Aluminium related changes in brain histology: Protection by calcium and nifedipine

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Aluminium (Al; 50 mg AlCl₃/kg body wt/day) treatment caused a marked change in histological picture of normal brain as indicated by an increased number of vacuolated spaces. These changes returned to normal partially by simultaneous treatment with nifedipine (0.7 mg/kg body wt/day) and completely by similar treatment with 50 ppm calcium (CaCl₂; 12.5 mg/kg body wt/day). Neither nifedipine nor calcium treatment alone altered the normal histological condition. The histological changes could not be correlated with the decrease in calcineurin activities in brain as nifedipine decreases calcineurin activity without any histological changes. Hence the histological changes may be considered as specific for Al and not due to a general decrease in calcineurin activity.

Experimentally aluminium (Al) is a well established neurotoxic agent. Application of alumina gel to cerebral cortex induces epileptic foci. Al has also been associated with several neurodegenerative disorders notably Alzheimer’s disease (AD) and dialysis encephalopathy. Brain Al level has been reported to be in the range of 30-250 μM in these disorders whereas, in control subjects it is around 16 μM only and it is likely that the localised concentration is even higher. As reviewed by Peri, normal brain Al level is 2 μg/g dry wt and it is increased by 1.4 fold in AD and depositions is mainly found in the hippocampal region. Al has been found to be concentrated in the neurofibrillar tangles and amyloid plaques, two of the hallmarks of AD. In Guam and Kii islands, it has been observed that a number of people were suffering from a disease known as amyotrophic lateral sclerosis (ALS), which was associated with high Al and less Ca content in drinking water. These patients showed 3-4 times higher concentrations of Al in their tangle bearing neurons of hippocampus than the tangle-free Guamanian control patients.

As suggested by Jope and Johnson, altered Ca²⁺ homeostasis may be one of the mechanisms of Al neurotoxicity. Intracellular Ca²⁺ is a trigger for long term potentiation (LTP) and Al has been found to inhibit tetraethyl ammonium (TEA)- induced LTP in hippocampal slices of male Wistar rats. This observation supports the hypothesis that Al interferes mainly with the Ca²⁺ homeostasis in neurons and Ca²⁺ dependent processes underlying neuronal plasticity.

Al toxicity is however, accompanied by increase in total Ca level in brain and liver. Previously it was reported that 50 ppm CaCl₂ in drinking water can provide protection against Al toxicity in liver. It has been reported that Serine/Threonine phosphoprotein phosphatase 2B (PP2B) or calcineurin is inhibited by oral Al treatment of 50mg/kg body wt/day.

The aim of the present study is to find out whether there is any relationship between calcineurin inhibition and histopathological changes in brain after AlCl₃ oral treatment.

Animal treatment—Male Sprague Dawley rats (48) were maintained with ad libitum food and water in 12 hr L:D cycle. Rats were divided into 6 groups with 8 animals each. Group 1 was the control group treated with distilled water as drinking water. Group 2 was given AlCl₃ at a dose of 50 mg/kg body wt/day. Group 3 rats were treated with 50 ppm CaCl₂ (12.5 mg/kg body wt/day) along with the same dose of AlCl₃ and group 4 was given only 50 ppm CaCl₂. Group 5 rats were given an L type Ca²⁺ channel blocker, nifedipine (a dihydropyridine compound) at a dose of 0.7 mg/kg body wt/day simultaneously with AlCl₃. Group 6 rats were treated with the same dose of nifedipine only. The drugs were given orally and the doses of the drugs were chosen as per prescription for a 70 kg adult human being. All the groups were treated for 40 days. After 40 days of treatment the rats were sacrificed and their whole brains were collected at 0°C.

Histology of brain—Brains (cerebral cortex) were fixed in 10% formaldehyde-saline and embedded in paraffin after gradual dehydration with graded concentrations of alcohol (50, 70, 90 and 100%),
xylene and molten paraffin. Sections (5 μm thick) were cut and stained with hematoxylin and eosin.

Normal histological picture of inner layer of cerebral cortex shows larger polymorphic neuronal and sharply demarkated smaller neuroglial nuclei (Fig. 1). In the polymorphic cells, nucleus is surrounded by clear space. In the neuroglial cells, cytoplasm is not distinguishable and the nucleus is naked. Aluminium treatment (Fig. 2) resulted in an increase in number of vacuolated spaces in the matrix which could be due to disintegration or decrease of polymorphic cell nuclei. Neuroglial nuclei outlines get indistinct.

In the rats of group 3 (50 ppm CaCl₂ along with AlCl₃) the neuroglial nuclei outlines become distinct and the polymorphic cell nuclei look similar to the

Figs1-5—Histological observation of brain slices of rats treated for 40 days, orally with, 1—distilled water, (i) larger polymorphic neuronal cells: nucleus is surrounded by clear space; (ii) smaller neuroglial nuclei: cytoplasm is not distinguishable and nucleus is naked; 2—AlCl₃ (50 mg/kg body wt/day), (i) vacuolated space in matrix: due to disintegration of polymorphic cell nuclei; (ii) neuroglial nuclei outlines get indistinct; 3—AlCl₃ and 50 ppm CaCl₂, (i) control like polymorphic cell nuclei; (ii) distinct neuroglial nuclei outline; 4—AlCl₃ and nifedipine (0.7 mg/kg body wt/day) (i) and (ii) control like and disintegrated polymorphic cells respectively (ii) unchanged neuroglial nuclei; 5—nifedipine only (i) control like polymorphic cells and (ii) neuroglial cells. Figs1-5: E&H x 250.
Table I—Serine/threonine phosphoprotein phosphatase activity in Sprague Dawley rat brain after 40 days of treatment with AlCl₃ (50 mg/kg body wt/day), CaCl₂ (50 ppm) and nifedipine (0.7 mg/kg body wt/day).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of specific activity (µg Pi/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00 ± 5.38</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>30.74 ± 0.73³</td>
</tr>
<tr>
<td>AlCl₃ + nifedipine</td>
<td>129.59 ± 7.97</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>35.12 ± 4.58</td>
</tr>
<tr>
<td>AlCl₃ + CaCl₂</td>
<td>132.07 ± 5.79b</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>156.56 ± 13.04</td>
</tr>
</tbody>
</table>

*P values: *<0.05; ³<0.01; ⁵<0.001

The results of ANOVA are in accordance with Student's t test.

The histological and biochemical results of the present study are in agreement with each other for the rats treated with only AlCl₃, only 50 ppm CaCl₂ and simultaneously with AlCl₃ and 50 ppm CaCl₂. Nifedipine, being a Ca²⁺ channel blocker, reduced the calcineurin activity but maintained the normal histological condition. This observation suggests that the mere decrease in calcineurin activity caused by the reduction of cytosolic Ca²⁺ concentration is not responsible for the histological changes. The data also suggest that due to the presence of Al, the calcineurin activity may be affected by direct binding with Al. The histological changes may considered to be specific for Al treatment only and not due to a general decrease in calcineurin activity.

References