Influence of aluminium on neurotoxicity of lead in adult male albino rats

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Influence of aluminium on neurotoxicity of lead was studied in male albino rats. Aluminium enhanced the net deposition of lead in brain. This was further substantiated by higher levels of malondialdehyde (MDA), and lower activities of acetylcholinesterase enzyme in the brain homogenates of the rats treated with both lead and aluminium as compared to those of rats treated with lead only. In lead plus aluminium treated animals, a significant neurological deficit was observed when the animals were subjected to rota-rod, traction performance (TP) and tail immersion tests.

Keywords: Acetylcholinesterase, Aluminium, Brain, Lead toxicity, Malondialdehyde, Neurotoxicity

Lead, an environmental and occupational pollutant is reported to affect the nervous system both in animals and human beings. Investigators have put forth several mechanisms to account for neurotoxic effects of lead which include (a) inhibition of acetylcholinesterase enzyme activities of brain, (b) decrease in local concentration of trace minerals like copper, iron and zinc, (c) inhibition of membrane associated enzyme, Na⁺-K⁺-ATPase, (d) induction of lipid peroxidation in nervous tissue, and (e) alteration of calcium dependent systems.

Lead has extensively been studied for its interaction with a number of important elements and it has been revealed that calcium, iron and zinc when given along with lead, reduce the toxicity and retention of lead. Copper and vitamin D have been reported to enhance the overall toxicity and retention of lead. Momensin, an ionophore antibiotic, also potentiates the toxic effects of lead. A number of compounds have been reported to affect the neurotoxic effects of lead in one or other way, but there is no report concerning the influence of aluminium on neurotoxicity of lead. Since aluminium is extensively used in food processing and storage, and in pharmaceuticals mainly as antacids and phosphate binders, the chances of simultaneous exposure to lead and aluminium have become inevitable. Therefore, the present study was conducted to explore the influence of aluminium, if any, on neurotoxic effects of lead in male albino rats.

Materials and Methods

Twenty-four colony bred male albino rats (60 days old; weighing 75-85 g) were procured from the Laboratory Animal Resource Section of the Institute. The animals were maintained at 23°±2°C under 12 hr light/dark cycle and given water and food ad libitum. The feed was prepared by mixing wheat mash : 62 parts, maize mash : 30 parts, salt and mineral mixture: 0.5 parts of each, followed by boiling to form feed of soft consistency. In addition cow milk was added to feed @4 ml per rat. The animals were acclimatized to the laboratory conditions for two weeks and thereafter randomly grouped into four groups of six animals each. Principles of animal handling were strictly adhered to and the handling of the animals was made under the supervision of the animal ethics committee of the Institute. Lead acetate and aluminium chloride were procured from Qualigens Fine Chemicals, Mumbai, India, which were 99% pure. After acclimatization, the rats were exposed to lead acetate (2.5% solution in water) and/or aluminium chloride (2% solution in water) or de-ionized water only. The doses administered were selected on the basis of a separate acute toxicity study carried out in male albino rats earlier.

Neurotoxicity induced by the lead was evaluated by testing the integrity of sensory and motor functions of the experimental rats on day 45 and 90 of their treatment. For evaluating the motor functions, during the period of acclimatization, all the animals were trained to balance on rota-rod revolving (16 rpm). They were subjected to forced locomotor activity (FLA) on day 45 and 90 of treatment and time of stay on rotating rod was recorded. The time of 3 min was taken as cut-off point in this experiment to record the fraction of animals that were exhibiting motor deficit.

The animals were also subjected to traction performance. A stainless steel bar (2 mm diam) with
horizontal length of 10 cm was placed about 30 cm above the floor level. The experimental rats were kept in such a manner that their fore-paws gripped the horizontal bar. Hanging time of animals was recorded.

Sensory evaluation was done by recording the reaction of rats to tail-immersion in hot water. Rats were held in a position so as to allow their tail (5 cm) immersed in hot water (55°C±0.5°C) in a beaker. Time taken to withdraw the tail clearly out of water was recorded.

On day 90 of treatment, the animals were sacrificed under ether anaesthesia. Brain of each animal was collected, washed with normal saline, blotted dry and then weighed. About 500 mg of nervous tissue was used for determination of acetylcholinesterase enzyme (AChE) and lipid peroxidation product as malondialdehyde formed. AChE activity was expressed as nmole substrate hydrolysed hr⁻¹ g⁻¹ nervous tissue. Lipid peroxidation product was expressed as nmole malondialdehyde (MDA) formed hr⁻¹ g⁻¹ nervous tissue.

Data was subjected to correlation analysis.

Results
On day 45 and 90, treated rats exhibited a significant reduction in FLA (Table 1) as compared to controls. Proportion of animals with reduced FLA was also significantly higher as compared to controls.

A significant reduction in the traction performance (TP) was observed in rats treated with both lead acetate and aluminium chloride after 90 days of treatment (Table 2).

Reaction time to tail immersion of different treatment groups has been summarized in Table 2. Only lead plus aluminium treated rats exhibited a significant increase in reaction time to tail immersion on day 45 (Table 2). On day 90, the rats treated with lead acetate also exhibited a significant increase in reaction time to tail immersion as compared to controls. Significantly higher concentration of lead (5.32±1.0 µg nervous tissue) was observed in the animals that had been treated with both lead acetate and aluminium chloride over a period of 90 days (P<0.05). Lead concentration (4.16±0.53 µg/g nervous tissue) in the brain of animals that had been treated with lead acetate only was not statistically different from that of control animals (2.16±0.29 µg/g nervous tissue). No significant difference in the relative brain weights of treated rats was observed when compared with controls (Table 3).

The animals treated with aluminium chloride or lead acetate plus aluminium chloride exhibited a significant reduction in AChE activities (Table 4). MDA levels in the nervous tissue of the animals treated with lead acetate alone or lead plus aluminium chloride showed a significant increase compared to controls (Table 4). However, MDA levels were significantly higher in lead plus aluminium treated animals as compared to animals treated with lead acetate only. Correlation analysis revealed a negative correlation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time spent on rota-rod on 45th day of treatment (sec)</th>
<th>No. of animals with reduced FLA</th>
<th>Time spent on rota-rod on 90th day of treatment (sec)</th>
<th>No. of animals with reduced FLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>180.03 ± 0.03</td>
<td>0</td>
<td>180.33 ± 0.33</td>
<td>0</td>
</tr>
<tr>
<td>Al</td>
<td>149.33 ± 21.99</td>
<td>4*</td>
<td>43.10 ± 19.76***</td>
<td>4*</td>
</tr>
<tr>
<td>Pb</td>
<td>140.45 ± 20.95</td>
<td>4*</td>
<td>123.03 ± 25.06*</td>
<td>5**</td>
</tr>
<tr>
<td>Pb + Al</td>
<td>73.87 ± 23.44**</td>
<td>5**</td>
<td>85.22 ± 19.28**</td>
<td>5**</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; and ***P<0.001. For comparing the proportions, Chi²-test was used.
between lead content and AChE activity \((r=-0.74;\ d.f.=24-2; \ P<0.01)\) of nervous tissue. Similarly, lead content in nervous tissue of different treatment groups was positively correlated with higher MDA levels \((r=+0.98;\ d.f.=24-2, \ P<0.01)\) as observed in brain of the treated animals.

**Discussion**

Rationale behind application of 125mg/kg body weight dose level for lead acetate and 50 mg/kg for aluminium chloride was that these dose levels provide almost equal number of molecules \((2.3 \times 10^{20})\). Selection of these doses has been established based on acute toxicity study as reported earlier\(^{16}\). Sub-lethal doses of lead are reported to reduce FLA because a significant reduction in the swimming performance has been observed in rats earlier\(^{22}\). We have observed that lead-induced reduction in FLA was time dependent. This was because the treatment continued from day 45 to 90, and as a result there was a significant reduction in time of stay of lead treated rats at rota rod, even though the proportion of the affected rats on day 45 of treatment was significantly higher compared to controls. It may be suggested that maximum body burden (steady state) of lead was attained under long-term exposure and the effects described were therefore, associated with body burden rather than daily intake. With lead plus aluminium treatment time spent on the rotarod was significantly reduced only after 45 days of treatment. This indicated that levels of lead that affected FLA were attained quite earlier in the presence of aluminium than when the lead was used alone. This was confirmed in the present study by observing significantly higher levels of lead in brain of those animals that were given lead plus aluminium for a period of 90 days. On the contrary, the animals treated with lead alone did not exhibit higher content of lead in the brain compared to control rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Traction performance (sec)</th>
<th>Reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.0±5.2</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>Al</td>
<td>37.3±4.8</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Pb</td>
<td>26.7±4.9</td>
<td>3.1±0.4</td>
</tr>
<tr>
<td>Pb+Al</td>
<td>32.5±6.4</td>
<td>3.8±0.3**</td>
</tr>
</tbody>
</table>

*\(P<0.05\) and **\(P<0.01\); comparison with respect to control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Relative brain wt. (g/100 g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>De-ionized water only</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>Al</td>
<td>100</td>
<td>0.60±0.03</td>
</tr>
<tr>
<td>Pb</td>
<td>125</td>
<td>0.67±0.08</td>
</tr>
<tr>
<td>Pb+Al</td>
<td>125</td>
<td>0.64±0.06</td>
</tr>
</tbody>
</table>

**Table 2 — Traction performance and reaction time (tail-immersion test) of male albino rats treated with lead acetate (Pb) and aluminium chloride (Al) over a period of 90 days**

**Table 3 — Relative brain weights of the male albino rats treated with lead acetate (Pb) and/or aluminium chloride (Al) over a period of 90 days**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AChE activity (µmole acetyl thiocohline hydrolysed/hr/g nervous tissue)</th>
<th>LPO product (nmole malondialdehyde formed/g nervous tissue/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.46±9.01</td>
<td>27.26±0.92</td>
</tr>
<tr>
<td>Al</td>
<td>69.63±4.23*</td>
<td>27.56±0.67</td>
</tr>
<tr>
<td>Pb</td>
<td>73.97±4.83</td>
<td>36.20±1.37**</td>
</tr>
<tr>
<td>Pb+Al</td>
<td>58.74±3.82*</td>
<td>40.60±1.43**</td>
</tr>
<tr>
<td>F value</td>
<td>4.735</td>
<td>33.35</td>
</tr>
</tbody>
</table>

*\(P<0.05\) and **\(P<0.01\); comparison with respect to control. \(P<0.05\) for a v/tr. b.

Lead and aluminium when given alone did not affect TP of rats. Such results have also been reported earlier\(^{18}\) wherein one drug has been indicated to affect FLA or TP efficiently than other. This observation may be attributed to the fact that threshold level for affecting FLA may be different from the threshold level that affects TP. Since TP is also dependent on state of muscle relaxation, the effect of two metal radicals in the periphery could not be ruled out.

After 45 days of treatment, reaction time to noxious thermal stimuli was significantly increased in the
animals treated with both lead plus aluminium. When the treatment continued from day 45 to 90, lead treated animals also exhibited a significant increase in their reaction time to tail immersion. This further supported our statement that the threshold level for affecting the sensory or motor functions were attained by lead quite earlier in the presence of aluminium. Lead is known to affect the response latencies to noxious stimuli by involving mu-opioid receptor system which is more vulnerable to lead toxicosis during adult life.\textsuperscript{23-24} It also appeared that threshold level of lead that effectively reduced TP was attained in presence of aluminium over a period of 90 days. It is also known that lead changes K\textsuperscript{+} level in neurons and interferes with the depolarization of nerve endings. This results in change in nerve conduction velocities which is responsible for altered reaction to noxious stimuli under the condition of lead intoxication.\textsuperscript{25}

Lead treatment only did not depress AChE activities, but concomitant administration of lead plus aluminium resulted in significant decline in AChE activities of brain. Similarly, lipid peroxidative damage was quite higher in lead plus aluminium treated animals, as was indicated by formation of significantly higher concentration of malondialdehyde in the nervous tissue of treated group compared to that of controls or only lead treated animals. Since correlation analysis revealed a negative correlation between lead and AChE activities in brain, and a positive correlation between lead and malondialdehyde in the nervous tissue, it implied that observed effects were due to lead content that accumulated in brain. Malondialdehyde measurement serves as an index of brain damage induced by lead ions through the process of lipid peroxidation.\textsuperscript{26} It was thus clear that more brain damage occurred by lead in presence of aluminium.

From this study, it could be concluded that aluminium chloride enhanced attenuation of higher concentration of lead in brain, probably because of one of the following reasons, (a) that aluminium altered the permeability of blood brain barrier to either lead or carrier biomolecule of lead, as aluminium is known to alter the permeability of blood-brain-barrier to some biomolecules like prolactin, cortisol, growth hormone, rat utilizing hormone and thyroxine by directly affecting its integrity,\textsuperscript{27} and (b) persistent exposure to aluminium might have reduced the net absorption of phosphates from gut with resultant mobilization of phosphorus from the skeletal stores towards blood. Since lead gets deposited in bones as lead phosphate, mobilization of phosphorus from bones might have also mobilized lead towards blood and then to other soft tissues. Thus, increase in lead concentration of brain achieved in the presence of aluminium ultimately resulted in reduced AChE activities in brain and increased lipid peroxidative damage, with resultant decline in the motor performance or sensory perception.

References