Effect of aluminum exposure on superoxide and peroxide handling capacities by liver, kidney, testis and temporal cortex in rat

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Oxidant imbalance is one of the causative mechanisms of aluminum-induced neurotoxicity. In this study, we investigated aluminum-induced oxidant imbalance in non-neuronal tissues (liver, kidney and testis) and temporal cortex in rats. The differences in adaptations to superoxide and peroxide handling capacities (SPHC) of studied organs due to aluminum insult were also evaluated. Male Wistar rats were exposed to aluminum (10 mg/Kg body wt/day) for 4 weeks through orogastric intubation. Liver showed significant decrease in reduced glutathione level, while significant alteration in lipid peroxidation was observed in temporal cortex in aluminium-exposed animals. Superoxide dismutase activity was significantly altered in liver and temporal cortex and catalase activity significantly reduced in the liver due to aluminum exposure, while glutathione reductase and glutathione peroxidase activities were altered in all the tested organs. Among the organs, glutathione-independent SPHC was relatively higher in liver and kidney, while glutathione-dependent SPHC was relatively higher in testis and temporal cortex. As compared to control, aluminum-exposed rats demonstrated reduction in glutathione-dependent SPHC in temporal cortex and increment of the same in testis, while increment in glutathione-independent SPHC was observed in liver. In conclusion, aluminum-induced alteration in oxidant handling capacity could be the cause of oxidative stress both in the neuronal and non-neuronal tissues.

Keywords: Aluminum, Catalase, GPx, GR, GSH, Kidney, Liver, SOD, Superoxide peroxide handling capacity, Temporal cortex, Testis.

Omnipresence of aluminum and its extensive use in contemporary life, supported by common disbelief of ‘biological inertness’, has made aluminum exposure unavoidable. In addition, occupational, environmental, iatrogenic and accidental exposure of aluminum enhances the body burden. Interestingly, patients receiving parenteral nutrition are subjected to unavoidable aluminum burden. Most of the oral aluminum remains unabsorbed and a small fraction exits through bile and urine.

A toxicokinetic study has revealed that liver and kidney are preferred target organs of aluminum along with brain. Aluminum accumulation in liver and kidney after oral dose has been detected using high angle annular dark field scanning electron microscopy. Being metal, aluminum can bind to almost all types of biomolecules and causes toxic effect. In addition to liver and kidney, histopathological studies have demonstrated toxic effects of aluminum in testis. In general, oxidative stress is associated with aluminum-induced neurotoxicity. Being a non-redox metal, the involvement of aluminum in these oxidant imbalances has raised concern.

In the present study, we have investigated the aluminum-induced oxidant imbalance in neuronal (temporal cortex) and non-neuronal tissues (liver, kidney and testis) in rats. The differences in adaptations in superoxide and peroxide handling capacity (SPHC) of the studied organs due to aluminum insult are also evaluated.

Materials and Methods

Materials

Trichloroacetic acid (TCA) (Thomas Baker, Mumbai, India), thiobarbituric acid (TBA), 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB, HiMedia Lab. Pvt. Ltd., Mumbai, India) and 1-chloro-2,4-dinitrobenzene (CDNB Aldrich, Milwaukee, WI) were used. All other chemicals were purchased from Sisco Research Laboratory, India; Sigma Chemical Co., St. Louis, USA and E. Merck, India.

Animals and treatment

Male albino Wistar strain rats (6 weeks old, average body weight of 120 g) were used in the study. The animals were housed in polypropylene cages inside a pathogen-free well ventilated room.
maintained under standard husbandry conditions with regulated temperature (22 ± 2°C) and 12 h light-dark cycle. The animals had free access of standard diet of rat chow and water ad libitum.

The rats were allowed to acclimatize for 2 weeks and divided into two groups: aluminum-treated group (Al\textsubscript{+}): three rats were treated with aluminum (10 mg/kg bw/day) in gum acacia for 4 weeks through orogastric intubation, and control group (Al\textsubscript{0}): two rats were treated with gum acacia during the same period. This dose and duration of aluminum exposure has already demonstrated not to produce oxidative imbalance in cerebrum and cerebellum on its own, but make them vulnerable to exogenous oxidative stressors\textsuperscript{6,7}. The experimental protocol was approved by the Institutional Animal Ethics Committee of NRI Medical College & General Hospital in accordance with the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines.

**Tissue collection and biochemical assay**

At the end of experimental period, rats were sacrificed after overnight fast. Liver, kidneys, testes and brain were removed, washed with ice-cold saline, blotted dry, weighed and immediately transferred to the ice chamber. Tissue samples were homogenized in phosphate buffered saline (PBS) for biochemical estimations. The reduced glutathione (GSH) content and level of thiobarbituric acid reactive substances (TBARS)\textsuperscript{6} and the activities of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) were assayed\textsuperscript{8}.

**Statistical analysis**

Data were analyzed through Mann Whitney U test. Level of significance was evaluated from the table of critical U values of both \(\alpha = 0.05\) and 0.1.

**Results**

The GSH content of non-neuronal tissues decreased, whereas that of neuronal tissue increased in response to aluminum exposure and the change was found to be significant in liver (Table 1). The aluminum-induced alteration in TBARS level was significantly increased in temporal cortex (39.8%) only (Table 1). The SOD activity decreased in all non-neuronal tissues, but elevated significantly in temporal cortex (Fig. 1). The CAT activity altered significantly in liver (21%). The GR activity decreased maximum in testis (22%), while GPx activity decreased maximally in kidney (35%) (Fig. 1); these changes were found insignificant. In aluminum-exposed animals, glutathione-independent SPHC was higher in liver, while glutathione-dependent SPHC was higher in testis and lower in temporal cortex; however, neither of tested organs showed significant alteration in both types of SPHC in comparison to those of control animals (Fig. 2).

**Discussion**

Brain is the highly vulnerable organ for aluminum-induced oxidative stress,\textsuperscript{6,7} oxidative stress in liver and

<table>
<thead>
<tr>
<th>Organs</th>
<th>GSH content (mg of GSH/g wet tissue)</th>
<th>Lipid peroxidation (µmoles of TBARS/g wet tissue)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(\text{Al_0})</td>
<td>(\text{Al_+})</td>
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<tr>
<td>Non-neuronal tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.81 ± 0.05</td>
<td>1.58 ± 0.06*</td>
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<tr>
<td>Kidney</td>
<td>1.42 ± 0.10</td>
<td>1.34 ± 0.04</td>
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<tr>
<td>Testis</td>
<td>1.39 ± 0.13</td>
<td>1.36 ± 0.13</td>
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<tr>
<td>Temporal cortex</td>
<td>1.24 ± 0.09</td>
<td>1.32 ± 0.07</td>
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<tr>
<td></td>
<td></td>
<td>(\text{Al_0})</td>
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<td></td>
<td></td>
<td>13.83 ± 2.18</td>
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<td></td>
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<td>12.96 ± 2.09</td>
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<td>12.77 ± 2.16</td>
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<td>32.27 ± 4.25</td>
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<td>15.01 ± 2.40</td>
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<td></td>
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<td>12.56 ± 2.55</td>
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<td>11.15 ± 2.56</td>
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<td></td>
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<td>45.10 ± 1.98*</td>
</tr>
</tbody>
</table>

* indicates that the calculated lower Ui for \(\text{Al_0}\) and \(\text{Al_+}\) values was smaller than the critical U (\(\alpha = 0.05\)), respectively
kidney could also be crucial. The oxidant levels of these organs are dependent on the SPHC. Like brain, testis is a lipid-rich organ and is protected by blood testis barrier.

Significant lowering of GSH level in liver of aluminum-exposed rats in the present study corroborated with the other findings. Although the earlier study has also found significant decrease in GSH level in kidney of aluminum-exposed rats, no statistically significant reduction was observed in GSH level of kidney and testis in this study. Insignificant reduction in GR activity in liver, kidney and testis (Fig. 1) suggested the possibility of insufficient conversion of GSSG to GSH. Thus, aluminum might cause oxidative stress through consumption of available GSH and/or less formation of GSH by restricting GR in non-neuronal tissues.

Interestingly, temporal cortex showed insignificant rise in both GSH content and GR activity, suggesting better glutathione-mediated handling of oxidative stress in comparison to non-neuronal tissues.

The alteration in cellular redox state is also manifested by lipid peroxidation. The brain tissue is vulnerable to lipid peroxidation due to its composition and metabolic uniqueness, and aluminum-induced TBARS level elevation has been evidenced. Significant rise in lipid peroxidation in liver, kidney and brain has been observed due to aluminum exposure in earlier study. In another study, increase in TBARS level in testis has been reported in 70 day-aluminum exposed rats. However, we observed increased lipid peroxidation in temporal cortex, but not in non-neuronal tissue of aluminum-exposed animals (Table 1).

The SOD activity of all the tested non-neuronal tissues was reduced in aluminum-exposed animals, while significant ($\alpha = 0.05$) increase in SOD activity was observed in brain (Fig. 1). Earlier, reduction in testicular SOD activity has also been found in aluminum-exposed animals. Significant reduction in CAT and SOD activities in the liver and kidneys of aluminum-exposed rats in our study (Fig. 1) was in agreement with earlier findings. These observations also emphasized the tissue-specific responses towards aluminum-induced oxidant imbalance in neuronal and other tissues.

Hydrogen peroxide produced from the dismutation activity of SOD is neutralized either by GSH-independent CAT or by GSH-dependent GPx. Insignificant reduction in GPx activity of temporal cortex of aluminum-exposed animals in this study was in agreement with already reported observations. We also observed insignificant reduction in GPx activity in liver and kidney in aluminum-exposed animals (Fig. 1). This suggested oxidant imbalance in the organs due to aluminum exposure which was manifested by impact at different levels of cellular antioxidative mechanisms. Insignificant increase of both CAT and GPx activities in testis of aluminum exposed rats reconfirmed the differential response in non-neuronal tissues to aluminum, which might be due to specified accumulation preference of aluminum in these organs.

A rough estimation of tissue capability to cope with the oxidative stress can be made by the ratio of CAT and SOD activities, while, GPx/SOD ratio indicates resistance to oxidative damage in response to chemical stress. Both these ratios are indicator of tissue capability to oppose the oxidant imbalance; however, GPx/SOD depends on GSH to defy the threats of peroxide and superoxide, even as CAT/SOD do the same without depending on GSH. Among the tested organs, liver and kidney demonstrated relatively higher glutathione-independent SPHC, whereas glutathione-dependent SPHC was higher in testis and temporal cortex. In the current study, no significant SPHC alteration was observed due to aluminum exposure (Fig. 2). Nevertheless, aluminum-treated animals exhibited prominent increase in glutathione-independent SPHC in liver, increase in glutathione-dependent SPHC in testis and decrease in glutathione-dependent SPHC in temporal cortex. These observations suggested organ-wise differences in aluminum-induced alterations in SPHC.

All the tested organs were facing oxidant imbalance because of aluminum exposure and the response of these organs towards handling stress was...
not uniform. Like that of cerebrum and cerebellum, non-neuronal tissues may also show differences in oxidative vulnerability. Though the small sample size was in agreement with the calculated number of animals based on earlier study, non-parametric test was used for statistical calculation to entail the significance of the study. However, authoritative inference can be derived using more number of animals.

In conclusion, the study demonstrated possible non-neuronal aluminum toxicity due to oxidant imbalance. The differential response of non-neuronal tissues (liver, kidney and testis) to aluminum-induced oxidative stress might be due to inconsistent alteration in oxidant handling capacity.

Acknowledgement

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References