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UROPORPHYRIN ACCUMULATION IN THE BONE MARROW OF RATS EXPOSED TO LEAD

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The effect of lead on heme biosynthesis has been well documented in both human and experimental lead poisoning. Classic signs include significantly elevated excretion of δ-aminolevulinic acid (ALA) and coproporphyrin in the urine [1] and marked accumulation of protoporphyrin-IX in erythrocytes[2]. Such effects on various precursors of heme biosynthesis have been largely attributed to the inhibition by lead of the enzymes ALA-dehydratase and ferrochelatase [3]. However, lead poisoning may also be characterized by increases in the urinary excretion of porphobilinogen (PBG) [4,5], possibly resulting from an inhibition of the conversion of PBG to uroporphyrinogen. Furthermore, experiments conducted by Piper and Tephly [6] indicate that erythrocytic uroporphyrinogen I synthetase is potentially more sensitive to lead inhibition than hepatic uroporphyrinogen I synthetase, suggesting that the source of PBG excretion following lead poisoning may be from erythropoietic tissue such as bone marrow.

The objective of this project was to compare the effect of lead on the tissue levels and isomer composition of uroporphyrin in bone marrow, erythrocytes, liver and kidney. The initial choice for measurement of uroporphyrin levels and isomer composition was made because of the reported indications that the biosynthesis of uroporphyrinogen may be potentially rate-limiting for the biosynthesis of heme [7,8]. Thus, alterations of uroporphyrin levels and/or its isomer composition might be expected to be an early, sensitive response to potential tissue specific effects of lead exposure.

MATERIALS AND METHODS

Treatment of animals. Adult, male Sprague-Dawley rats (Sasco, Omaha, NE, 170-200 g) were housed in hanging cages in groups of three and were exposed to a 12-hr light/dark cycle (6:00 a.m. lights on; 6:00 p.m. lights off). Food (Purina Laboratory Rodent Chow, Ralston-Purina Co., St. Louis, MO) and water were provided *ad lib*. To induce lead poisoning, lead acetate (30 mg/kg), dissolved in 0.9% NaCl, was administered by i.p. injection at days 0 and 2 followed by decapitation at 7 days. Controls received equivalent volumes of 0.9% NaCl (4 ml/kg).

Preparation of tissues for porphyrin analysis. Kidneys and livers were rapidly excised, washed and perfused with 0.9% NaCl at 5°. Blood was collected in heparinized tubes and centrifuged at 2000 *g* for 15 min at 5°. The plasma and leukocytes were discarded. Erythrocytes were suspended twice with 0.9% NaCl at 5° followed by centrifugation at 1000 *g* for 10 min after each suspension. Bone marrow was flushed with 0.9% NaCl at 5° from the femurs of each animal and centrifuged at 1000 *g* for 10 min at 5°. The supernatant fraction was discarded and the cells of each marrow pellet were suspended twice with 0.9% NaCl and then centrifuged at 1000 *g* for 10 min at 5° after each suspension. Each tissue sample (approximately 1.0 to 1.5 g wet weight) was homogenized in 10 ml of 3 N HCl using a Potter-Elvehjem glass homog-

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enizer equipped with a motor-driven Teflon pestle. The homogenate was centrifuged at 1000 g for 15 min to remove cellular debris. The supernatant fraction was then centrifuged at 27,000 g for 30 min and the resulting supernatant fraction utilized for porphyrin analysis. Aliquots of the 27,000 g supernatant fraction, containing the porphyrins, were passed through a C18 bonded disposable solid phase extraction column from Analytichem, Inc. (Harbor City, CA) in order to isolate the porphyrins on the column's C18 packing material. Uroporphyrin was quantitatively eluted with 2 ml of HPLC-grade acetonitrile which was then evaporated to dryness under a nitrogen stream followed by reconstitution of the porphyrin in 0.4 ml of 1 M acetic acid. Uroporphyrin isomers were separated and quantitated by reverse phase HPLC according to the method of Wright and Lim [9]. All analyses were performed on a Waters Associates liquid chromatograph (model 6000A solvent delivery system and Rheodyne 7125 injector) interfaced with a Schoeffel model FS 970 fluorometer and equipped with a C18 bonded column (spherical 5 mm, 4.5 mm inner diameter x 25 cm) from IBM Instruments, Inc. (Danbury, CT). A C18 bonded-guard column (spherical 10 mm, 4.6 mm inner diameter x 3 cm) from Brownlee Labs (Santa Clara, CA) was located between the pump and the analytical column.

Statistical analyses. The significance of differences between means was performed by Student's t-test. The level of significance was chosen as $P<0.05$.

RESULTS AND DISCUSSION

The effect of lead on total uroporphyrin and its isomeric composition in bone marrow tissue is depicted in Fig. 1.

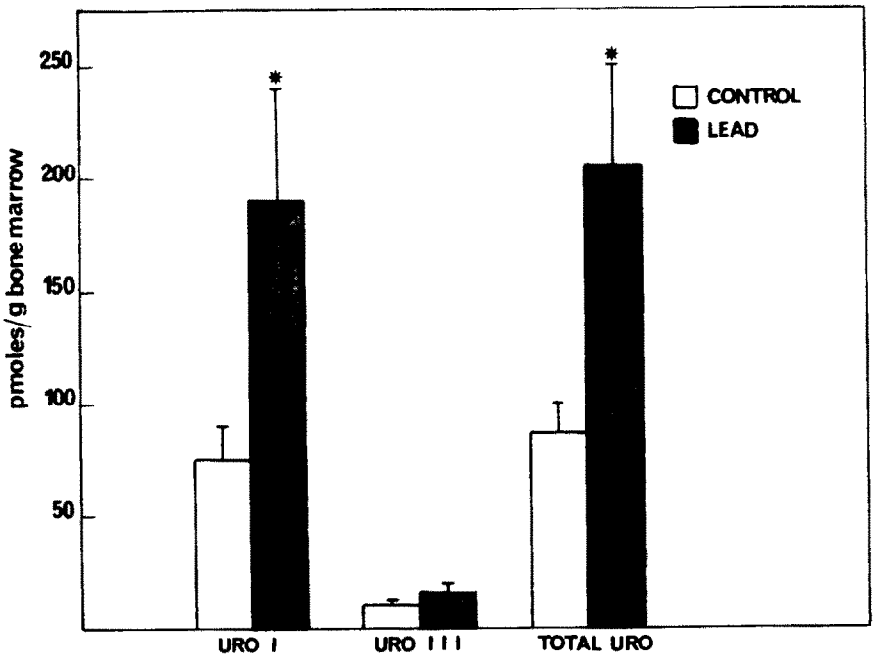

Fig. 1. Effect of lead on total uroporphyrin and its isomeric composition in rat bone marrow. Lead acetate (30 mg/kg) was administered i.p. at days 0 and 2 followed by sacrifice at day 7. Each bar represents the mean ± S.E.M. for three rats. *Significant difference ($P<0.05$) between lead-treated and control rats.

Total uroporphyrin was significantly higher than controls, and it was composed primarily of uroporphyrin I. These results suggest a sensitivity of bone marrow uroporphyrinogen III cosynthase to lead. Alterations of either uroporphyrin levels or isomer composition were not observed in liver, kidney or erythrocytes following administration of lead acetate as shown in Table 1. These findings suggest that the formation of uroporphyrinogen and the subsequent biosynthesis of heme in bone marrow may be more sensitive to lead acetate exposure than in liver, kidney or erythrocytes.

Table 1. Effect of lead on total uroporphyrin and its isomeric composition in liver, kidney and erythrocytes

Tissue	Control (pmoles/g tissue) I	III	Total	Lead (pmoles/g tissue) I	III	Total
Liver	54.0 ± 3.0	50.0 ± 4.0	104.0 ± 3.0	50.0 ± 5.0	53.0 ± 3.0	103.0 ± 4.1
Kidney	67.0 ± 4.0	77.0 ± 7.0	144.0 ± 5.0	65.0 ± 6.0	75.0 ± 7.0	140.0 ± 6.7
Erythrocytes	2.3 ± 0.2	1.6 ± 0.2	3.9 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	4.0 ± 0.3

Rats received lead acetate (30 mg/kg, i.p) at days 0 and 2 followed by sacrifice at day 7. Uroporphyrin tissue levels were determined as described in Materials and Methods. Each value is the mean ± S.E.M. of three determinations.

Further research studies are being conducted in order to understand the selective inhibition of the formation of uroporphyrinogen and the subsequent biosynthesis of heme in bone marrow following exposure to lead acetate.

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