Roles of N-acetylcysteine, Methionine, Vitamin C and Vitamin E as Antioxidants Against Lead Toxicity in Rats

Madiha, M. Abdel-Kader; Abeer, A. Afify; Amany, M. Hegazy

Food Science & Nutrition Dept., National Research Center, Dokki, Cairo, Egypt

Abstract: Humans are exposed to a number of toxic elements in the environment. Lead, widely used in industry, is a great environmental health problem of both humans and animals. The present study was designed to investigate the protective effect of sulphur-containing antioxidants (N-acetylcysteine (NAC) and methionine (Meth.) as well as vitamin C and vitamin E) against the lead toxicity. Experiments were carried out on rats given lead acetate in their drinking water for 4 weeks (1000 ppm) with or without relatively high doses of the mentioned antioxidants. Several biochemical parameters representing different organs function were followed. The results showed significant decrease in hemoglobin (Hb) in the lead (Pb) group. Whereas malondialdehyde (MDA) levels were significantly higher compared to the normal control group. Pb- NAC and Pb-Meth. groups were found to be very effective in increasing Hb levels when compared to those in other Pb- antioxidant groups. Marked reduction was observed in MDA concentrations in Pb- antioxidant groups when compared to Pb- group. Meanwhile the vitamin C treatment group had non significantly decrease. There was a decrease below Pb- control group values in erythrocyte superoxide dismutase (SOD) and glutathione peroxidases (GSH-Px) levels in the Pb- antioxidant groups. The plasma Vit. C levels in the Pb- antioxidant rat groups were lower than the normal control rats, but not significantly. A significant increase could be noted in Vit.A contents in plasma from the Pb- NAC and Pb- Meth. supplemented groups compared to Pb- group. Plasma Vit.E. contents were markedly higher in rats given Pb- Vit. E or Pb in conjunction with NAC when compared to Pb- control group. A noticeable increase in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as liver functions occurred due to lead acetate in the drinking water. In conclusion, the results in this study indicate that the oxidative stress induced by lead is reduced by the all investigated antioxidants. Also the hazards due to ingestion of lead-acetate can be partially corrected if the intake from sulphur-containing compounds and vitamins is increased.

Key words: Lead toxicity, oxidative stress, N-acetylcysteine, methionine, vitamin C, vitamin E.

INTRODUCTION

Lead is one of the most abundant heavy metals on earth. It has been widely used throughout human history, posing a serious health problem to susceptible populations, such as children or occupationally exposed people (Jung-Hun et al., 2007). It commonly used in industrialized countries, adversely affects human and animal physiological, biochemical and behavioral functions such as kidney dysfunction and impairment of liver function. Lead intoxication is a complex disorder that affects several cells and organs, including functional and structural alteration of erythrocytes (Koller et al., 2004; Calderon- Salinas et al., 1999). One of the most prominent effects of lead is exerted on the biosynthesis of hem, the prosthetic group present hemoglobin, cytochromes, catalases and peroxidases (Needleman, 2004). Some researchers have investigated the benefit of antioxidants in preventing lead toxicity, the mechanisms of antioxidant nutrients being effective via rebalancing the impaired pro-oxidant / antioxidant ratio are not completely clear. Antioxidant nutrients including, vitamin E, vitamin C, vitamin B6, B-carotene, zinc, and selenium (Kedziora- Kornatowska et al.,2003; Elayat and Bakheet, 2010). The strong scientific interest in the role of antioxidants has expanded the focus of research from reducing the oxidative stress of lead exposure to improving the pro-oxidant / antioxidant balance of cells. Thiol-containing compounds bind lead at their-SH (thio) groups and have antioxidant features. Therefore, thiol-containing antioxidants may be useful as a component of an effective treatment for lead poisoning. Several previous studies have suggested that lead- induced oxidative damage in red blood cells may result from direct
interaction of lead with their membranes, inducing lipid peroxidation (Sandhir et al., 1994; Caylak et al., 2008). N-Acetylcysteine (NAC) has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining interacelluar glutathione levels and scavenging reactive oxygen species (Ereal et al., 1996). In addition, NAC also has some chelating action on lead (Aruoma et al., 1989). Methionine acts a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role in detoxification (Reed, 1990). In addition, methionine has been shown to chelate lead and remove it from tissues (Patra et al., 2001). Both vitamin C and vitamin E decreased lipid peroxidation and augmented the activities of antioxidant enzymes studied in the kidneys and liver of rats. (levander et al., 1980; Ribarov et al., 1981; Kedziora- Kornatowska et al., 2003).

Little information is available in the literature regarding the antioxidant activity of thiol-containing amino acids (N-Acetylcysteine and methionine). Therefore, the present study was undertaken to investigate the antioxidant activity of these sulphur containing amino acids of rats received lead acetate in their drinking water for 4 weeks. Also the antioxidant activity of vitamin C and E in the same rats was studied.

MATERIALS AND METHODS

Animals:
Sprague-Dawley rats were procured from the Central Animal House, National Research Center. They were housed in stainless steel cages in a temperature-controlled room (20-22°C) with a 12 hours light and 12 hours dark exposure.

Experimental Design:
Thirty sex Sprague- Dawley rats with an average weight (170- 200 g) were divided into 6 groups of 6 rats each. All groups were given only standard rat feed and water during the 1st week (Table 1). After this period the animals were fed to the following diets: Group (1) basal diet + Sodium acetate (1000 ppm ) in their drinking water, group (2) basal diet + lead acetate (1000 ppm) in their drinking water, group (3) basal diet + N- acetyl cysteine (800ppm), group (4) basal diet + methionine (100ppm), group (5) basal diet +vitamin C (500ppm), group (6) basal diet + vitamin E (20ppm).The experiment continued for 4 weeks.

Analytical Measurements:
Parameters measured were, blood hemoglobin determined according to the method of Leong et al(2003), malondialdehyde( MAD)was determined as described by Ohkawa et al(1979), super oxide dismutase(SOD) was estimated as described by Roth and Gilbert(1984), glutathione peroxidases (GPx) was determined according to the method of kraus and Ganther (1980), aspartate aminotransferase (AST) was determined by the method of Friedman et al.(1980), alanine aminotransferase (ALT) was estimated as described by International Federation of Clinical Chemistry (1980), Vit.C determined by the method of Jagota and Dani (1982), Vit. E was determined according to Desai and Machlin (1985), Vit.A was estimated as described by Neeld and Pearson (1963), uric acid content was determined according to the method described by Barham, D.and Trinder, P. (1972). creatinine was estimated by the method of Larsen (1972).

Statistics:
All values are reported as mean± standard error. One way ANOVA analysis was used to compare between groups with statistical significance set at p<0.05.

Results:
Animals given lead acetate in drinking water for 4 weeks had significantly lower Hb concentration when compared with the normal control and other Pb antioxidant groups (Table 2). Although NAC and methionine were found to be very effective in increasing Hb levels when compared to those in other lead groups. Vit.C and E supplements did not appear to change Hb concentration. Blood Hb levels of supplemented groups with vitamin C and vitamin E were higher than that of lead group, but lower than those of NAC and Meth. and normal control groups.

Table (2) shows the results of serum MDA concentration in all tested groups. The MDA level of lead group was significantly higher than the control group values. Significant reduction was observed in MDA concentrations in all lead- antioxidant groups when compared to lead group. No significant decrease of MDA level was noted, in Vit. C treatment group, when compared with the normal control group.
Table 1: Composition of experimental diets (g./100g.).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>14.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixture (AIN-93)</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture (AIN-93)</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Starch</td>
<td>62.3</td>
</tr>
</tbody>
</table>

The control diet content according to AOAC, (2000).
* Salt mixture and vitamin mixture according to Reeves et al., 1993.

Table 2: Effect of lead on blood hemoglobin, serum MDA, whole blood GSH and Erythrocytes SOD.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>MDA (nmol/ml)</th>
<th>GSH-Px (nmol/L)</th>
<th>SOD (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (without Pb)</td>
<td>15.9 ± 0.396 *</td>
<td>5.03 ± 0.554 **</td>
<td>0.667 ± 0.055 *</td>
<td>1121.8 ± 49.3 *</td>
</tr>
<tr>
<td>Positive control (with Pb)</td>
<td>12.8 ± 0.373 b</td>
<td>6.31 ± 0.377 * a</td>
<td>0.619 ± 0.026 a</td>
<td>2177.3 ± 173.1 *</td>
</tr>
<tr>
<td>Pb + NAC</td>
<td>15.8 ± 0.567 a</td>
<td>3.39 ± 0.332 bc</td>
<td>0.552 ± 0.025 b</td>
<td>1465.3 ± 100.1 b</td>
</tr>
<tr>
<td>Pb + Meth.</td>
<td>15.4 ± 0.488 a</td>
<td>3.06 ± 0.111 a</td>
<td>0.313 ± 0.011 a</td>
<td>1277.1 ± 99.8 b</td>
</tr>
<tr>
<td>Pb + Vit. C</td>
<td>14.6 ± 0.351 a</td>
<td>4.26 ± 0.409 b</td>
<td>0.429 ± 0.038 a</td>
<td>1499.7 ± 50.2 a</td>
</tr>
<tr>
<td>Pb + Vit. E</td>
<td>14.9 ± 0.229 a</td>
<td>3.79 ± 0.347 a</td>
<td>0.623 ± 0.033 a</td>
<td>1437.8 ± 64.1 a</td>
</tr>
</tbody>
</table>

All values represent mean ± S.E. for 6 samples.

The activity of antioxidant enzymes SOD and GSH-Px in RBCs are shown in the same table. Significant decreases in SOD activity of erythrocytes were recorded in lead- Meth. group as compared with control group, but these effects were not significantly different than the other lead- antioxidant groups. Supplementing lead with the all tested antioxidants especially lead-Meth. and lead- Vit.C lowered the GSH-Px activities in RBCs as compared with the Pb- control group. Meanwhile the other investigated antioxidants did not.

The plasma vitamin C, A and E-levels are given in table (3). The plasma Vit. C levels in the Pb-antioxidant rat groups were lower than the normal control rats, but not significantly. Although no significant decrease was observed in the Pb- group and Pb-NAC as well as Pb- Meth. groups. A significant increase could be noted in Vit. A contents in plasma from the Pb- NAC and Pb- Meth. supplemented groups compared to Pb-group. Meanwhile Vit.C supplemented group decreased Vit.A levels than the normal control group and increased Vit.A concentration than the Pb- group. Plasma Vit.E. Contents were significantly higher in rats given Pb- Vit. E or Pb in conjunction with NAC when compared to Pb- control group. While the other antioxidant supplemented groups showed no significant differences between them when compared to normal control group.

Table (4) shows the change in the activity of AST and ALT of rats in the different groups. As shown in this table, a significant increase in these liver functions occurred due to lead acetate in the drinking water. The activity of AST and ALT in normal control group were 74.0±3.67 and 20.2±0.638 respectively increased to 93.6±7.06 and 24.6±2.89 respectively in Pb- control group. The all investigated antioxidants caused a significant decrease in the activity of these enzymes, when compared with those of Pb- group. Uric acid and creatinine levels of the same rats are given in the same table. Lead- group rats showed significantly increase in serum uric acid and creatinine values. Mean values of serum uric acid and creatinine showed non significant changes in the all Pb- antioxidant groups except Pb- Meth. and Pb- Vit. E groups respectively, when compared with the control groups. Significant decrease in uric acid concentration was recorded in Pb-Meth. group, compared to control groups.

Discussion:

Lead poisoning occurring in humans and animals either due to occupational or environmental exposure has become a great public problem lead alters virtually all biochemical processes and organ systems. Everyone is exposed to lead in air, household dust, food, drinking water and various consumer products. Acute and chronic lead exposure can seriously affect human health. It is a confirmed multi-target toxicant with effects on the gastrointestinal, haematopoietic, cardiovascular, nervous, immune, reproductive and excretory system (Daggett et al, 1998; Al-Neamy et al, 2001; D’Souza et al, 2007).

Oxidative stress also leads to lipid peroxidation in RBC membranes, autooxidation of hemoglobin and limited repair processes, leading to decrease survival (Rice-Evans and Baysal, 1987; Caylak et al., 2008).

The present study was designed to investigate oxidative stress parameters (Hb and MDA in serum, SOD and GSH-Px activities in erythrocyte hemolysate, and Vit.C,A and E in plasma as well as uric acid and creatinine in rats administered lead in conjunction with sulphur containing compounds (NAC and Methionine) as well as vitamins (C and E) as antioxidants.

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Most researches on lead toxicity in rats utilizes 4-5 weeks as lead exposure period in which they receive only water or therapeutic antioxidant compounds. In our study, lead and preventative antioxidant compounds were administered simultaneously in water to rats during the 4 weeks.

One of the most prominent effects of lead is exerted on the biosynthesis of heme, the prosthetic group present in hemoglobin (EL-Missiry, 2000; Lavicoli et al., 2003; Needleman, 2004). Our results demonstrated that administration of lead acetate in the drinking water for 4 weeks induced a toxic effect on RBCs as indicated by a significant reduction in Hb concentrations, similar to other reports (Caylak et al., 2008; Elayat and Bakheet 2010).

Lead induced peroxidation results in the formation of aldehydic by-products such as MDA. In the present study, the increased MDA levels in serum of lead-exposed rats were significant confirming previous studies (Gurer et al., 1998, Kedziora-Kornatowska et al., 2003; Caylak et al., 2008). In lead intoxication, lead induces generation of reactive oxygen species such as hydrogen peroxide (H$_2$O$_2$), superoxide ion (O$_2^-$), singlet oxygen and hydroxyl radical (HO$^\cdot$). SOD and GSH-Px play an important role in protecting the cells against the toxic effects of O$_2^-$ and peroxides as well as H$_2$O$_2$, respectively, and may be upregulated in response to lead exposure (Caylak et al., 2008). Different effect of lead on the activities of SOD and GSH-Px was previously noticed. Sivaprasad et al., (2004) reported a drop of SOD and GSH-Px activities in lead exposed rats, while Soltaninejad et al., (2003) found higher activity of SOD. No significant effects of exposure to lead on the activities of SOD and GSH-Px were indicated (Caylak et al., 2008). In this work SOD has been observed elevated, whereas GSH-Px was decreased, when compared to normal control group.

We also determined the non enzymatic antioxidant status, e.g vitamins C, A and E in plasma of rats exposed to lead. In lead administrated rats; there is a decrease in these vitamin levels, similar to other previously reports (Attri et al., 2003; Caylak et al., 2008).

In conclusion, the hematological system is a major target of low-level lead exposure. The results obtained from this study showed that sulphur-containing antioxidants and vitamins supplementation proved to be beneficial to compensate hazards due to lead toxicity. The antioxidants preparation including the studied ones is necessary. The extrapolation of these findings to humans still needs investigation.
REFERENCES


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