Bull. Environ. Contam. Toxicol. (1989) 42:476–481

Bulletin of Environmental Contamination and Toxicology

Delta Aminolevulinic Acid Dehydratase and Ferrochelatase Activities during Chronic Lead Exposure in Mice

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Acute lead poisoning and chronic lead intoxication have occured among human beings since antiquity. Prevention of chronic lead toxicity is actually a matter of concern in the scientific community. Sources of exposure to lead are diverse and include: food and beverages (Batschelet et al. 1979) air from combustion of lead containing auto exhausts (Quinche et al. 1969), industrial emmissions (Simier & Chabert 1983), lead based paints (Siegel 1963) etc.

Lead stores in the body and its toxic effects on the nervous system, the kidney and the blood are well known (Albahary 1972, Kao 1973, Waldrom 1966, Moore et al. 1980). The metal can interfere in several steps in the heme biosynthesis pathway in the bone marrow resulting in anemia.

In this study, three biological markers of lead intoxication : blood aminolevulinic acid dehydratase (ALA-D) ; Free erythrocyte protoporphyrin (FEP) levels and blood Zinc protoporphyrin (ZPP) levels have been studied. This markers reflect the activity of two enzymes of this pathway: ALA-D in the smooth endoplasmic reticulum and ferrochelatase in the mitochondria. ALA-D is an enzyme within the erythrocyte that catalyzes the conversion of aminolevulinic acid to porphobilinogen and is a very sensitive parameter for the diagnosis of subclinical lead intoxication (Bonsignore 1973, Chiba 1976). Ferrochelatase catalyzes the incorporation of the ferrous ion into the porphyrin ring structure, and the depression of its activity results in depressed heme formation and accumulation of protoporphyrin IX (Nordman & Devars du Mayne 1984) mainly in the form of ZPP.

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According to the norms of the European Communities (28/7/82) the concentration of ZPP and lead in blood, should be monitored among lead exposed workers to prevent toxicity. In this work, lead accumulation in tissues (bone, liver, kidney and brain), ALA-D activity, FEP, ZPP and blood lead levels were monitored in Balb C + mice who were given 366 mg/L of lead in the drinking water during six months, until blood lead levels similar to those of some occupationally exposed populations were obtained.

MATERIALS AND METHODS

Sexually mature (9- week old) Balb C+ mice were purchased from IFFA-CREDO. They were housed in a room with a 12 hours light-dark cycle at 25ºC. They were fed standard laboratory chow (Panlab).To avoid over crowding, five animals were placed in each cage. The control group (N=30) was given deionized water and the treated group (N=30) a solution of lead acetate (Merck 7374) in deionized water. The concentration of lead acetate in drinking water was 366mg/L corresponding to an intake of 0.97 ± 0.12 mg of lead/animal/day. The duration of the exposure to lead was 6 months. Animals (5 controls and 5 treated) were anaesthetized with an intraperitoneal injection of sodium thiopental (Abbott Laboratoires, Spain) each month prior to sacrifice, and tissues (brain, liver, kidney and bone) were excised and frozen at -40º C until analysis. The blood was taken by cardiac puncture and was collected in polypropylene tubes, previously washed with HNO_3 10% (Merck Suprapur 441), and after treated with heparin.

Organs were digested with HNO_3 (Merck Suprapur 441) in pressure reactors (Phaxe 2005) at 75ºC during 24 hours and finally diluted (1/5) with deionised water. 1 mL of whole blood was diluted (1/5) with $(NH_4)_2HPO_4$ (5 g/L in Triton X-100 0.1% m/v).

Lead analyses of blood and organs were performed with an atomic absorption spectrophotometer (Perkin Elmer 3030) with graphite furnace and Zeeman background correction.

No differences were found between the results obtained with a standard addition method and those obtained with an external calibration method so the later was used due to its greater simplicity. Results were expressed in µg/g (wet tissue basis).

ZPP concentration in blood was analysed with a spectrofluorimeter (Perkin Elmer 3000) (Lamola et al. 1975).

ALA-D activity in blood was determined with a spectrophothometer (Gilford S III) (Granik et al. 1972).

Free erythrocyte porphyrin concentration in blood was determined with a spectrofluorimeter (Perkin Elmer 3000) (Piomelli et al. 1973) .

RESULTS AND DISCUSSION

Lead accumulation kinetics in organs during the six months of the study can be found in figures 1,2.

Lead concentration in the organs (kidney, liver and bone) rose significantly ($P < 0.05$ according to student´s t test) in the first month, and increased progessively reaching concentrations at the end of the study of 5.5 ± 0.4 µg/g in the kidney, 12.3 ± 0.3 µg/g in the liver, and 48.6 ± 4.0 µg/g in the bone. Lead concentration in brain did not increase significantly ($P > 0.05$) until the fourth month of treatment and finally reached a concentration of 0.45 ± 0.05 µg/g significantly different from controls ($P < 0.05$).

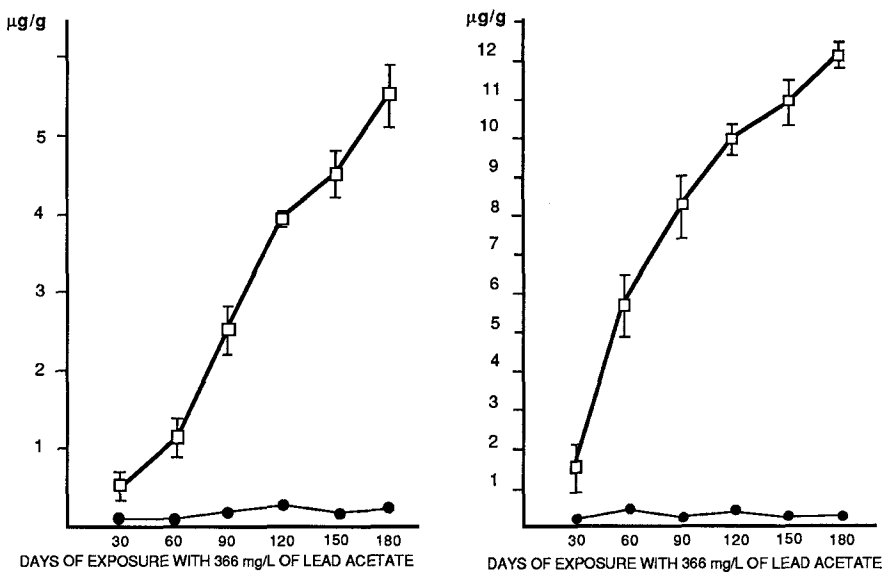

FIGURE 1. CONCENTRATION OF LEAD IN KIDNEY (LEFT) AND LIVER (RIGHT), (MEAN ± DS ; N= 5) OF CONTROL ● AND TREATED MICE □ DURING THE STUDY TIME COURSE

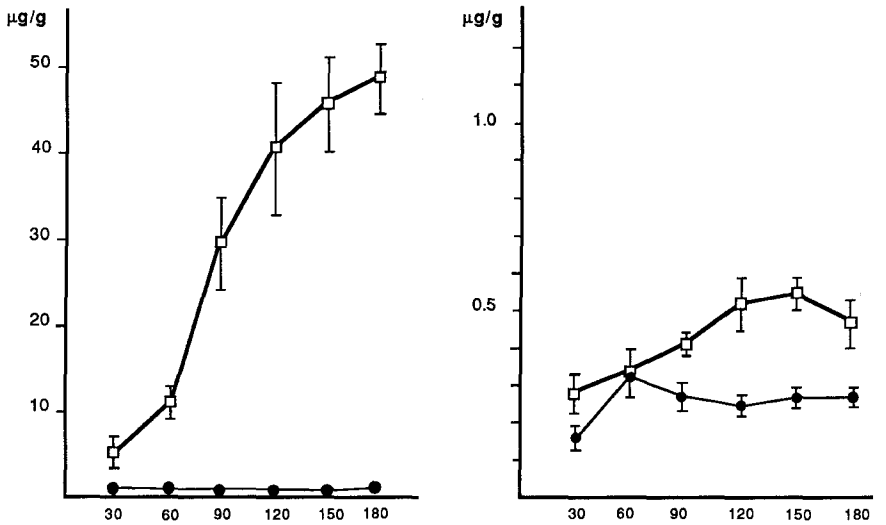

FIGURE 2. CONCENTRATION OF LEAD IN BONE (LEFT) AND BRAIN (RIGHT), (MEAN ± DS ; N= 5) OF CONTROL● AND TREATED MICE □ DURING THE STUDY TIME COURSE

ALA-D activity, FEP, ZPP and blood lead levels during the assay can be found in table I.

days	group	BLOOD LEAD	FEP	ZPP	ALA-D
30	CONTROL	5.0 ± 0.1	61.7 ± 3.8	4.5 ± 0.3	21.5 ± 3.1
	TREATED	61.6 ± 4.7	43.3 ± 4.9	3.1 ± 0.4	5.9 ± 3.9
60	CONTROL	5.0 ± 0.2	71.6 ± 4.6	4.9 ± 0.3	21.4 ± 2.7
	TREATED	56.9 ± 2.7	57.6 ± 12.6	3.7 ± 0.8	9.2 ± 2.2
90	CONTROL	4.9 ± 0.3	66.6 ± 7.5	4.7 ± 0.8	19.3 ± 2.6
	TREATED	67.3 ± 5.9	71.6 ± 9.4	5.2 ± 2.2	8.6 ± 1.9
120	CONTROL	5.9 ± 0.5	51.5 ± 4.5	3.8 ± 0.3	22.3 ± 3.2
	TREATED	58.4 ± 2.9	51.0 ± 18.0	3.4 ± 0.9	7.7 ± 0.9
150	CONTROL	6.5 ± 0.5	67.0 ± 11.5	4.7 ± 1.2	20.5 ± 2.4
	TREATED	48.2 ± 0.25	57.6 ± 4.1	4.1 ± 0.6	7.5 ± 2.4
180	CONTROL	4.5 ± 0.8	67.0 ± 16.8	4.7 ± 0.8	21.4 ± 3.2
	TREATED	60.0 ± 1.5	69.6 ± 5.8	5.1 ± 0.4	6.8 ± 1.7

TABLE I : CONCENTRATION OF LEAD IN BLOOD (µg/dL) , FEP (µg/g), ZPP (µg/g Hb), AND ALA-D ACTIVITY (U/L) DURING THE 6 MONTHS OF THE ASSAY. THE RESULTS ARE THE MEAN ± SD OF FIVE ANIMALS.

Lead concentration in blood in the treated animals rose significantly ($P < 0.001$) in the first month and was stable during the rest of the study with concentrations ranging from 48 to 67 µg/ dL ($X = 58 \pm 6.2$ µg/ dL).

ALA-D activity decreased significantly ($P < 0.001$) in the first month reaching values of 5.9 ± 3.8 U/L (controls : 21.5 ± 3.1 U/L). The difference was maintained during the assay without significant modifications.

FEP levels were unaffected by lead treatment and the same for the ZPP levels.

The difference beetwen the accumulation pattern in brain and that of the other organs studied (liver, bone, kidney) could be caused, in part, to effectiveness of the blood-brain barrier during the first period of the lead intake.

Lead concentration in the blood of the treated animals did not show modifications after the initial rise. This suggests that this parameter does not indicate lead accumulation in tissues but only the degree of lead recently absorbed. The same appears to be the case of the ALA- D which was reduced following the onset of lead intake but didn´t show a further reduction during the rest of the study.

ZPP and FEP levels were not affected in the treated animals suggesting that in conditions of chronic lead intake (the animals did not show overt signs of toxicity) there is not a reduction in the activity of ferrochelatase.

The different behavoir of ALA-D and ferrochelatase in this type of lead intoxication could be due to different sensitivities of these two enzymes to lead or to a different enzymes are located (ferrochelatase in the mitochondria, ALA-D in the cytoplasm).

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Received July 18,1988; accepted September 20 ,1988.