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Antioxidant effects of methionine, α-lipoic acid, *N*-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats

Emrah Caylak[a], Metin Aytekin[b,*], Ihsan Halifeoglu[a]

[a]*Department of Biochemistry and Clinical Biochemistry, Firat University, Medical School, Elazig, Turkey*
[b]*Departments of Pathobiology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA*

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Abstract

Lead, widely used in industry, is a great environmental health problem. Many studies have examined its effects on the health of both humans and animals. Experimental studies have shown that sulphur-containing antioxidants have beneficial effects against the detrimental properties of lead. The present study was designed to investigate markers of oxidative stress (hemoglobin (Hb) in whole blood, malondialdehyde (MDA) in sera; superoxidase dismutase (SOD) and glutathione peroxidise (GSH-Px) in erythrocyte hemolysate and vitamins A and E in plasma) in rats given lead (2000 ppm) with or without sulphur-containing antioxidants (L-methionine (Met) (100 mg/kg/day), *N*-acetylcysteine (NAC) (800 mg/kg/day), L-homocysteine (Hcy) (25 mg/kg/day), lipoic acid (LA) (50 mg/kg/day)) in their water for 5 weeks. In the lead group, Hb and plasma vitamin E levels were significantly lower whereas MDA levels were significantly higher compared to controls ($p<0.05$). Hb levels in lead–methionine and lead–LA groups were significantly higher than the lead group ($p<0.01$). MDA levels were reduced in all groups compared to the lead group ($p<0.01$). There was a decrease below control values in erythrocyte SOD ($p<0.01$) and GSH-Px ($p<0.05$) levels in the lead–LA group. Plasma vitamin A levels were significantly high in lead-methionine group compared to lead group ($p<0.01$). In conclusion, the data suggests that oxidative stress induced by lead is reduced by sulphur-containing compounds.

Keywords: Lead; Oxidative stress; Methionine; Lipoic acid; *N*-Acetylcysteine; Homocysteine

Introduction

Lead, commonly used in industrialized countries, adversely affects human and animal physiological, biochemical, and behavioral functions. The mechanism of lead toxicity may be due, in part, to disruption of the prooxidant/antioxidant balance, leading to tissue injury via oxidative damage to critical biomolecules such as lipids, proteins, and DNA. The hematological system is the major target of low level lead exposure. 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 (Hermes-Lima et al., 1991; Sandhir et al., 1994) and inhibiting heme and hemoglobin synthesis (Warren et al., 1998).

The strong scientific interest in the role of antioxidants has expanded the focus of research from reducing

*Corresponding author. Tel.: +1 216 4456542; fax: +1 216 636 0104.
E-mail address: aytekim@ccf.org (M. Aytekin).

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the oxidative stress of lead exposure to improving the prooxidant/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.

Methionine acts a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role in detoxification (Reed and Orrenius, 1977; Reed, 1990). In addition, methionine has been shown to chelate lead and remove it from tissues (Patra et al., 2001). α-Lipoic acid (LA), is a coenzyme of pyruvate and the α-ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle (Patel and Roche, 1990), and has metal chelating, free radical scavenging, and antioxidant-regenerating abilities (Packer et al., 1995). *N*-Acetylcysteine (NAC) has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining intracellular glutathione levels and scavenging reactive oxygen species (Ercal et al., 1996). In addition, NAC also has some chelating action on lead (Aruoma et al., 1989). Homocysteine (Hcy), a thiol formed by demethylation of methionine, is at moderately high levels, a known independent risk factor for atherosclerosis and increased vascular dysfunction (Refsum and Ueland, 1998). However, according to some authors, Hcy, contains a thiol group, displays an antioxidant effect on cellular systems at micromolar levels (Zappacosta et al., 2000).

In the present study, we investigated the beneficial effects of thiol-containing antioxidants on altered oxidative stress parameters and antioxidant enzyme levels with lead treatment. We determined malondialdehyde (MDA) levels as an indicator of lipid peroxidation. Oxidative stress status was described by determination of hemoglobin (Hb), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and vitamins A and E levels. We also investigated whether Hcy has an antioxidant effect in response to lead exposure.

Methods

Chemicals

Lead acetate and NAC were purchased from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma (St. Louis, MO, USA). HPLC grade reagents were used in vitamins A and E analysis.

Animals

Wistar-albino male rats were procured from Firat University Experimental Research Unit Animals weighed 150–200 g. Animals were utilized as per the permission from the Medical Faculty Animal Ethics Committee. They were fed a standard rat pellet diet and had free access to water. The rats were housed in stainless steel cages in a temperature-controlled room (20–22 °C) with a 12 h light and 12 h dark exposure.

Experimental design

The animals were randomized into six groups of 10 animals each. All groups were given only standard rat feed and water during the 1st week. After this period of adjustment to their environment, group I ($n = 10$) served as the control and was given only standard rat chow and water for 5 weeks. Group II ($n = 10$) received 2000 ppm lead acetate in their drinking water for 5 weeks. Group III ($n = 10$) received 2000 ppm lead acetate in their drinking water for 5 weeks and 100 mg/kg/day methionine dissolved in water and administered in their drinking water. Group IV ($n = 10$) was treated like group III, except that it received 25 mg/kg/day i.p. LA dissolved in a 1:1 ratio with ethyl alcohol and administered intraperitorically for 5 weeks. At the end of the study, two rats had died from peritonitis. Group V ($n = 10$) received water containing 2000 ppm lead acetate and 800 mg/kg/day NAC. Group VI ($n = 10$) received 2000 ppm lead acetate and 50 mg/kg/day Hcy in their water. The rats were housed in separate cages to ensure the correct dose was received. At the end of the 5th week, the animals were sacrificed by cervical decapitation. The blood samples were collected in lead free tubes using heparin and EDTA as anticoagulant or tubes without anticoagulants. Plasma and serum were removed by centrifugation for 10 min at 3000 rpm. The red blood cells were washed three times with an equal volume of cold saline. The samples were maintained at −20 °C before performing assays (not longer than 7 days).

Assays

Hb determination

Hb concentrations in whole blood were spectrophotometrically analyzed by the cyanomethemoglobin method (Leong et al., 2003). Blood samples (20 μl) were mixed with 5 ml Drabkin's solution (0.1% sodium bicarbonate, 0.005% potassium cyanide, and 0.02% potassium ferricyanide) for hemoglobin determination. Hb standard was purchased from Sigma (St. Louis, MO, USA).

MDA determination

MDA concentrations were measured as TBARS (thiobarbituric acid reactive substances) according to a modified version of Satoh's (1978) and Yagi's (1984)