Research Article

Treatment with vitamin C and metal chelators reverses lead-induced DNA damage in liver and brain tissues of albino rats

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ABSTRACT: Lead toxicity resulting from occupational or environmental exposure has long been treated with chelating agents and/or antioxidants. The ameliorative effects of these chelators on the genotoxicity of lead and possible damage on the DNA are yet to be fully investigated. In this study, the efficacy of conventional chelators (namely; 2, 3-mesodimecarpsuccinic acid (DMSA), D-Penicillamine and Calcium disodium ethylene di amine tetra acetate (CaNa$_2$EDTA) with vitamin C were compared in the tissues of experimental rats exposed to lead. Twenty four hours after the last treatment, animals were sacrificed under anaesthesia, the brain and the liver of the animals were then harvested, mopped dry and stored on ice. Quantitative DNA fragmentation was determined spectrophotometrically using the diphenylamine reaction. Result showed a 4-fold increase in the percentage damage observed in the brain of animals administered lead for 12 weeks, with a 2-fold increase observed in the liver over the control group. Treatment with Vitamin c and CaNa$_2$EDTA for 5 days and 10 days significantly reduced the fragmentation percentage in the liver. While CaNa$_2$EDTA seem to have no significant ameliorative effect in the brain tissue. Treatment with D-Penicillamine also showed significant ameliorative effect in both tissues. Ten days treatment with succimer however proved more effective than the 5 days treatment. The results of this experiment did suggest a gradual reversal in oxidative DNA damage following withdrawal from exposure and the most effective treatment observed with vitamin c in both tissues.

KEYWORDS: Lead toxicity; DNA damage; Vitamin C; Metal chelators
INTRODUCTION

Lead is a potent neurotoxin that accumulates in soft tissues and bone over time; interferes with a variety of body processes and it is toxic to many organs and tissues including the heart, bones, intestines, kidney, spleen, brain reproductive and nervous systems (Gurer and Ercal, 2000). Many heavy metals, including lead, are known to induce over-production of reactive oxygen species (ROS), decrease availability of antioxidant reserves to respond to the resultant damage and consequently enhance lipid peroxidation (Maleacka et al., 2001).

Lead damages cellular materials, alters cellular genetics and it interferes with bio-elements, whose role is critical for several physiological processes. While the toxic effects of lead have been recognized for millennia, it has remained a significant public health concern due to its continued use and toxicological potential. Lead is still widely used in many industrial processes and it is very persistent in the environment. Although toxic effects caused by occupational exposure to lead have been extensively studied, there are still conflicting results regarding its genotoxicity. Occupational lead poisoning is presently becoming the most common disease of environmental origin, which is increasing very rapidly in developing countries (Porru and Alessio, 1996; Ademuyiwa et al., 2007).

Chelators have long been used in the treatment of lead poisoning and various chelators are prescribed according to the blood lead concentration of the patient. The therapeutic approach is to increase the excretion of lead by chelation (Gurer and Ercal, 2000). The chelating agents for treating lead poisoning include Ethylene diaminetetraacetic acid (EDTA), which is injected intravenously, Succimer and D-Penicillamine which are administered orally (Menkes, 2006).

The possible adverse effects of these conventional chelators on DNA are yet to be explored. Ability to bind plasma protein, precipitate encephalopathy, cause proteinuria and hematuria are some of the shortcomings, which have received wide publications, of the conventional chelators. A perusal of literature shows that possible genotoxic effects of these conventional chelators have not received attention. Studies on the genotoxic effects of lead poisoning using nonconventional markers are limited and controversial (Vaglenov et al., 2001; Fracasso et al., 2002). It is therefore expedient to determine the extent of damage that lead and possibly the chelators can cause to the genetic system using nonconventional biomarkers as indicators and seek to know the most effective ameliorative agent that is capable of reducing or reversing these damages with minimal adverse effect, if any.

MATERIALS AND METHODS

Experimental Design

A total of 110 albino rats were broadly divided into 3 groups. Control (n=15), administered normal saline for 12 weeks, lead groups (n=55), were administered 150mg/Kg body weight lead for 12 weeks and the treatment group (n=40). The control group and a part of lead group (L1) (n=15) were subdivided into 3 groups (12 weeks, 12 weeks and 5 days, 12 weeks and 10 days). The remaining group (L2) was divided into 8 groups (n=5) and were treated with either succimer or D-Penicillamine (oral: 30 mg/kg body weight); vitamin C (oral: 150 mg/Kg body weight) or CaNa2EDTA (IV: 80 mg/Kg body weight) in two divided doses of 5 days each. The treatment group was subdivided into 8 groups (n=5) and were treated as control for the L2 group.

Quantitative DNA Fragmentation Assay in Tissues

DNA fragmentation was quantitatively determined using the diphenylamine reagent. This method was introduced by Burton in 1955. This assay is based on the reaction of DNA with diphenylamine. When DNA is treated with diphenylamine in acidic conditions, a blue compound is formed with the sharp observation maximum at 595 nm. Typically, a 10% tissue homogenate was prepared by homogenizing 0.2 g of the tissue in 1.8 ml of ice-cold lysis buffer using a power driven Teflon pestle in a glass homogenizing cup maintained at 4°C (Model RQ- 127A: RPM 8000: Clearance 2.5mm; REMI Motors Ltd: Mumbai, India). The homogenate was then centrifuged for 15 minutes at 6000 rpm. The supernatant was then transferred to a labelled conical glass tube. To the pellet in the microcentrifuge tube was added 0.65 ml of 5% (w/v) TCA and to the supernatant in the conical glass, 1.5 ml of 10% (w/v) TCA was added. Both samples were precipitated overnight at 4 °C. The supernatant in the conical glass was then centrifuged for 10 minutes at 2500 g at room temperature. The supernatant was then removed and 0.65 ml of 5% (w/v) TCA was added to the pellet. Two blank tubes with 0.65 ml of 5 ml of 5% (w/v) TCA each were prepared and treated with the rest of the samples.

A hole was made through the top of each microcentrifuge tube and each conical tube was covered with marble. Both sets of tubes were boiled for 15 minutes in 100 °C water bath. The tubes were cooled to room temperature and centrifuged for 5 minutes at 2500 g at that temperature. Each supernatant (0.25 ml) (from both glass and microcentrifuge tubes) were transferred to labelled round bottom glass tubes. Diphenylamine reagent (0.5 ml) was added to each tube and incubated for ≥ 4 hours at 37 °C with foil covering each tube. Absorbance was read at 600 nm in a spectrophotometer.
Percentage fragmented DNA is expressed as:
\[(As/As+p) \times 100\]

where \(As\) is Absorbance of the supernatant),
\(As+p\) is Absorbance of Supernatant + Pellet

**Statistical Analysis**

The results obtained are expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by turkey’s multiple range test was used to analyse the results. Values with \(p<0.05\) were regarded as being significant, using the Statistical Package for Social Sciences (SPSS) version 17.0.

**RESULTS AND DISCUSSION**

Lead has been known to be an environmental pollutant and its toxicity has also been associated with health hazards (Patrick, 2006). The liver acts as chief player in detoxification process and is one of the target organs affected by lead toxicity owing to its storage in the liver (Shalan et al., 2005). In this study a significant increase is observed in the percentage DNA damage in the liver and brain of lead treated groups and it is observed that there was a gradual decrease in the damage in both tissue following withdrawal from exposure. The role played by natural compounds in the modulation of the toxic effects of lead is little known.

In this study, we examined the effect of vitamin C treatment on lead toxicity as measured via DNA fragmentation in tissues of rats. Our findings demonstrate the efficacy of vitamin C in treating lead toxicity. The complete data set from this investigation are shown in Tables 1 and 2, and further simplified in graphical forms in Figures 1-8. Treatment with vitamin C and CaNa₂EDTA for 5 days and 10 days significantly decreased the percentage DNA fragmentation effectively in the liver and brain. However, treatment for 10 days was not significantly different from the pattern observed in 5 days treatment in the brain.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Vit C Liver</th>
<th>DPA Liver</th>
<th>DMSA Liver</th>
<th>CaNa₂EDTA Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control 12 weeks</td>
<td>47.134 ± 4.41*</td>
<td>47.134 ± 4.41*</td>
<td>47.134 ± 4.41*</td>
<td>47.134 ± 4.41*</td>
</tr>
<tr>
<td>II</td>
<td>Normal Control 12 weeks 5 days</td>
<td>43.068 ± 1.08*</td>
<td>43.068 ± 1.08*</td>
<td>43.068 ± 1.08*</td>
<td>43.068 ± 1.08*</td>
</tr>
<tr>
<td>III</td>
<td>Normal control 12 weeks 10 days</td>
<td>43.681 ± 1.93*</td>
<td>43.681 ± 1.93*</td>
<td>43.681 ± 1.93*</td>
<td>43.681 ± 1.93*</td>
</tr>
<tr>
<td>IV</td>
<td>Lead 12 weeks</td>
<td>79.464 ± 3.32*</td>
<td>79.464 ± 3.32*</td>
<td>79.464 ± 3.32*</td>
<td>79.464 ± 3.32*</td>
</tr>
<tr>
<td>V</td>
<td>Lead 12 weeks 5 days</td>
<td>69.511 ± 5.45*</td>
<td>69.511 ± 5.45*</td>
<td>69.511 ± 5.45*</td>
<td>69.511 ± 5.45*</td>
</tr>
<tr>
<td>VI</td>
<td>Lead 12 weeks 10 days</td>
<td>60.599 ± 2.89</td>
<td>60.599 ± 2.89</td>
<td>60.599 ± 2.89</td>
<td>60.599 ± 2.89</td>
</tr>
<tr>
<td>VII</td>
<td>Treatment Only 5 days</td>
<td>43.677 ± 0.95*</td>
<td>40.709 ± 3.60*</td>
<td>36.772 ± 2.04*</td>
<td>41.422 ± 112*</td>
</tr>
<tr>
<td>VIII</td>
<td>Treatment Only 12 days</td>
<td>83.946 ± 1.49*</td>
<td>32.167 ± 3.73*</td>
<td>29.120 ± 1.32*</td>
<td>34.551 ± 2.49*</td>
</tr>
<tr>
<td>IX</td>
<td>Lead + Treatment 12 weeks 5 days</td>
<td>61.820 ± 2.86</td>
<td>54.345 ± 5.24*</td>
<td>55.542 ± 5.03*</td>
<td>59.819 ± 5.18*</td>
</tr>
<tr>
<td>X</td>
<td>Lead + Treatment 12 weeks 10 days</td>
<td>44.775 ± 9.81</td>
<td>42.082 ± 7.21*</td>
<td>42.697 ± 3.58*</td>
<td>48.386 ± 3.42*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± standard error of mean; \(n = 5\). Significantly different (\(p < 0.05\)).
DNA fragmentation in the liver and brain are shown in Figures 3 and 4 respectively. The results showed a similar pattern to what was observe with Vitamin C. Significantly, treatment with D-penicillamine did not have an adverse effect on its own when compared with the control.

The ameliorative effects of succimer on tissue damage measured by DNA fragmentation in the liver and brain are shown in Figures 5 and 6 respectively. The results showed a similar pattern to what was observe with Vitamin C and D-penicillamine above.

Figures 7 and 8 showed the results of the experiments in which 80 mg/Kg CaNa2EDTA was used as a potential corrective remedy for lead-induced toxicity in the liver and brain respectively. The results presented showed that it had a similar effect as vitamin C, D-penicillamine, and succimer. Several chelation therapeutic strategies have been proposed, including supplementation with antioxidants and up-regulation of endogenous anti-oxidative defense system for lead induced oxidative stress in various body organs (Raafat et al., 2011; Hamadouche et al., 2012; Dewanjee, et al., 2013). Ameliorative effect observed in the liver and of the group treated with D-penicillamine and succimer in which 10 days treatment showed a more significant decrease compared with 5 days treatment.

However, the mechanism of actions of these chelating agents and antioxidants is still indistinct. Although it has been shown that meso-2,3-dimercaptosuccinic acid is 95 % plasma protein bound, most likely by virtue of binding on one of its sulfhydryl groups to a cysteine residue on albumin, leaving the other –SH available to chelate metals (Kalia and Flora, 2005). Studies have suggested that D-Penicillamine administration can increase the urinary excretion of lead because of complexes which it forms with this heavy metal (González-Ramírez et al., 1990). In this study, D-Penicillamine was able to ameliorate the effect of lead in the liver and brain.

Succimer in this study shows a more ameliorative effect in brain tissues compared to other chelators used. An examination of published data describing the effect of chelating agent treatment on brain levels indicates that succimer produces a reduction in brain levels under all conditioned examined (Jones et al., 1994). Studies have shown that Succimer can cross blood brain barrier of mice, limiting its use to extracting heavy metals from other parts of the body other than the central nervous system (Aasath et al., 1995).

Table 2: Effect of Vitamin C and Chelators on the treatment on lead-induced DNA fragmentation in brain tissue. Values are expressed as Mean ± standard error of mean; n = 5. Significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Vlt C Brain</th>
<th>DPA Brain</th>
<th>DMSA Brain</th>
<th>CaNa2EDTA Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>13.05 ± 1.13 *</td>
<td>13.05 ± 1.13 *</td>
<td>13.05 ± 1.13 *</td>
<td>13.05 ± 1.13 *</td>
</tr>
<tr>
<td>II</td>
<td>Normal</td>
<td>12.53 ± 0.95 *</td>
<td>12.53 ± 0.95 *</td>
<td>12.53 ± 0.95 *</td>
<td>12.53 ± 0.95 *</td>
</tr>
<tr>
<td>III</td>
<td>Normal</td>
<td>11.34 ± 1.03 *</td>
<td>11.34 ± 1.03 *</td>
<td>11.34 ± 1.03 *</td>
<td>11.34 ± 1.03 *</td>
</tr>
<tr>
<td>IV</td>
<td>Lead</td>
<td>41.31 ± 3.94 *</td>
<td>41.31 ± 3.94 *</td>
<td>41.31 ± 3.94 *</td>
<td>41.31 ± 3.94 *</td>
</tr>
<tr>
<td>V</td>
<td>Lead</td>
<td>37.27 ± 3.19 *</td>
<td>37.27 ± 3.19 *</td>
<td>37.27 ± 3.19 *</td>
<td>37.27 ± 3.19 *</td>
</tr>
<tr>
<td>VI</td>
<td>Lead</td>
<td>33.70 ± 3.62 *</td>
<td>33.70 ± 3.62 *</td>
<td>33.70 ± 3.62 *</td>
<td>33.70 ± 3.62 *</td>
</tr>
<tr>
<td>VII</td>
<td>Treatment</td>
<td>21.49 ± 5.23 *</td>
<td>21.49 ± 5.23 *</td>
<td>21.49 ± 5.23 *</td>
<td>21.49 ± 5.23 *</td>
</tr>
<tr>
<td>VIII</td>
<td>Treatment</td>
<td>16.91 ± 1.75 *</td>
<td>16.91 ± 1.75 *</td>
<td>16.91 ± 1.75 *</td>
<td>16.91 ± 1.75 *</td>
</tr>
<tr>
<td>IX</td>
<td>Lead +</td>
<td>30.93 ± 1.63 *</td>
<td>28.48 ± 4.23 *</td>
<td>25.26 ± 4.45 *</td>
<td>30.93 ± 5.36 *</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>28.77 ± 2.94 *</td>
<td>23.39 ± 2.58 *</td>
<td>19.74 ± 3.79 *</td>
<td>28.81 ± 3.42 *</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± standard error of mean; n = 5. Significantly different (p < 0.05).
Figure 1: Effect of Vitamin C treatment on lead-induced DNA fragmentation in liver tissue of albino rats.

Figure 2: Effect of Vitamin C treatment on lead-induced DNA fragmentation in brain tissue of albino rats.
Figure 3: Effect of D-Penicillamine treatment on lead-induced DNA fragmentation in liver tissue of albino rats.

Figure 4: Effect of D-Penicillamine treatment on lead-induced DNA fragmentation in brain tissue of albino rats.
Figure 5: Effect of Succimer treatment on lead-induced DNA fragmentation in liver tissue of albino rats.

Figure 6: Effect of Succimer treatment on lead-induced DNA fragmentation in brain tissue of albino rats.
Figure 7: Effect of CaNa$_2$EDTA treatment on lead-induced DNA fragmentation in liver tissue of albino rats.

Figure 8: Effect of CaNa$_2$EDTA treatment on lead-induced DNA fragmentation in brain tissue of albino rats.
Conclusion

The results obtained in this study indicated that, lead administration induced toxicity in rats as reflected on elevation of percentage DNA damage in the brain and liver. Oral administration of these conventional chelators caused significant amelioration in damage in tissues. The ability of these chelators to reduce DNA fragmentation caused as a result of lead toxicity may relate to its antioxidant actions via free radical scavenging mechanism. Therefore, supplementation of diets with ascorbic acid (vitamin C) may be recommended to improve the body burden of lead and provide protection against toxic effects. Also, co-administration of vitamins combined with chelating agents may have better beneficial role and protective effects against lead intoxication. However, efforts are needed for the choice of appropriate dose, duration of treatment, and possible side-effects on major organs.

REFERENCES


