Estimation of Brain Lead Content Levels in Lead Induced Swiss Albino Mice Pups

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ABSTRACT

Lead is ubiquitous in our environment but has no physiologic role in biological systems. The present study was undertaken to estimate the Lead content in mice brain. GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders and lead because of its cumulative property it is capable of exerting toxic effects at any level of exposure. Random breed Swiss albino mice were used for the present study. Sexually mature male and females weighing 25-30 gm were put in breeding cages in the ratio of 2:1 (6 female: 3 male) and were provided standard diet and water ad libitum. The cages were checked every day in the morning and females showing vaginal plug were isolated. The pregnant female was housed in an individual cage and was started on diet containing 4.5% lead nitrate and lead acetate trihydrate respectively along water ad libitum. An animal model of lead poisoning was developed in which suckling mice were exposed to lead nitrate and lead acetate trihydrate from birth indirectly through their mothers and then directly after weaning. Comparable litters that received normal standard diet and water ad libitum were studied concurrently as controls. Estimation of lead content in mice offspring was measured on 30 days of age. Estimation of lead was carried out by flameless atomic absorption spectroscopy. All the experimental work was approved by the Institutional Animal Ethics Committee (Ref. No. IAEC/257). There was 87-fold increase (ppm dry weight) in the Cerebral Cortex lead and 83-fold increase (ppm dry weight) in the Cerebellum lead. This was similar to the concentration of lead in brain reported in six cases of human pediatric lead encephalopathy leading to death.

Keywords: Brain Lead Content, Lead nitrate, Encephalopathy, Plumbism.

INTRODUCTION

It has been known since ancient times that lead may cause poisoning in man1, but only within this century have extensive studies of the problem been focused. Lead is ubiquitous in our environment but has no physiologic role in biological systems. Its effects are pervasive yet often subtle, with consequences ranging from cognitive impairment in children to peripheral neuropathy in adults. Exposure of lead can take place either through inhalation of dust, fumes, vapors2, or ingestion of contaminated foods or drinks. Because of its cumulative property it is capable of exerting toxic effects at any level of exposure. Toxic effect of lead on the body is known as Plumbism. The brain is exceptionally sensitive to the effects of lead poisoning3, and it is the young-from birth to about 7 years of age who show the most serious brain damage following lead poisoning. The clinical manifestations of lead poisoning are well defined and include headache, incoordination, tremor, twitching, convulsion, paralysis, coma and death5.

In behavioral neurosciences such as neurobiology and biopsychology, animal models enable investigation of brain–behavior relations, with the aim of gaining insight into human behavior and its underlying neuronal and neuroendocrinological processes. Species with behavioral or psychological repertoires similar to humans so that the results of experiments with these animal models may throw light on seemingly related behavior in human beings3–4. Thus, whereas animals are in most instances intended as model of humans, one animal species may also serve as model organism for another species. Studies, however, which explicitly compared behavior across species, are rare in neuroscience5.

MATERIALS AND METHODS

Animals

Swiss Albino mice of either sex (young, age 10-12 weeks, 20-25g) were used for the study. Animals were housed in polypropylene cages and maintained under standard laboratory environmental conditions; temperature 25 ± 2°C, 12h dark cycle and 50±15 relative humidity with free access to food and water ad libitum. Animals were acclimatized to laboratory condition before the test. Each group consisted of six (n=6) animals. All the experiments were carried out during light period (08:00-16:00). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional Animal Ethical Committee of Banasthali University, Rajasthan approved the protocol of the study (Ref. No.IAEC/257).

Development of Model

Random breed Swiss albino mice were used for the present study. Sexually mature male and females weighing 25-30 gm were put in breeding cages in the ratio of 2:1 (6 female: 3 male) and were provided standard diet and water ad libitum. The cages were checked every day in the morning and females showing vaginal plug were isolated. The pregnant female was housed in an individual cage and was started on diet containing 4.5% lead nitrate and water ad libitum. An animal model of lead poisoning...
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**Lead Analysis**

In present study, tissue and biological fluids were used without additional processing other than as described below. Estimation of lead was carried out by flameless atomic absorption spectroscopy with a Perkin-Elmer model 403AA fitted with a Heated Graphite Atomizer (HGA-70). The volume to be analyzed was injected into the carbon rod and the oven programmed for 80sec drying time at 600°C, 3min ashing at 4700°C, and 16 sec atomization at 1560°C. The lead lamp was a high intensity lamp, operated at 8MA with a slit opening of 3 mm, at wavelength 3833 in the ultraviolet band, with a final attenuation at 0.25. New standard curves, ranging from 0.3 to 0.9ng of lead, were made for each new set of assays. The concentration of lead in each test sample was estimated from the standard curve.

**Statistical analysis**

Values are expressed as mean ± SEM from 6 animals. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Tukey-kramer test. p<0.05 was considered significant.

**RESULTS AND CONCLUSION**

The content of lead in the cerebellum and cerebrum of 30 day-old animals is presented in Table 1. There was an 82.85-fold increase (ppm dry weight) in the cerebellum lead and an 87.25 fold increase in lead of the cerebral cortex. Since the cerebellum and cerebral cortex contain 82% water the wet tissue concentrations of lead are about 12.53 ppm and 5.98 ppm, respectively. This was similar to the concentration of lead in brain reported in six cases of human pediatric lead encephalopathy leading to death (12-27 ppm wet weight).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Condition</th>
<th>Dry Weight (mg)</th>
<th>Concentration (ppm)</th>
<th>Poisoned (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral Cortex</td>
<td>Control</td>
<td>218.75 ± 10.71</td>
<td>0.37 ± 0.11</td>
<td>32.23 ± 3.39</td>
</tr>
<tr>
<td></td>
<td>Poisoned</td>
<td>188.60 ± 10.07</td>
<td>0.83 ± 1.11</td>
<td>68.57 ± 22.77</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Control</td>
<td>43.99 ± 4.20</td>
<td>0.83 ± 1.11</td>
<td>68.57 ± 22.77</td>
</tr>
<tr>
<td></td>
<td>Poisoned</td>
<td>38.35 ± 1.21</td>
<td>0.83 ± 1.11</td>
<td>68.57 ± 22.77</td>
</tr>
</tbody>
</table>

*Nourished by mothers on normal diet (control) and diet containing 4.5% lead nitrate (poisoned). Each value is the mean and standard deviation of tissue samples from six mice.

**REFERENCES**