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Health effects in children aged 3–6 years induced by environmental lead exposure

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Abstract

Objectives: To investigate the involvement of oxidative damage in lead-induced toxicity in children aged 3–6 years and to enlighten whether oxidative stress indicators are correlated with the known indices of lead toxicity.
Methods: Blood samples were collected from 408 subjects (217 boys and 191 girls) in the urban kindergartens. The age range of the subjects was 3–6 years. Blood lead levels (BLLs) were analyzed by flameless atomic absorption spectrophotometry. Activities of δ-aminolevulinic acid dehydratase (ALAD), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and contents of glutathione (GSH) in erythrocyte and levels of plasma malondialdehyde (MDA) were analyzed spectrophotometrically in these children.
Results: Children with BLLs $>$or $=100\,\mu g/L$ had significantly decreased erythrocyte ALAD activities and increased plasma MDA levels compared to the children with BLLs $<100\,\mu g/L$. No significant changes were observed in erythrocyte SOD and GSH-Px activities and GSH levels associated with elevated BLLs in these children.
Conclusion: Present data indicate that oxidative damage could be induced by lead in children with BLLs $>$or $=100\,\mu g/L$, and this may partly be attributed to the inhibited ALAD activities. Statistically significant changes of oxidative stress parameters in preschool children while BLLs were more than $100\,\mu g/L$ could be implicated that oxidative damage might contribute to lead-induced intellectual impairment.

Keywords: Blood lead levels; Preschool children; Oxidative damage; Aminolevulinic acid dehydratase

1. Introduction

Lead is an element that has no known physiologic function in humans but adversely affects a variety of fundamental biochemical processes. Children aged <6 years are particularly susceptible to lead poisoning because they absorb far more lead from their environments than adults and their central nervous systems are still developing. Based on the accumulating evidence of lead toxicity at low concentrations, lead poisoning adversely affects children worldwide on the raised hearing threshold and decreased intelligence quotient (Lanphear et al., 2002; Lidsky and Schneider, 2003). By 1991, the US Centers for Disease Control and Prevention recommended $100\,\mu g/L$ of blood lead levels (BLLs) in preschool children as the threshold of lead poisoning. However, the biochemical changes in the body of children with BLLs around $100\,\mu g/L$ were poorly understood.

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Lead could induce oxidative damage by disrupted prooxidant/antioxidant balance in animals including humans. Reduced glutathione (GSH) levels and reduced glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in tissues or in blood are most commonly used to evaluate lead-induced oxidative damage (Pande and Flora, 2002; Patra et al., 2001; Villeda-Hernandez et al., 2001). δ-Aminolevulinic acid dehydratase (ALAD) is the second enzyme in the heme biosynthesis pathway and catalyzes condensation of two molecules of δ-aminolevulinic acid (ALA) to a porphobilinogen. ALAD is a zinc metalloenzyme possessing thiol (SH) groups, which are essential for its activity. Lead, because of its affinity for SH groups and replacement of zinc, is known to inhibit ALAD activity, resulting in accumulation of ALA. ALAD has been suggested as a sensitive index of the effect of lead exposure (Gurer-Orhan et al., 2004). Accumulated ALA also has been shown to involve lead-induced oxidative damage by causing formation of reactive species. This possibility implies that inhibited ALAD activity might be a promising indicator of lead-induced oxidative damage in the body.

The present study was designed to investigate the changes of oxidative stress in lead-induced toxicity in preschool children. To achieve this goal selected oxidative stress parameters along with some indices of lead poisoning were determined in blood of preschool children. Malondialdehyde (MDA) levels were determined as an endpoint of lipid peroxidation. BLLs and ALAD activity were used as indices of lead toxicity. The correlation between the oxidative stress indicators and the well-known indices of lead toxicity were then analyzed.

2. Materials and methods

2.1. Subjects

A total of 408 children, 217 boys and 191 girls, in Anshan city, a northeast city famous for steel refinery in China, were selected. The children examined were volunteers from five urban kindergartens. The age range of these subjects was 3–6 years. The sex and age distributions are shown in Table 1.

Table 1
Sex and age distributions of preschool children examined

Age group (months)	Sex		
	Male	Female	Total
31–42	10	7	17
43–54	77	63	140
55–66	85	86	171
67–78	45	35	80
Total	217	191	408

2.2. Sampling

After cleaning the skin with double-distilled water and alcohol, a trained phlebotomist drew 1 mL of venous blood from each child and transferred it into a metal-free heparin tube, which was stored at 4 °C. These tubes were brought in a box of ice for analysis from the survey sites to our laboratory in the same day. Aliquots of blood samples were separated immediately upon arrival at our laboratory: 0.4 mL of blood was centrifuged and plasma was separated for MDA analysis; 50 μL of blood for SOD was washed with normal saline and the red cells were left for further analysis; 50 μL of blood for GSH was hemolyzed and determined immediately; 100 μL of blood was separated for lead determination; the remaining part was used for ALAD and GSH-Px analysis. All aliquots were kept in −80 °C freezer until analysis.

2.3. Analysis procedures

BLLs. Atomic absorption spectrophotometry-graphite furnace (Varian spectra-AA 40P; USA) was used following a standardized analytical method (Honda et al., 1989) with an accuracy of ±5 μg/L. Instrumental parameters used for sample analysis were drying for 65 s between 85 and 120 °C, charring for 30 s between 300 and 480 °C, atomization for 3 s at 1850 °C, cleaning for 4 s at 2700 °C. Photometry was performed at a wavelength of 283.3 nm and using a lead hollow cathode lamp with a current supply of 7.5 mA, taking advantage of Zeeman background correction. Duplicate determinations were carried out for each sample and the average was taken as a measure. Quality control was performed by determination of the reference samples from the US CDC as participation in the CDC Proficiency Testing Program. The test results were in good agreement with the reference values.

δ-ALAD. Determination was by the method of Berlin and Schaller (1974). Briefly, 20 μl of whole blood was added into 0.48 mL redistilled water. The reaction was started by adding substrate (δ-ALA) into the hemolysate and incubated for 60 min at 38 °C. The reaction product (porphobilinogen) was determined using modified Ehrlich's reagents and measured at 555 nm. Enzyme activity was evaluated by determining the amount of porphobilinogen formed at 38 °C. One unit of ALAD activity was defined as increase of absorbance per 0.1 by 1 mL of blood. Results were reported in U/g hemoglobin. Hemoglobin concentration in blood was measured by the cyanmethemoglobin method using Drabkin's reagent.