 mg tissue)	80.24 ± 3.22	94.31 ± 2.18 [a]	90.50 ± 3.49	79.96 ± 2.01 [b c]
Alkaline phosphatase (Units / 100 mg tissue)	54.80 ± 2.69	50.23 ± 3.17	52.52 ± 3.54	41.28 ± 3.62 [a]
Aspartate aminotransferase (µg pyruvate produced / hr / mg tissue)	8.38 ± 0.14	8.06 ± 0.22	7.52 ± 0.28 [a]	8.61 ± 0.15 [c]
Alanine aminotransferase (µg pyruvate produced / hr / mg tissue)	3.58 ± 0.23	4.32 ± 0.30	3.74 ± 0.28	3.72 ± 0.22

a = statistically significant ($p < 0.05$) when compared with normal control,
b = statistically significant ($p < 0.05$) when compared with normal aluminum exposed ,
c = statistically significant ($p < 0.05$) when compared with protein malnourished control.

Figure 3

Table 2: Impact of aluminum on oxidative stress parameters of brain in normal and protein malnourished rats.

Parameters	Normal		Protein malnourished	
	Control	Aluminum exposed	Control	Aluminum exposed
Reduced glutathione (µmoles / g tissue)	3.03 ± 0.26	3.22 ± 0.41	2.61 ± 0.18	2.77 ± 0.14
Thiobarbituric acid reactive substances (µmoles / g tissue)	44.79 ± 2.17	41.43 ± 5.23	39.31 ± 3.14	40.75 ± 4.51

No significant impact of protein malnutrition as well as aluminum exposure have been observed in respect to brain weight. Brain weights (g/100g body weight) of aluminum-exposed animals (1.24 0.06) of adequate protein-fed group were found to be insignificantly lower than that of control animals (1.38 0.07) of the same group. Likewise, the control animals from the protein-malnourished group also showed only insignificantly higher brain weights when compared

with control animals of the same group (1.56 0.06 vs 1.46 0.09). However, the aluminum-exposed animals, those are maintained on normal protein diet showed a significant high level of aluminum content (4.92 0.70 µmoles / 100mg wet weight) of brain in comparison to the brain aluminum content (2.86 0.54 moles / 100mg wet weight) of animals of the same group but without aluminum exposure. On the other hand, the observed higher brain aluminum content (4.62 0.53 moles / 100mg wet weight) of aluminum-exposed animals was not statistically significant over the brain aluminum content (2.88 0.86 moles / 100mg wet weight) of control animals of the protein-malnourished group. In response to aluminum exposure the acid phosphatase activity of brain was found to be increased significantly (17.53 %) in the normal protein group but decreased significantly (13.18 %) in the protein malnourished group (Table 1 and Figure 2). Whereas the diminished alkaline phosphatase activity of brain of the aluminum-exposed protein malnourished group of rats was found significant only when compared with the normal control (Table 1). Protein malnutrition caused a significant decrease in aspartate aminotransferases activity but when this is associated with aluminum insult the activity of the enzyme was found to be increased (Table 1). No significant change was observed in the alanine aminotransferases activity (Table 1), reduced glutathione content and lipid peroxidation (Table 2) of brain. Significant decreases in DNA and RNA contents of brain were observed in protein malnourished aluminum-exposed group when compared with normal aluminum-exposed group (Table 3).

Figure 4

Table 3: Impact of aluminum on nucleic acids and protein contents of brain in normal and protein malnourished rats.

Parameters	Normal		Protein malnourished	
	Control	Aluminum exposed	Control	Aluminum exposed
DNA (µg / 100 mg tissue)	75.99 ± 6.75	81.28 ± 4.19	70.66 ± 3.38	63.06 ± 3.11 [b]
RNA (mg / g tissue)	1.43 ± 0.10	1.60 ± 0.15	1.45 ± 0.08	1.14 ± 0.10 [b c]
Protein (mg / 100 mg tissue)	9.37 ± 0.18	9.45 ± 0.41	10.13 ± 0.75	9.80 ± 0.13

b = statistically significant ($p < 0.05$) when compared with normal aluminum exposed ,
c = statistically significant ($p < 0.05$) when compared with protein malnourished control.

Figure 5

Figure 2: Comparison of percentage changes (in comparison to respective control) of enzyme activities of brain in response to aluminum exposure. ACP = Acid phosphatase, ALP = Alkaline phosphatase, AsAT = Aspartate aminotransferases, AlAT = Alanine aminotransferases,

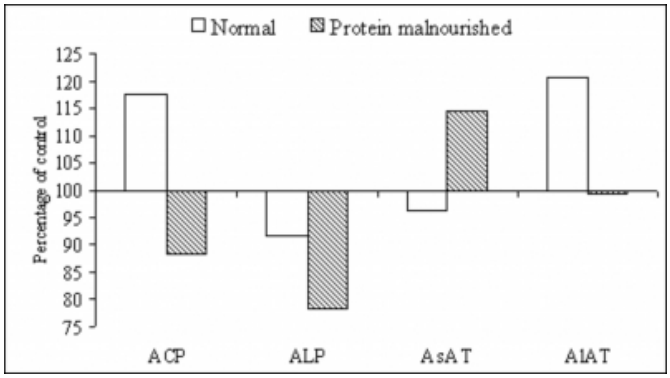
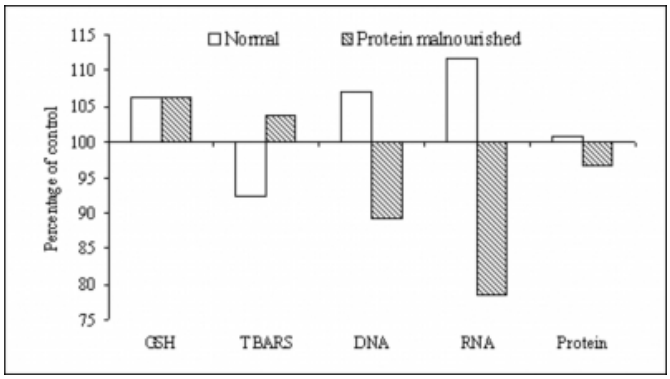


Figure 6

Figure 3: Comparison of percentage changes (in comparison to respective control) of oxidative stress parameters, nucleic acids and protein of brain in response to aluminum exposure. GSH = Reduced glutathione, TBARS = Thiobarbituric acid reactive substances.



In addition to these the RNA content was significantly reduced in response to aluminum exposure when the animals were maintained with low protein diet. The brain protein content was also found to remain unaltered in response to aluminum exposure as well as protein malnutrition (Table 3).

DISCUSSION

Several developmental studies have shown that the effect of aluminum on the body weight gain and maturation is controversial and such effects depend on the species of the subject as well as the compound of aluminum used ($_{14}$). The present study demonstrates that the brain weight of rats, receiving either normal protein diet or restricted protein diet is independent of aluminum treatment. This is evident from

the fact that there is no significant difference between the control and aluminum-exposed animals in terms of brain weight in both dietary regimens.

Aluminum treatment of rats fed on either an adequate or an inadequate protein diet increased the aluminum content in the brain. Rat brain aluminum content was significantly increased in the aluminum-exposed rats of the normal protein diet group but this increase was insignificant in the low protein diet group.

In the present investigation, the aluminum treatment caused a significant elevation of the acid phosphatase (ACP) activity in the normal group. This observation is in agreement with the earlier observations of the altered activities of specific lysosomal hydrolytic enzymes in non-neural ([15]) and neuronal tissue ([16]) due to aluminum administration. Form this observation it can be suggested that aluminum-induced increased ACP activity of brain may be an indication of lysosomal damage. The elevated activity of lysosomal enzymes in various conditions was suggested to be due to increased synthesis of the enzymes ([16]) that may be true for ACP also. Earlier it has been suggested that this elevated ACP activity can be regarded as one of the protective mechanisms against aluminum insult ([16,17]). Hence, the significant decrement in the ACP activity of the brain of protein malnourished rats in response to aluminum exposure indicate either absence of sufficient insult caused by aluminum or failure of the brain to respond against the insult. Thus the dietary protein causes a significant variation in the aluminum-induced responses of the brain.

Similarly a significant diminution of the alkaline phosphatase (ALP) activity has been observed only in the protein malnourished group of rats, when they are exposed to aluminum. The aluminum-exposed rats of the normal diet group also showed decreased activity of ALP but that is not statistically significant.

Both the tested transaminases have been observed to be unaltered in response to aluminum insult when the rats were maintained with normal protein diet. However, the protein malnourished rats showed significant decrease in aspartate aminotransferases (AsAT) activity. Colombo et al observed reduction in the brain aspartic acid content as well as increase in the brain and liver glutamic acid content in the rats maintained with low protein diet in comparison to those of normal protein diet ([18]). In addition to these, they have recorded the changes in the aminotransferases activities and suggested that these may be an adaptive response to dietary protein imbalance ([18]). Hence, the observed changes in brain AsAT may be and indication of adaptive responses of brain. This decrement in brain AsAT activity of the protein malnourished group was not observed when the animals were exposed simultaneously with the aluminum, rather the AsAT activity of brain found to be increased significantly in comparison to the protein malnourished control animals. On the other hand, the AlAT activity of both the dietary regimens remain unaltered in response to aluminum treatement. The interaction of gluatamate and / or aspartate with aluminum have been shown previously ([19,20]). Thus, from the present study it may be suggested that both {a}brain amino acid pool status and {b}interloping between aluminum and specific amino acids are involved in the responsiveness of brain towards the aluminum insult.

The chemical basis for the promotion of free radical production by aluminum salts is obscure ([21]). However, a number of publications suggesting the oxidative damage caused by aluminum [directly or indirectly] made the fact very much debatable ([6]). There are no significant changes in reduced glutathione and thiobarbituric acid reactive substances contents of brain in any of the dietary regimens of rats in response to aluminum exposure. Hence the present dose and duration of aluminum treatment, irrespective of the dietary protein status, did not cause oxidative stress that might disturb the cellular antioxidative mechanisms.

Significant reductions in the brain DNA and RNA contents were observed when the protein malnourished aluminum exposed animals were compared with the normal protein fed aluminum exposed animals. However, within the dietary regimens, aluminum exposure did not alter the brain DNA content significantly. But in case of RNA, the content of brain of the protein malnourished rats was found to be reduced significantly in response to aluminum exposure. The results suggested that, the decreased DNA content may be indicative of cellular destrophy, whereas the alteration in RNA contents suggesting that the aluminum does affect the nucleic acid machinery of brain cells.

The results depicted in table 1 and figure 1 indicated that the dietary protein has a significant influence upon the response of aluminum on the brain. This might significantly alter the behavioral toxicity of aluminum. For the confirmation, further studies on the impact of dietary protein status on aluminum-induced behavioral modifications is required.

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