

Research article

## Response of regional brain glutamate transaminases of rat to aluminum in protein malnutrition

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### Abstract

**Background:** The mechanism of aluminum-induced neurotoxicity is not clear. The involvement of glutamate in the aluminium-induced neurocomplications has been suggested. Brain glutamate levels also found to be altered in protein malnutrition. Alterations in glutamate levels as well as glutamate- $\alpha$ -decarboxylase in different regions of rat brain has been reported in response to aluminum exposure. Thus the study of glutamate metabolising enzymes in different brain regions of rats maintained on either normal or restricted protein diet may be of importance for understanding the neurotoxicity properties of aluminium.

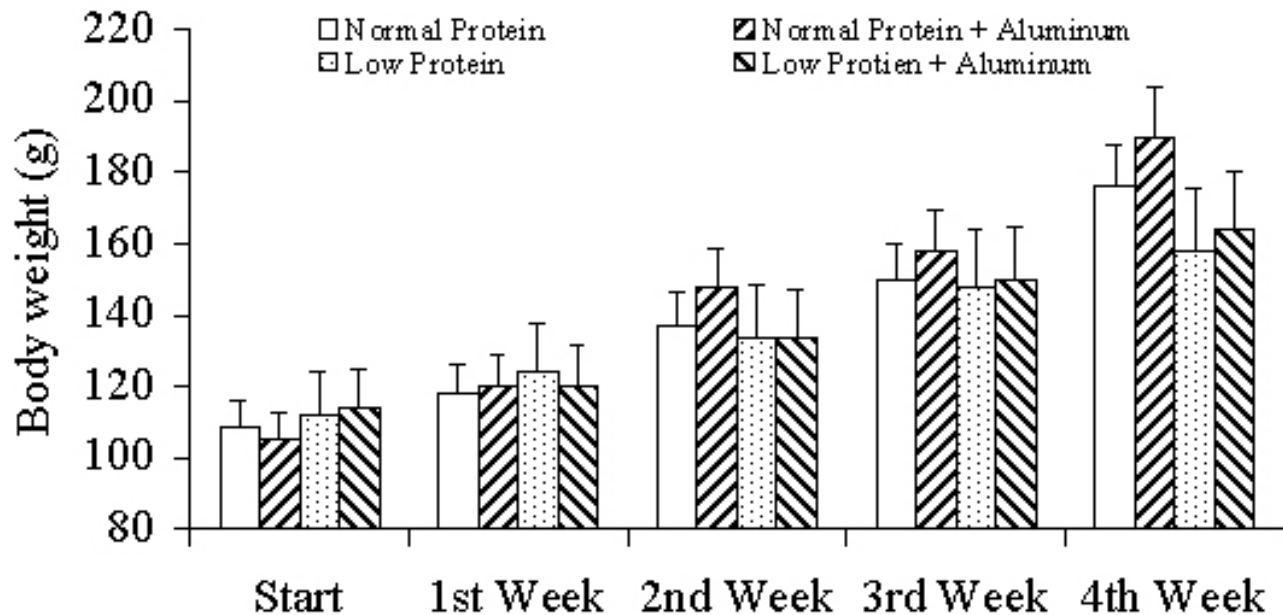
**Results:** Dietary protein restrictions does not have an significant impact on regional aluminum content of the brain. The interaction of aluminum intoxication and protein restriction is significant in the thalamic area and the midbrain-hippocampal region in cases of glutamate oxaloacetate transaminase. In the case of glutamate pyruvate transaminase, this interaction is significant only in thalamic area.

**Conclusion:** The metabolism of amino acids, as indicated by activities of specific transaminases, of brain is altered in response to aluminum exposure. These alterations are region specific and are dependent on dietary protein intake or manipulation of the brain amino acid homeostasis.

### Background

The ubiquity of numerous aluminum compounds found in soil, water and air ensure that we live in an aluminum-rich environment. The entrance aluminum to human body is carefully refrained; however, the metal gains access to the body. The so-called 'biological inertness' and other chemical properties of aluminum render maximum use of this metal in our every day living. The toxic consequences in human after aluminum exposure are now well established [1,2]. The brain is the most sensitive organ to show aluminum-induced disorders [3]. Despite several hypotheses, the mechanism of aluminum-induced neurotoxicity is not clear [4,5]. There are suggestions that glutamate may be involved in the aluminum-induced

neurocomplications. The excitotoxic effect of glutamate is believed to be the cause of several neurodegenerative processes [6] and several enzymes with the capacity to degrade glutamate have been suggested as possible neuroprotectants [7]. It has been reported that aluminum impairs the glutamatergic neurotransmission [8] and mediates glutamate-induced neurotoxicity in organotypic cultures [9]. On the other hand, glutamate is an important metabolic intermediate of the brain and it has potential aluminum binding capacity [10]. In our earlier studies, we have shown that aluminum causes alteration in glutamate levels and glutamate  $\alpha$ -decarboxylase activities of different brain regions [11]. In addition to this, we had also shown dietary protein restrictions to alter the aluminum-



**Figure 1**

Week wise body weight of rats during the period of treatment. Each data point represents the mean of six observations  $\pm$  SEM.

induced neurological responses in several brain regions [3,12]. Thus the present investigation is aimed to study the impact of aluminum as well as dietary protein restriction on transaminases of the brain, which are related to the glutamate metabolism.

## Results

### Body weights

The body weights of the rats during the period of treatment (0–4<sup>th</sup> week) are presented in figure 1. A slightly higher gain in body weight (on the 4<sup>th</sup> week) was observed in the aluminum-treated rats of both the normal and low protein dietary groups. However, these differences in body weight are not statistically significant. During the course of the treatment, a decrement in food intake was observed in the aluminum-treated rats of both dietary groups (Figure 2).

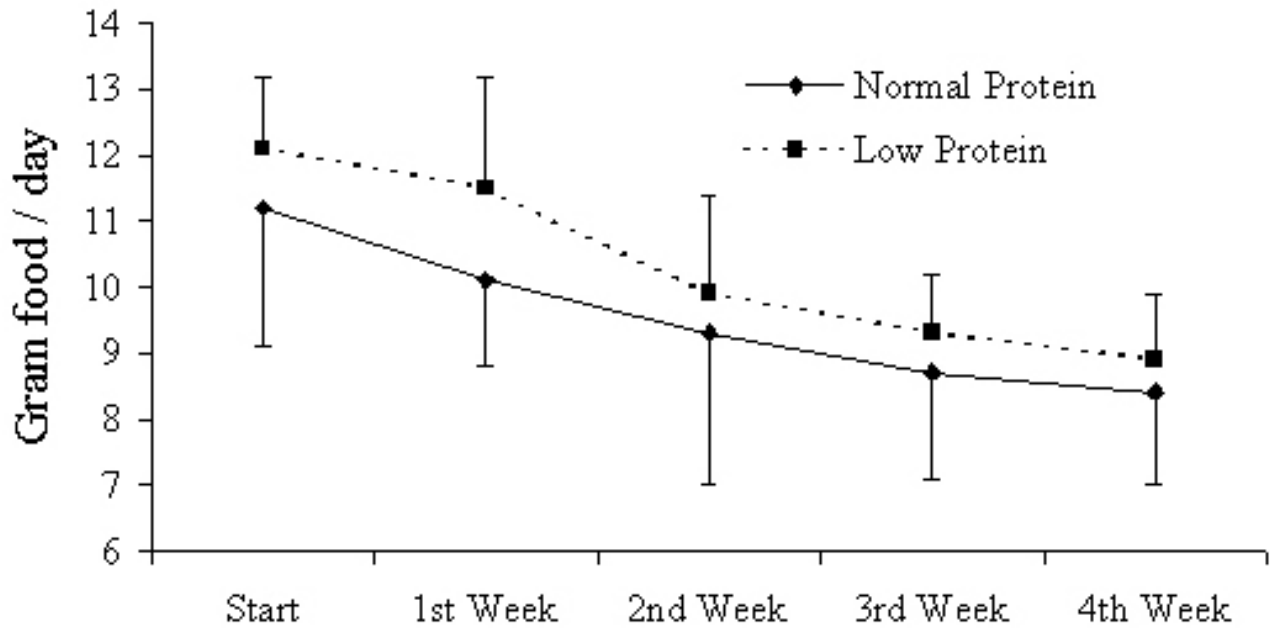
### Aluminum content

Changes in the aluminum content in different brain regions on exposure to aluminum are given in table 1. The present treatment increases the aluminum content of the cerebrum, thalamic area, midbrain-hippocampal region and cerebellum by 70.6%, 115.6%, 123.3% and 53.5% respectively in normal protein-fed group of rats, whereas 59.4%, 93.3%, 107.7% and 53.2% respectively in low protein-fed group of rats. Though there is significant amount of added components for treatment effects in all

of the tested brain regions, Scheffe's F test for multiple comparisons showed that aluminum exposure significantly increased the aluminum content of cerebrum, thalamic area and midbrain-hippocampal region of normal protein group and thalamic area and midbrain-hippocampal region of rats maintained on low protein diet. However, two factor ANOVA (with replication) showed that only effects of aluminum treatment were significant in all the four brain regions.

### Glutamate oxaloacetate transaminase

The glutamate oxaloacetate transaminase (GOT) activities of different brain regions of all groups of rats are presented in table 2. The cerebrum, thalamic area, midbrain-hippocampal region and cerebellum of low protein-fed group of rats showed 9.7%, 17.9%, 20.8% and 13.6% increase in GOT activities in response to aluminum exposure. On the other hand, only the cerebellum of the normal protein-fed group of rats showed 1.6% increase in GOT activity when exposed to aluminum. Regional GOT activities of thalamic area and midbrain-hippocampal region of rats maintained on normal protein diet were reduced by 10% and 11.5% respectively. Changes in the GOT activity of only the cerebrum and midbrain-hippocampal regions of inadequately protein-fed rats were found to be statistically significant by Scheffe's F test for multiple comparisons. However, significant impacts of treatment were observed in all the brain regions (ANOVA single factor). Two factor



**Figure 2**  
Week wise food intakes of rats during the period of treatment. Each data point represents the mean of six observations  $\pm$  SEM.

**Table 1: Regional aluminum content ( $\mu$ moles / 100 mg tissue) in rat brain.**

Brain regions	Groups of animals				Statistical calculation (F)	
	Normal protein		Low protein		ANOVA Single factor	ANOVA Two factors (with replication)
	Control	Aluminum exposed	Control	Aluminum exposed		
Cerebrum	2.86 $\pm$ 0.47	4.88 $\pm$ 0.44*	2.91 $\pm$ 0.54	4.64 $\pm$ 0.21	6.30 #	Protein : 0.05 Aluminum : 18.75 # Interaction : 0.11
Thalamic area	4.16 $\pm$ 0.52	8.97 $\pm$ 0.71*	4.96 $\pm$ 0.42	9.59 $\pm$ 0.52*	24.69 #	Protein : 1.65 Aluminum : 72.39 # Interaction : 0.03
Midbrain-hippocampal region	4.17 $\pm$ 0.42	9.31 $\pm$ 0.62*	4.13 $\pm$ 0.25	8.58 $\pm$ 0.68*	28.47 #	Protein : 0.55 Aluminum : 84.39# Interaction : 0.45
Cerebellum	3.10 $\pm$ 0.52	4.76 $\pm$ 0.64	2.82 $\pm$ 0.34	4.32 $\pm$ 0.44	3.56#	Protein : 0.51 Aluminum : 10.14# Interaction : 0.03

Data are means of six observations  $\pm$  SEM. \* indicates significant difference ( $p < 0.05$ ) in comparison to respective control. # indicates calculated F is higher than the critical F ( $\alpha = 0.05$ ).

ANOVA with replication showed that aluminum exposure contributed significantly on the changes of the GOT activity of cerebellum only, but the interactions of impact of dietary protein deficiency and aluminum exposure were

significant in thalamic area as well as midbrain-hippocampal region.

**Table 2: Regional glutamate oxaloacetate transaminase activity ( $\mu\text{g}$  pyruvate produced / hr / mg tissue) in rat brain.**

Brain regions	Groups of animals				Statistical calculation (F)	
	Normal protein		Low protein		ANOVA Single factor	ANOVA Two factors (with replication)
	Control	Aluminum exposed	Control	Aluminum exposed		
Cerebrum	8.42 $\pm$ 0.37	8.38 $\pm$ 0.35	7.69 $\pm$ 0.25	8.44 $\pm$ 0.25*	9.03 #	Protein : 1.14 Aluminum : 1.31 Interaction : 1.62
Thalamic area	8.48 $\pm$ 0.16	7.64 $\pm$ 0.28	7.39 $\pm$ 0.32	8.71 $\pm$ 0.52	3.41 #	Protein : 0.00 Aluminum : 0.49 Interaction : 9.75 #
Midbrain-hippocampal region	8.50 $\pm$ 0.18	7.52 $\pm$ 0.31	7.25 $\pm$ 0.27	8.76 $\pm$ 0.20*	9.03 #	Protein : 0.00 Aluminum : 1.15 Interaction : 25.95 #
Cerebellum	8.08 $\pm$ 0.25	8.21 $\pm$ 0.21	7.67 $\pm$ 0.23	8.71 $\pm$ 0.28	3.12#	Protein : 0.04 Aluminum : 5.86# Interaction : 3.46

Data are means of six observations  $\pm$  SEM. \* indicates significant difference ( $p < 0.05$ ) in comparison to respective control. # indicates calculated F is higher than the critical F ( $\alpha = 0.05$ ).

**Table 3: Regional glutamate pyruvate transaminase activity ( $\mu\text{g}$  pyruvate produced / hr / mg tissue) in rat brain.**

Brain regions	Groups of animals				Statistical calculation (F)	
	Normal protein		Low protein		ANOVA Single factor	ANOVA Two factors (with replication)
	Control	Aluminum exposed	Control	Aluminum exposed		
Cerebrum	3.41 $\pm$ 0.21	4.25 $\pm$ 0.30	3.68 $\pm$ 0.25	3.69 $\pm$ 0.20	2.07	Protein : 0.35 Aluminum : 2.97 Interaction : 2.88
Thalamic area	2.49 $\pm$ 0.19	3.77 $\pm$ 0.19*	3.41 $\pm$ 0.16	3.48 $\pm$ 0.18	9.23#	Protein : 2.96 Aluminum : 13.78# Interaction : 10.96#
Midbrain-hippocampal region	3.34 $\pm$ 0.19	3.34 $\pm$ 0.14	3.15 $\pm$ 0.07	2.71 $\pm$ 0.15	4.10#	Protein : 7.71# Aluminum : 2.29 Interaction : 2.29
Cerebellum	4.91 $\pm$ 0.09	5.43 $\pm$ 0.22	4.58 $\pm$ 0.10	5.06 $\pm$ 0.13	5.88#	Protein : 5.84# Aluminum : 11.80# Interaction : 0.02

Data are means of six observations  $\pm$  SEM. \* indicates significant difference ( $p < 0.05$ ) in comparison to respective control. # indicates calculated F is higher than the critical F ( $\alpha = 0.05$ ).

**Glutamate pyruvate transaminase**

Table 3 represents the regional GPT activities of the different groups of rats. Elevated activities of GPT were observed in the cerebellum (24.6%), thalamic area (51.4%) and cerebellum of aluminum-exposed animals of normal protein-fed group. In the protein-restricted group, the GPT activities were found to be increased in the cerebel-

lum (10.5%) and decreased in midbrain-hippocampal region (14%). Though a significant impact of treatments were observed in all the brain regions except the cerebrum (single factor ANOVA), only changes in the thalamic area of normal protein diet group was found to be significant by Scheffe's F test. Two factor ANOVA (with replication) showed that impact of aluminum is significant in thalam-

ic area and cerebellum, impact of protein restriction is significant in midbrain-hippocampal region and cerebellum, and the interaction of both protein restriction and aluminum treatment is significant only in thalamic area.

The correlations between the region wise changes in the level of aluminum and activities of transaminases in response to aluminum exposure (of both adequately protein-fed rats and inadequately protein-fed rats) have been shown in table 4. Only significantly negative correlation was observed between changes in aluminum level and alteration of GOT activities in adequately protein-fed group of rats.

**Table 4: Correlation between regions of brain in terms of transaminase activity and aluminum content.**

Correlation between	Normal protein	Low protein
Aluminum : GOT	-1.00*	0.91
Aluminum : GPT	0.16	0.90
GOT : GPT	-0.10	-0.60

\* Indicates significant correlation ( $p < 0.05$ )

## Discussion

Aluminum treatment of rats fed on either normal protein or low protein diet increased the aluminum content in all the brain regions. The midbrain-hippocampal region of the aluminum-exposed rat brain showed highest level of accumulation of aluminum. This observation is in agreement with the available reports indicating hippocampus to be the susceptible brain region for accumulation of aluminum [15–17]. However, the regional differences in increment of aluminum level on exposure to aluminum are comparable in both the dietary regimens. Protein deficiency is not significantly impinging on regional aluminum level of brain (Table 1).

The observation that glutamate oxaloacetate transaminase (GOT) is present in rat cerebral homogenate at about the same concentrations as in liver suggests its importance in the brain amino acid pool homeostasis [18]. The GOT activity is found in both mitochondrial and soluble fractions of the brain. The enzyme in mitochondria is latent and can be 'activated' by various procedures that disrupt the mitochondrial structure. The critical role of mitochondrial dysfunction in the pathogenesis of age-related nerve cell degeneration has been suggested in several occasions [19,20]. On the other hand, coexposure of aluminum and glutamate is reported to produce morphological abnormalities of mitochondrial structures in pyramidal neurons

of hippocampal formation, which are not observed when exposed to aluminum or glutamate alone [9]. Toninello *et al* [21] also showed that aluminum is an inducer of mitochondrial permeability transition.

No significant change in GOT activity has been observed in any of the brain regions in response to aluminum exposure when the animals were maintained on normal protein diet. Favarato and Zatta [22] also did not observe any alteration in serum GOT activity in response to aluminum. However, regional specificity was observed in aluminum-induced elevation of brain GOT activity, in the inadequately protein-fed animals. Increased GOT activity may be connected with an increased transport of NADH from the cytosol to mitochondria [23]

In the present investigation the regional variation of normal GOT activity is not prominent but the response to aluminum insult shows significant regional specificity. Thus it may be suggested that aluminum exposure causes region specific alteration in amino acid metabolism. Hence, altered amino acid distribution pattern within the brain might be expected in response to aluminum exposure.

The observed region-specific alterations may also be attributed to the level of accumulated aluminum in the respective regions. Diencephalon is reported to be the most vulnerable portion in case of accumulation in brain [17,24], which is also observed in the present investigation (Table 1).

Though glutamate pyruvate transaminase (GPT) is indicated to be distributed in both mitochondrial and soluble fractions of the brain, unlike GOT, it is less active in brain. However, Matthews *et al* [7] reported that both the enzymes degrade glutamate; though only GPT was able to reduce toxic (500  $\mu$ M) levels of glutamate into the physiologic (<20  $\mu$ M) range. Our earlier observation showed that aluminum exposure causes significant increase in glutamate content in different regions of brain while only thalamic area showed enhanced  $\gamma$ -amino butyrate content [11]. In the present investigation, the GPT activity is increased only in thalamic area of sufficient protein-fed group. These observations support region-wise specific sensitivity to aluminum exposure as indicated in nucleic acid content and nucleic acid metabolism [3]. On the other hand, in continuation with earlier studies [11] it can be suggested that either the enhanced glutamate level is not sufficient enough to trigger the response of GPT in these regions or other removal pathway(s) are activated. In addition to these, direct inhibition of GPT by aluminum and local concentration of pyruvate may also be involved in the present observation of GPT activity. The present investigation has revealed differential response of

enzymes in response to aluminum insult in various regions of the brain. This finding corroborates with the earlier observation of regional variation in enzyme activities of brain in response to aluminum exposure [12,25].

These transaminase reactions are reversible, but the equilibrium of the GOT and GPT reactions favour formation of aspartate and alanine respectively [26]. Increased aminotransferase activities might participate in the enhanced synthesis of excitatory amino acid neurotransmitters in the nervous system [24].

## Conclusions

These observations are suggestive of the fact that the metabolism of the amino acids in response to aluminum exposure is very much region specific. As amino acids in the brain compose a major part of the neurotransmitters, it is expected that, these alterations might also be involved in the aluminum-induced neurobehavioral impairments. These alterations are region specific and are dependent on dietary protein intake or manipulation of the brain amino acid homeostasis.

## Methods

### Animal maintenance and aluminum exposure

Male albino rats of Wistar strain weighing 100–120 g were divided into four groups of almost equal average body weight. The animals of two groups were maintained on the diet containing 18% protein (casein) while the remaining two groups were maintained on 6% protein (casein) diet as reported earlier [3]. One group of rats from each of the two dietary regimens received 4.2 mg/Kg body weight / day as aluminum chloride intraperitoneally for four weeks and the animals of the remaining groups serving as pair-fed controls received only the vehicle.

### Tissue collection

After the period of treatment, overnight fasted rats were sacrificed by cervical dislocation. The whole brain was removed, washed with ice-cold saline, blotted dry and immediately transferred to the ice chamber. Cerebrum, thalamic area, midbrain-hippocampal region and cerebellum were separated following of the dissection of the frozen brain after thawing (making soft) in cold, under dissecting microscope [3].

### Biochemical estimation

The aluminum content of each tissue region was estimated by Atomic Absorption Spectrometer after digested with acid mixture, containing nitric acid, sulphuric acid and perchloric acid in the ratio of 6:1:1, over a regulated heater [12]. The activities of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were measured through spectrophotometric determination of 2,4 dinitrophenylhydrazone of pyruvate [13].

## Authors' contributions

PN carried out the literature survey, experimental works and statistical calculation.

AKC conceived of the study, and participated in its design and coordination.

All authors read and approved the final manuscript.

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