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The Effect of Lead on Erythrocyte Glucose-6-Phosphate Dehydrogenase Activity in Rats

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Background: Exposure to lead damages the biological systems, developing oxidative stress. Glucose-6-phosphate dehydrogenase (G6PD) produces NADPH in pentose phosphate pathway. This molecule protects tissues from oxidative stress. There are conflicting reports in the literatures of the effects of lead on G6PD activity.
Objectives: The aim of this investigation was to evaluate the effect of lead on erythrocyte G6PD activity in rats given lead acetate in their drinking water.
Materials and Methods: In this study 14 albino rats were divided into two groups of seven animals. The treated group was exposed to 2% lead acetate in the drinking water during eight weeks. The control group was kept in the similar condition as the test group; however this group was not exposed to lead acetate. The blood lead level was measured with an atomic absorption spectrophotometer. The G6PD activity was determined by kinetic method. The hemoglobin content was determined by Drabkin's method. Malondialdehyde (MDA) in rats' plasma was measured with the thiobarbituric acid test using HPLC.
Results: The blood lead concentration in treated group was increased when compared to control group ($P < 0.05$). A significant decrease in hemoglobin level was noted in lead-treated animals ($P < 0.05$). The G6PD activity in erythrocytes of rats received lead acetate increased up to 81% compared to the control group ($P < 0.05$). The G6PD/hemoglobin ratio in control and treatment groups was 17.7 ± 3.6 U/g and 44.9 ± 4.4 U/g, respectively. A significant increase in the plasma MDA level was also observed in the exposed group ($P < 0.05$).
Conclusions: The results of this study showed that exposure to lead increased the activity of G6PD in the rat erythrocytes, perhaps resulting in an up regulation of the enzyme to detoxify lead.

Keywords: Glucose-6-Phosphate Dehydrogenase; Malondialdehyde; Lead; Oxidative Stress; Rats

1. Background

Lead is a toxic heavy metal which damages the biological systems. The toxic effect of lead and its compounds in animals and humans have been investigated for many years. Exposure to lead is associated with a number of morphological, physiological and biochemical abnormalities. Lead toxicity was observed in acute or chronic forms and its magnitude depends on many parameters. It can accumulate in some organs and results in kidney failure and abdominal pain. Also, lead diffuses in bone matrix and replaces calcium. Lead toxicity especially in children damages the nerve system and causes a number of behavioral abnormalities. It inhibits delta-aminolevulinic acid (ALA) dehydratase and ferrochelatase enzymes in the heme biosynthesis pathway and causes blood disease (1, 2). Several mechanisms have been suggested for lead toxicity on biological systems (3, 4). Lead is mainly located in erythrocytes in blood and only 1% of lead is remained in serum. Recent studies have proposed that lead is capable of causing oxidative stress in erythrocytes. It induces an imbalance between generation and removal of reactive oxygen species (ROS) in cells and tissues that it causes damage to DNA, proteins and membranes. It is suggested that lead affects the activity of enzymes responsible for maintenance of redox balance in organisms (5, 6).

G6PD (EC 1.1.1.49) is the first enzyme in the pentose phosphate metabolic pathway. This enzyme supplies cells with most of the NADPH through the oxidation of

Implication for health policy/practice/research/medical education:
From the data presented in the current study, it appears that exposure to lead could increase the activity of G6PD and G6PD/hemoglobin ratio in the rat erythrocytes in vivo.

glucose-6-phosphate to 6-phosphogluconate. NADPH has important role in much functions of body and it has critical function in antioxidant system and protects body from molecules that produce oxidative stress (7). There are conflicting reports in the literature of the effects of lead on G6PD activity (8-10).

2. Objectives

The present study was planned to evaluate the erythrocyte G6PD activity in rats given lead acetate in their drinking water.

3. Materials and Methods

Lead acetate was procured from BDH Chemicals (India). All other analytical grade laboratory chemicals and reagents were purchased from Merck or Sigma. Male albino rats weighing 75 ± 10 g were obtained from the Razi Institute (Karaj, Iran) and were housed in stainless steel cages in a temperature-controlled room (22°C) with a 12-h light/dark cycle. All animal experiments were carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC) in such a way to minimize the number of animals and their suffering. They were fed with standard rat chow. The animals were randomly divided into two groups each of seven animals. Group I served as control given only standard rat chow and water. Group II received standard rat chow and 2% lead acetate in its drinking water. Blood samples were taken after eight weeks. Blood lead was measured with an atomic absorption spectrophotometer (Avanta PM; GBC, Australia) equipped with a deuterium background corrector and a graphite furnace (GF3000, GBC) and a furnace autosampler (PAL3000, GBC). A wavelength of 283.3 nm and a spectral slit width of 1 nm were used (11). The G6PD activity was determined in the basis of NADPH production at 340 nm and 37oC by kinetic method. The assay mixture contained 10 mM magnesium chloride, 0.2 mM NADP+, and 1 mM Glucose 6-phosphate in 100 mM Tris-hydrochlorid buffer solution at pH 8.0. Assays were carried out in duplicate and the activities were followed up for 60 second. One unit of activity (U) is defined as the amount of enzyme required to reduce 1 μmol/min of NADP+ under the assay conditions (12).

The hemoglobin content was determined spectrophotometrically using Drabkin's method (13).

The effect of lead on lipid peroxidation was evaluated by measuring the amount of malondialdehyde (MDA) produced in rats' plasma with the thiobarbituric acid (TBA) test using the HPLC method. The HPLC equipment used in the present study was the Cecil System (Adept; Cecil, UK), which included a Cecil CE4200 UV-Vis detector. Plasma MDA concentration was determined by the modified methods of Seljeskog et al. (14) and Ohkawa et al. (15), based on TBA reactivity. Briefly, 0.1 mL plasma that was prepared by adding ethylenediaminetetraacetic acid (EDTA), 0.2 mL of 8.1% SDS and 0.004 mL butylated hydroxytoluene (BHT) were added to the tubes and mixed. Then, 3 mL of 1.6% TBA in acetate buffer (pH 3.0) was added and the tubes were capped tightly. The tubes were incubated at 95°C in a water bath for 60 minutes and then cooled on ice water bath (0°C). The mixture was centrifuged (4000 g) for 20 min and the supernatant was taken. After this, 20 μL of the pink chromogen was injected onto a C18 column (6.0 mm x 250 mm) in a reverse phase HPLC system. The mobile phase consisted of 11% acetonitrile, 17% methanol and 72% phosphoric acid (50 mM, pH 6.8). The samples were eluted isocratically at a flow rate of 1 mL/min with the detection wavelength set at 532 nm. The standard curve was made from 1,1,3,3-tetraethoxypropane. The data are expressed as "mean ± SD". Analysis of data was performed using t-test to compare the mean values between the different groups. The measurements were performed in duplicate and the mean scores were used for statistical calculations. Statistical differences with $P < 0.05$ were considered as significant.

4. Results

Table 1 shows the blood lead concentration and hemoglobin content, erythrocyte G6PD activity and G6PD/hemoglobin ratio in both the control and lead-treated animals.

Within the animal group given lead acetate, the average value obtained for blood lead concentration (1905 ± 412 μg/L) was increased significantly after eight-week study when compared to control group (117 ± 43 μg/L) ($P < 0.05$). A significant decrease in hemoglobin level was noted in animal group treated with lead (10.5 ± 1.1 g/dL). The erythrocyte G6PD activity in group given lead acetate through drinking water increased to 81% compared to the control group ($P < 0.05$). These results indicate an up regulation of the enzyme associated with detoxification in lead toxicity. Additionally, comparison of the G6PD/hemoglobin ratio between control (17.7 ± 3.6 U/g) and treated groups (44.9 ± 4.4 U/g) suggested a significant increase in the ratio in lead-exposed animals ($P < 0.05$). Figure 1 shows the plasma MDA level in both the control (17.1 ± 2.3 μM) and lead-exposed (26.8 ± 4.3 μM) animals. The plasma MDA levels in group received lead, increased significantly to 57% as compared to the control group ($P < 0.05$).

5. Discussion

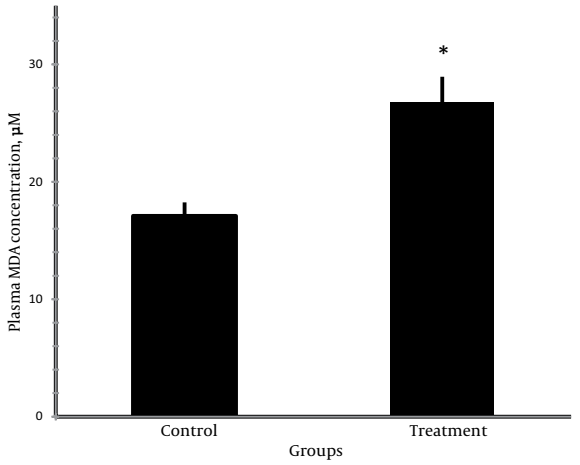
In the present study, we observed that the G6PD activity in the rat erythrocytes and the G6PD/hemoglobin ratio were significantly increased in lead-treated group as compared to the control group. This observation is in agreement with a report by Sadhu et al. in which a higher activity of G6PD in erythrocytes of the workers occupationally exposed to lead was found (8). Exposure to lead resulted in physiological, biochemical, and behavioral dysfunctions in animals and humans. It affects

Table 1. Effect of Lead Acetate (2%) Given in Drinking Water on Blood Hemoglobin Content (g/dL) and Blood Lead Concentration (μg/L), Erythrocyte G6PD Activity (U/L) and G6PD/Hemoglobin Ratio (U/g) of Control and Treatment Groups of Rats [a]

	Control Group	Treatment Group
Blood lead concentration, μg/L	117 ± 43	1905 ± 412 [b]
Hemoglobin content, g/dL	14.7 ± 0.9	10.5 ± 1.1 [b]
Erythrocyte G6PD activity, U/L	2590 ± 510	4680 ± 624 [b]
G6PD/hemoglobin ratio, U/g	17.7 ± 3.6	44.9 ± 4.4 [b]

[a] Data are presented as Mean ± SD
[b] Significantly different from control group ($P < 0.05$).

Figure 1. Plasma MDA Concentrations (μM) in Rats of Control and 2% Lead Acetate in Drinking Water Groups



All values are represented as mean ± SD; * Significantly different from control group ($P < 0.05$).

the haemopoietic system, central and peripheral nervous systems, kidneys, liver, and reproductive systems. It is mentioned that lead can participate in production of free radicals and reactive oxygen species (ROS) that react with cellular macromolecules and influence their functions (16, 17). Also, it is proven that lead inhibits the ALA dehydratase within the heme biosynthesis pathway and accumulates ALA. Autooxidation of ALA changes oxyheamoglobin to metheamoglobin that results in the formation of hydrogen peroxide (18). Overproduction of these species results in oxidative stress, a situation in which a number of pathological conditions proceeds. The increase in plasma MDA in the lead-exposed animals is in agreement with previously published results (6, 19). MDA is by far the most popular biomarker of oxidative damages and has served as an indicator of free radical damages. It is produced from the breakdown of polyunsaturated fatty acids (20). MDA has been recognized as an important indicator of lipid peroxidation and oxidative stress-related disease states and therefore, lead exposure may result in increasing cell susceptibility to oxidative attack by altering the membrane integrity and fatty acids composition of cells.

G6PD catalyzes the first reaction in the pentose phosphate pathway and supplying NADPH as a donor of reducing equivalents for glutathione reductase which reduces oxidized glutathione. G6PD activity would be expected to increase in response to increase of oxygen active forms and hydroperoxides. NADPH/NADP+ ratio regulates the G6PD activity and the pentose phosphate pathway (7). Although the pathway has not been studied extensively in lead poisoning, we may mention increased utilization of NADPH for oxygen active forms and hydroperoxide metabolism during lead poisoning would appear to provide a plausible explanation for the stimulation of G6PD activity in erythrocytes of lead-treated animals. The effect of lead in our study is not consistent with that of Lachant et al. in which it was reported that the inhibitory effect of lead ions on G6PD activity in the freshly hemolized erythrocytes after earlier incubation with lead ions (9). They suggested lead acts as noncompetitive G6PD inhibitor in relation to glucose-6-phosphate and NADP+. It is known that lead can influence on biomolecular activity directly and indirectly in molecular level. Some toxic effects of lead may be result on conformational changes of the biomolecules. Lead can bind with the negatively charged portions of the molecules such as sulphydryl, carbonyl and imidasole groups. This binding result in conformational changes of the biomolecules and it decreases their activity. G6PD has many sulfhydryl groups in its active site and therefore, lead may influence its activity. It is reported that lead can affect G6PD activity through the formation of a lead-sulfhydryl complex and inhibits it in vitro (21), nevertheless, the presence of some molecules such as glutathione in erythrocytes may protect G6PD from the effect of lead in vivo. Moniuszko-Jakoniuk and his coworkers reported that reduced-glutathione (GSH) level decreases in lead exposure and it implicates oxidative stress in lead toxicity (22). GSH is produced from oxidized-glutathione by glutathione reductase enzyme and NADPH molecule is necessary for this reaction. However, there are more complex effects of lead on G6PD. In conclusion, three different effects could be suggested for the stimulation of G6PD activity in erythrocytes of lead-treated animals. First, exposure to lead can increase the demand of NADPH molecule because of its free radicals producing characteristic. The second one is the capability of GSH and the other molecules such that it opposes the inhibitory effect of lead on G6PD that can affect the binding of lead with sulphydryl groups of G6PD. Finally, the third and the last is due to the reduction of GSH in

erythrocytes during exposure to lead that stimulates the enzymes involving the production of NADPH molecule such as G6PD.

In brief, we observed the increase in the activity of G6PD, an antioxidative enzyme, in lead exposed animals and it could be assumed as the defense of the organism against the increase of the oxygen active forms in lead toxicity.

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Author's Contribution

All authors helped for designing, analyzing the results and writing the current manuscript.

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