Thalamic superoxide and peroxide handling capacity (SPHC): An experimental study with aluminum, ethanol and tocopherol in rats

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Superoxide and peroxide handling capacity (SPHC) is an important determinant of oxidative stress. Neurotoxic impacts of aluminum are associated with oxidant imbalance. Here, we studied the influence of aluminum on oxidative stress parameters, antioxidative enzymes and SPHC of thalamic area on pro-oxidant (ethanol) and antioxidant (α-tocopherol) exposure. Two sets of male Wistar rats were divided into 8 groups (6 each) and exposed to aluminum (10 mg/Kg body wt.), ethanol (0.6 g/Kg body wt.) and α-tocopherol (5 IU/day) for 4 wk, each having respective control group. Levels of reduced glutathione (GSH), lipid peroxidation (TBARS) along with activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) of thalamic area were estimated for each group. Glutathione-independent superoxide peroxide handling capacity (GI-SPHC) and glutathione-dependent superoxide peroxide handling capacity (GD-SPHC) were calculated from the GPx, CAT and SOD values. Concomitant exposure to aluminum and ethanol demonstrated significant increase in SOD activity and significant decrease in GPx activity compared to the control group, while lone aluminum-exposed rats showed raised GR activity, without alterations in GPx and SOD activities. However, significant reduction of both GI- and GD- SPHC were found in ethanol-exposed groups. α-Tocopherol supplementation could resist most of the alterations. In addition, current antioxidant exposure reduced the inherent GD-SPHC, and thus, made thalamic area more vulnerable to oxidant threat. The present study corroborates the thalamic susceptibility to aluminum-augmented oxidant imbalance and suggests cautious use of antioxidant supplementation against neurodegenerative disorders.

Keywords: Antioxidants, Brain, Catalase, Lipid peroxidation, Neurodegenerative disorders, Neurotoxicity, Oxidant imbalance, Oxidative stress, Thalamus

Oxidative stress is a state with either increased oxidants level or decreased antioxidants level in the cellular microenvironment. Physiological and pathological conditions pose threats by creating oxidative stress. To combat with this, cells are inherently equipped with mechanisms including antioxidant molecules and antioxidative enzymes. Exogenous application of oxidant modulators i.e., antioxidants and pro-oxidants, can cause alteration of this crucial oxidant homeostasis. Ethanol is a known pro-oxidant while α-tocopherol is an antioxidant, and both of them are neuroeffective⁷. Aluminum, on the other hand, is a known neurotoxicant and associated with oxidative stress in brain regions⁵ while itself being redox inactive in physiological pH.

Although, no useful involvement of aluminum has been reported in natural biological functions, it is being used in many household, industrial and medicative purposes⁴,⁵. Because of its exceptional physico-chemical properties and abundance in ecology, exposure to aluminum is inevitable; while, its presence in body is undesirable. Aluminum gets accumulated in different brain regions. Thalamus is one such region that accumulates high amount of aluminum both during development⁵ and in adult rodents⁶. Thalamus is also reported to have higher level of aluminum accumulation along with parieto-rolandic cortex and cerebellar cortex in a typical case of dialytic dementia in a patient treated with aluminum gels⁷.

Regional variations in antioxidant parameters and altered oxidant status have been reported in chronic and acute aluminum exposures⁸,⁹. Incidentally, most prominent increase in SOD activity was with thalamus and hippocampus upon acute administration of aluminum¹⁰. In a recent study, highest baseline GSH concentration and potentially higher GSH recycle rate in thalamus of F344 rats have been reported by Pang et al.¹¹.
With ethanol as pro-oxidant\textsuperscript{12}, aluminum-induced oxidative stress is already evidenced to be dependent on the oxidant status and region-specific\textsuperscript{13,14}. Ethanol is a positive inducer of age-related deterioration of neuronal cells\textsuperscript{15}, and aging could markedly induce the aluminum-induced oxidative stress\textsuperscript{16}. On the other hand, aluminum has been found to enhance the lipid peroxidation and inhibit some key enzymes in thalamus where normal aging does not have any effect\textsuperscript{17}.

Exposure to aluminum alters the redox homeostasis by diminishing the free radical scavenging activities\textsuperscript{18,19}. However, dietary antioxidants come handy to overcome this problem\textsuperscript{18}, lipid soluble \(\alpha\)-tocopherol in particular, due to its ability to break the reactive oxygen species (ROS)-induced chain reactions\textsuperscript{20} and neuroprotection properties by suppressing glial activation process\textsuperscript{21}.

Thalamic region, in spite of being one of the highest sites of aluminum accumulation, susceptible to aluminum-induced oxidative stress but less vulnerable to normal aging, its aluminum-induced neurodegeneration is not much studied. In addition, cytochrome P450, an enzyme involved in ethanol catabolism is found to be inducible at thalamus\textsuperscript{22}. With this background, we investigated aluminum-induced alterations in SPHC of rat thalamus, with concurrent exposure to ethanol and \(\alpha\)-tocopherol.

**Materials and Methods**

**Materials**—All chemicals were of analytical grade and purchased from Sigma India, SRL India, Merck India and Qualigen, India.

**Animal maintenance and treatments**—The experimental protocol was approved by the Institute Animal Ethics Committee. Two sets of male albino Wistar rats (each set composed of 24) weighing 100-120 g were obtained, maintained and treated in the Central Animal House of NRI Medical College & General Hospital, and the procedures were performed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India)\textsuperscript{23}. After one week of acclimatization, rats were randomly divided (with the help of Random Allocation Software Version 1.0, May 2004) into 4 groups (containing 6 animals in each group), namely \(\text{Al}_0\text{Et}_0\) (vehicle treatments); \(\text{Al}_1\text{Et}_0\) (only @ 10 mg aluminum /Kg body wt.); \(\text{Al}_0\text{Et}_1\) (only 0.6 g ethanol/Kg body wt.); and \(\text{Al}_1\text{Et}_1\) (exposed to both aluminum and ethanol).

The treatment was continued for four weeks. Both ethanol and aluminum treatments were carried out through oral feeding. Ethanol or distilled water was given in the morning session while aluminum or vehicle was given in the evening session daily. Because of inconclusive toxicokinetic interactions of ethanol and aluminum, different treatment sessions were maintained\textsuperscript{24}. Morning sessions were preferred for ethanol exposures to avoid impact of ethanol on food intake. In another set of experiments all these groups were exposed to \(\alpha\)-tocopherol supplementation (5 IU/day).

**Tissue collection and biochemical assays**—After the period of treatment, overnight fasted rats were sacrificed by cervical dislocation. The whole brain was removed, washed with ice-cold saline. Under dissection microscope, the thalamic area was separated immediately and preserved in the ice-chamber for biochemical processing. The homogenized brain tissues were used for determination of reduced glutathione (GSH) content, lipid peroxidation, activities of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) as done earlier\textsuperscript{13}. Ratio of activities as CAT/SOD and GPx/SOD were used to calculate GSH-independent SPHC and GSH-dependent SPHC, respectively, as detailed earlier\textsuperscript{2,23}. Aluminum contents in thalamic areas were measured spectrophotometrically using cloud extraction procedure\textsuperscript{25} with separate set of animals having similar treatments of aluminum, ethanol and tocopherol.

**Statistical analysis**—The collected data were presented in Box and whiskers plot indicating Median, lower and upper quartile, range and outliers (if any). Variance and difference between the groups were analyzed by Kruskal-Wallis test and Wilcoxon Rank Sum test, respectively, accepting the probability of 5% or less as significant. Spearman’s rho test was employed to find out the correlation between level of GSH and activities of GPx and GR of thalamic area.

**Results**

Fig. 1a depicts the level of GSH and TBARS in thalamic area of four groups of rats which received two types of treatment protocols, with \(\alpha\)-tocopherol or without \(\alpha\)-tocopherol supplementation. Only the \(\alpha\)-tocopherol supplemented set demonstrated significant difference in thalamic TBARS levels between the \(\text{Al}_1\text{Et}_0\) group and \(\text{Al}_1\text{Et}_1\) group, even though for thalamic GSH levels all the groups were found identical in both set of experimentations.
The differences in SOD activity of thalamic area of tested groups were evidenced by significant chi square value of Kruskal-Wallis test (**P** < 0.05) for the set without α-tocopherol supplementation. The levels of SOD activities in thalamic area of Al_{0}Et_{0} and AlEt_{0} groups were found to be higher compared to that of Al_{0}Et_{0}; while, AlEt_{0} group demonstrated higher SOD activity in thalamic area than that of Al_{0}Et_{0} group (Fig. 1b). However, no difference between the groups of α-tocopherol supplemented set was indicated by the statistical analyses.

On the other hand, the catalase activity of thalamic area of Al_{0}Et_{0} group was significantly lower compared to that of Al_{0}Et_{0} group and AlEt_{0} group (Fig. 1b), even though Kruskal-Wallis test found that all the groups were identical in both sets of experimentations, with or without α-tocopherol supplementation.

In terms of GPx and GR activities of thalamic area, experimental groups which did not receive α-tocopherol supplementation demonstrated non-identical distribution of data levels. These observations were supported by significantly lowered GPx activities in thalamic area of Et_{0} groups (Fig. 1c). The Al_{0}Et_{0} group evinced significantly higher GPx activity of thalamic area when compared with AlEt_{0} group and AlEt_{0} group, while the same was significantly lower in Al_{0}Et_{0} group than the AlEt_{0} group (Fig. 1c). On the other hand, GR activity of thalamic area of Al_{0}Et_{0} group was higher than the
other groups (Fig. 1c), however, differences were found statistically significant against Al₀Et₀ and AlₐEtₐ groups.

Superoxide and peroxide handling capacity (SPHC) of thalamic area of ethanol-exposed (Et₀) groups which did not receive α-tocopherol supplementation were significantly lower compared to AlₐEt₀ animals. The differences between the tested groups were evidenced by non-identical distributions of thalamic SPHC values for both glutathione dependent (GD) and glutathione independent (GI) appraisals (Fig. 1d). Identical distribution GD-SPHC and GI-SPHC values for α-tocopherol-supplemented groups and non-identical distribution of those in other groups confirm the intergroup difference in α-tocopherol non-supplemented experimental set.

Both set of animals, α-tocopherol supplemented and non-supplemented, demonstrated significant chi square value for the aluminum content in thalamic area (Fig. 1e). The thalamic aluminum content was significantly higher in Al₁ animals compared to Al₀ animals irrespective of ethanol or α-tocopherol exposure (Fig. 1e). In addition, ethanol exposure did not produce statistically significant impact on thalamic aluminum content in either Al₀ or Al₁ animals of both set of experimentations.

Discussion

Capacity of a system to maintain the balance between oxidant and antioxidant processes is the headstone for oxidative status—maintaining it within physiological limit or allowing it to proceed toward pathological changes. Aluminum could induce oxidative stress in all types of brain cells. Present study explored the oxidative stress induction in thalamic area by aluminum itself, and in the presence of ethanol, providing a pro-oxidant ambience. In addition, whether the aluminum-induced alterations in thalamic oxidant imbalance could be prevented by antioxidant supplementation was also explored.

Kohila et al. did not find significant alteration of aluminum absorption even though they reviewed that ethanol can increase the neural membrane permeability for aluminum, possibly through increasing the membrane fluidity. Similarly, Abubakar et al. also did not find significant variation in regional brain aluminum levels in different doses of vitamin E exposure. Corroborating these earlier reports, increments in thalamic aluminum contents in the current context were also found to remain unaltered by the exposure to either ethanol or α-tocopherol (Fig. 1e). Nonetheless, pro-oxidant effects of ethanol and antioxidant effects of α-tocopherol on brain have been evidenced in several occasions especially, in relation to aluminum-induced neurodegeneration. Relatively lower levels of TBARS in the thalamic area of α-tocopherol supplemented Et₀ animals (Fig. 1a) indicated protection from oxidative stress faced by these animals. Statistically significant difference in thalamic TBARS level between Al₁Et₀ and Al₁Etₐ animals but not in between Al₀Et₀ and Al₀Etₐ animals receiving α-tocopherol supplementation (Fig. 1a) evinced that aluminum exposure could aggravate the ethanol-induced oxidative stress in thalamic area of brain. Augmentation of lipid peroxidation in brain was also noted while rats were co-treated with two pro-oxidants, fluoride and ethanol. On the contrary, similar difference was not observed in the experimental set without α-tocopherol supplementation. This might be due to masking effect of regular oxidative stress faced by thalamic area of brain. It is noteworthy that even though thalamus was reportedly less vulnerable to aging and demonstrated relatively lesser electrophysiological changes induced by aluminum exposure, the brain region could not resist the aluminum-induced lipid peroxidation to the same extent. Vitamin E supplementation has already been shown to compensate the total thiol content in testes and kidney reduced by aluminum exposure, while the total thiol content of brain, liver and plasma remained same in aluminum-exposed groups irrespective of vitamin E supplementation. Interestingly, all the tested organs showed significant increase in TBARS level which was reduced by vitamin E exposure, completely or partially. In this connection, brain regions like cortex and hippocampus, also behaved equally and showed unaltered GSH content while demonstrated vitamin E mediated partial protection against aluminum-induced rise in Fe²⁺-dependent and NADH-dependent lipid peroxidation. El-Gendy also reported similar impact of vitamin E supplementation in case of malonaldehyde and 4-hydroxynonenal contents in brain of aluminium-treated rats, however, vitamin E supplementation failed to protect the aluminum-induced decrease in GSH content of brain.

Aluminum-induced increases in SOD activities in mice cerebral cortex, striatum, cerebellum and medulla had been suggested to combat with additional
superoxide formation. On the contrary, brain SOD activity was insignificantly decreased by aluminum exposure, while significant decreases in SOD activities in liver, kidneys and testes along with vitamin E mediated protection were observed in aluminum-treated rats. In the absence of pro-oxidant dominance, aluminum was found to enhance the SOD activities of thalamic area only insignificantly, while the rise was statistically significant in the presence of concomitant exposure to ethanol (Fig. 1b). Importantly, reported consonance alterations in catalase activities were not observed in the thalamic area. Sánchez-Iglesias et al. also noted unparallel alterations in SOD and catalase of ventral midbrain and cortex of aluminum-treated rats. Similar to the reduction in level of lipid peroxidation, catalase activities of thalamic regions of all groups of animals with α-tocopherol supplementation were notably low compared to that of animals without α-tocopherol supplementation (Fig. 1b). In the presence of α-tocopherol supplementation, aluminum-exposed groups demonstrated lower catalase activities in thalamic areas compared to their counterparts, even though there was not much variation in terms of SOD activities of thalamus between the animals with or without α-tocopherol supplementation (Fig. 1b). The intergroup differences in thalamic SOD activities were note-worthily not observed in α-tocopherol supplementation group.

Corroborating the reported decrease in brain GPx activity by exposure to aluminum, most of the studied brain regions, including ventral midbrain, demonstrated significantly reduced GPx activity. The non-supplemented set demonstrated significant intergroup variations (as evidenced by Kruskal-Wallis test) in terms of GPx and GR activities of thalamic area (Fig. 1c). Aluminum-induced insignificant decreases in GPx activity were also found in cerebrum and cerebellum in similar studies where the difference became significant in the presence of concomitant exposure to ethanol as it was observed in the current context (Fig. 1c). Similarly, Kaur et al. also did not find difference between response of GPx in thalamus and responses of GPx in other brain regions to aluminum exposure. On the other hand, reports about aluminum-induced increase and decrease in brain GR activities are available. Though, demonstrating aluminum-induced decreases in both GPx and GR of whole brain, it has been suggested that the aluminum exposure might have caused either damage to these enzyme molecules or decline in the mRNA of these enzymes, a significant rise in thalamic GR was observed in response to only aluminum exposure. However, the increment was only insignificant in Al+ animals concomitantly exposed to ethanol (Fig. 1c). Nevertheless, this rise in thalamic GR could be a compensatory cellular adjustment to support the cellular GSH homeostasis and maintain the level of GSH (Fig. 1a) and GPx activity (Fig. 1c). On the other hand, vitamin E supplemented set of groups did not show any significant alterations in thalamic GPx and GR activities (Fig. 1c). Therefore, the α-tocopherol supplementation most likely prevented the compensatory adjustments and made the region susceptible to oxidant threat, as indicated by increased lipid peroxidation in the Al+Et+ group (Fig. 1a).

The notion is also supported by compromised glutathione-independent SPHC, where α-tocopherol supplementation had not only refrained the aluminum-induced alterations in thalamic area, but also reduced the regional performance to the same (Fig. 1d). In terms of glutathione-dependent SPHC, α-tocopherol supplementation had protected the region from aluminum and ethanol induced alterations (Fig. 1d).

Conclusion

Aluminum exposure in the present study, either along with concomitant insult with pro-oxidant (ethanol) or when supplemented with antioxidant (α-tocopherol) was not able to influence the oxidative stress parameters like GSH, lipid peroxidation, activities of SOD, catalase, GPx of thalamic area of rat brain. However, the GR of the particular region was elevated by aluminum exposure alone. On the other hand, thalamic area was sufficiently vulnerable to pro-oxidant (ethanol) and most cases could be protected by α-tocopherol supplementation. Data presented here indicate that aluminum exposure make thalamic area more vulnerable to oxidative stress by reducing its capacity to withstand the oxidant threat. Apparently, α-tocopherol supplementation used in the current study had a protective effect against oxidative stress; in fact, compromised glutathione independent SPHC indicates more susceptibility to oxidant threat only. Therefore, aluminum might augment oxidant threat and hence, vitamin E supplementation should be used against aluminum with caution. The results shown here may be useful for the mechanistic approach of aluminum-induced neurodegenerative changes and strategies to combat it.
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**References**


