Bull. Environ. Contam. Toxicol. (1997) 59:822-827

Bulletin of Environmental Contamination and Toxicology

Biomphalaria glabrata: Relevance of Albino Organisms as a Useful Tool for Environmental Lead Monitoring

N. R. Verrengia Guerrero,[1] M. N. Mozzarelli,[1] H. Giancarlo,[1] D. Nahabedian,[2] E. Wider[1]

[1]Department of Biological Chemistry, School of Science, Buenos Aires University, Fourth Floor Pab II, Ciudad Universitaria (1428), Buenos Aires, Argentina
[2]Department of Biological Science, School of Science, Buenos Aires University, Fourth Floor Pab II, Ciudad Universitaria (1428), Buenos Aires, Argentina

Received: 31 March 1997/Accepted: 18 August 1997

A great number of chemicals are continuously released into the environment causing pollution problems which are difficult to properly assess. The toxic effects of pollutants begin at the subcellular level as the result of noxious interactions with proteins, nucleic acids, organelles and cell membranes, causing disruptions in normal metabolic pathways and biochemical processes (Haux and Förlin 1988, Weinstein and Birk 1989). These types of biochemical effects may be considered early biomarkers of exposure and toxicity (Mayer *et al.* 1992). Therefore, during recent years, a great emphasis has focused on developing sensitive biomarkers as a useful tool for environmental studies (Haux and Förlin 1988, Mayer *et al.* 1992). Among them, quantification of enzyme activities in plants and animals may serve as valuable indicators of physiological alterations by chemicals (Mayer *et al* 1992, Stegeman *et al* 1992*).*

Studies have documented that several contaminants, both metal and organic compounds, are able to induce specific alterations in certain enzymes which are involved in heme biosynthesis (Stegeman *et al.* 1992). In this metabolic pathway, delta-aminolevulic dehydratase (ALA-D) (E.C.4.2.1.24) catalyses the condensation of two molecules of delta-aminolevulic acid (ALA) yielding porphobilinogen (PBG) plus water (Batlle *et al.* 1981). Lead inhibition of ALA-D activity has been demonstrated to correlate with the extent of metal exposure and absorption (Batlle *et al.* 1987, Stegeman *et al.* 1992). Several reports have found similar inhibition patterns in some aquatic species exposed to lead, such as fishes (Jackim 1974, Schmitt *et al.* 1993.)

Albino *Biomphalaria glabrata,* a pulmonate freshwater gastropod, has been recommended as a useful bioindicator organism for environmental studies (Münzinger 1987). These invertebrate molluscs also use hemoglobin as a respiratory pigment. To our knowledge, there is no information about enzyme activities of heme pathway in these organisms. Therefore, the aims of the present work were: a) to determine ALA-D activity in different tissues of albino *B. glabrata,* b) to investigate the modifications of enzyme activity in tissues of gastropods exposed to different levels of lead of environmental relevance employing acute bioassays (t=96 hr), and c) to correlate the extent of enzyme

Correspondence to: E. Wider

inhibition with metal bioaccumulation in those tissues.

MATERIALS AND METHODS

Adult albino gastropods *B. glabrata* were selected for this studies. The organisms were cultured under laboratory conditions in aereated glass aquariums (17-20 L) with dechlorinated tap water at a temperature of 20 ± 2 °C. *B. glabrata* were fed with lettuce leaves *ad libitum* (Fried et al. 1992) and were maintained under a 14/10 hr artificial light /dark cycle.

Eight animals of similar size (mean shell diameter 18 ± 2 mm, mean whole body weight 0.795 ± 0.100 g) were placed in small aquariums (3 L). Organisms were exposed to sublethal lead concentrations of 0.005, 0.025, 0.100 and 0.500 mg Pb/L employing acute bioassays (96 hr) without water renewal. Lead solutions for bioassays were prepared from appropriate dilutions of a lead nitrate stock solution containing 1,000 g Pb/L in dechlorinated tap water. Exposure levels were determined by atomic absorption spectrophotometry (AAS). For each level of exposure, three experiments were conducted for enzyme determinations and another three for lead accumulation analysis. Control organisms were exposed to dechlorinated tap water.

At the end of each bioassay, the animals were anesthetized by cooling them on ice during 6-8 minutes and sacrificed. Hemolymph was extracted by heart puncture and treated with Triton X-100 (final concentration 0.5%) to break cell membranes. Afterwards, the valves were removed and the whole body tissue was dissected under a stereoscopical microscope at 0 °C. Cephalopedal, lung, hepatopancreas and gonadal regions were pooled, due to their small size, and homogenized in 0.05 M Tris-HC1 buffer, pH = 7.4, containing 0.25 M sucrose (Stegeman *et al.* 1992). Homogenates were centrifuged at 100,000 x g for 60 minutes at 2 °C and the pellets were discarded. ALA-D activity was measured in the supernatants according to the method of Bustos *et al.* (1980). Enzymatic unit of ALA-D was defined as the amount of enzyme catalysing the formation of 1 µmol of PBG in 1 hr at 37 °C. Protein was determined using the method of Lowry *et al.* (1951) and bovine serum albumin as standard.

To determine the amount of lead accumulated in different tissues, samples were washed with double-distilled water, dried on filter paper, weighed and digested employing ultrapure concentrated nitric acid (2-3 mL per gram wet tissue) at 100-120 °C until the complete destruction of the organic material (Verrengia Guerrero 1995). The digested was diluted with 1% nitric acid solution (final volume = 5,0 mL) and filtered through filter paper to separate any remanent residue. Lead quantification was performed on the filtrate by flame AAS. Standard addition method was used to compensate for matrix effects. Appropriate standard solutions, in 1% nitric acid, were prepared by serial dilutions of a commercial stock solution containing 1,000 mg Pb/L. All reagents were from analytical grade.

RESULTS AND DISCUSSION

Table 1. shows the values of ALA-D activity in different tissues of control organisms of albino *B. glabrata*. Highest values were observed in gonadal and lung tissues, followed by cephalopedal region and then hepatopancreas. Enzyme activity was undetectable in the hemolymph. This result is in agreement with the fact that hemoglobin is not associated with red cells in the gastropod *B. glabrata,* but is found circulating as a free molecule in the hemolymph.

Table 1. ALA-D activity in control albino *B. glabrata*

Tissue	ALA-D* Units/mg prot.
Lung	40.5 ± 4.2
Hepatopancreas	8.5 ± 0.9
Cephalopedal	17.8± 2.0
Gonads	47.0 ± 1.1
Hemolymph	<0.2

(n = 3 experiments; enzyme activity was determined on 8 pooled tissues in each experiment)

* mean values ± standard deviation.

Figure 1 shows the percentage of enzyme activity in lung, hepatopancreas, cephalopedal and gonadal tissues of organisms exposed to lead concentrations of 0.005, 0.025, 0.100, and 0.500 mg Pb/ L. The highest level selected corresponds to the maximum permissible concentration of lead which can be present in industrial discharges released to freshwaters, according to the Argentine Law of Hazardous Wastes. At that level, ALA-D activity was very low in all the tissues analysed with the degree of enzyme inhibition varying between 82 and 87%.

In organisms exposed to 0.100 mg Pb/L, enzyme activity was lowest in hepatopancreas and cephalopedal regions. At 0.025 mg Pb/L, ALA-D activity was about 50% in lung, cephalopedal and gonadal tissues, but was much lower (about 30%) in the hepatopancreas. In gastropods treated with 0.005 mg Pb/L, no inhibition was found in lung and cephalopedal regions. At this lowest dose, enzyme activity was decreased about 10% in gonads while hepatopancreas showed a significant degree of inhibition of about 50%.

The results of lead accumulation in different tissues of *B. glabrata*, according to lead exposure, are shown in Table 2. At all the levels studied, the hepatopancreas concentrated the highest levels of metal after an acute treatment. Lower concentrations of lead bioaccumulation were observed in lung, cephalopedal and gonads.

According to the literature, significant inverse relationships and high

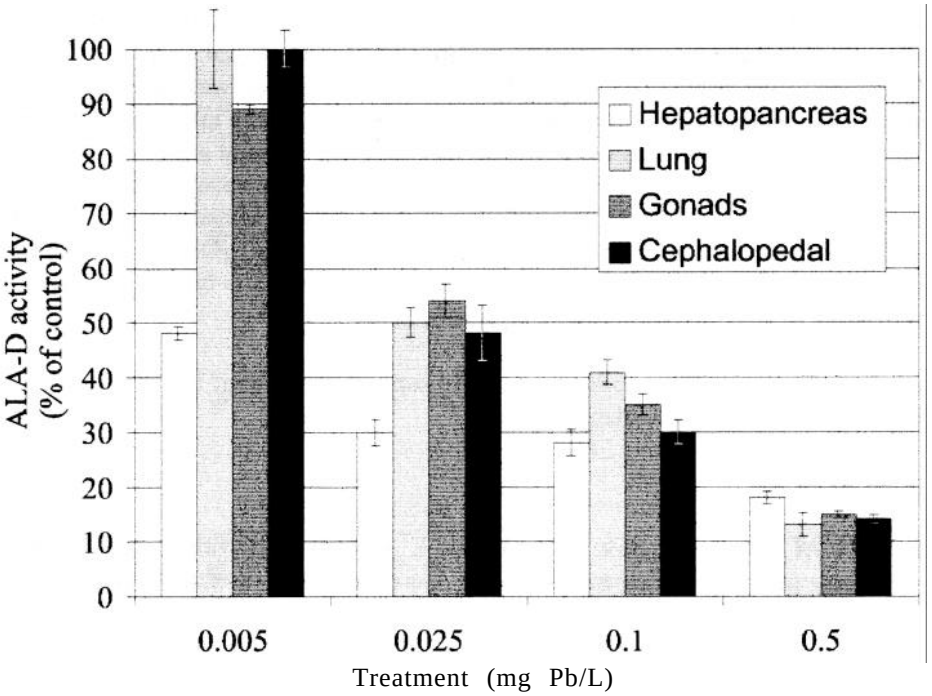

Figure 1. ALA-D Activity after acute lead treatment

concentratins factors have been demonstrated between ALA-D activity and erythrocyte lead levels in different vertebrates species (Hoffma *et al.* 1985, Schmit t*et al.* 1993, Bakall i*et al.* 1995). Red cells are generally absent in invertebrate organisms. Nevertheless, significant inverse relationships were also found in this study between ALA-D activity and tissue lead levels. The correlation factors were: $r^2 = 0.982$ for lung, 0.872 for gonads, 0.838 for cephalopedal, and 0.713 for hepatopancreas.

Table 2. Lead bioaccumulation in tissues o *B. glabrata* exposed to different metal levels.

Tissue	Levels of metal exposure (mg Pb /L)*			
	0.005	0.025	0.100	0.500
Lung	<0.1	3.1 ± 0.2	20.4 ± 3.3	51 ± 4
Hepatopancreas	3.9 ± 0.6	18.6 ± 3.3	72.0 ± 3.9	206 ± 23
Cephalopedal	<0.1	7.5 ± 1.3	17.4 ± 4.8	79 ± 13
Gonads	<0.1	2.7 ± 1.6	11.4 ± 1.6	59 ± 6

(n = 3 experiments; metal bioaccumulation was determined on 8 pooled tissues in each experiment)

* mean values ± standard deviation.

The results presented in this work have shown that ALA-D activity in tissues of albin o*B. glabrata* can be considered a sensitive biomarker of lead exposure. At the highest lead level selected (0.500 mg Pb/L), ALA-D inhibition was very high in all tissues analysed but also at the lowest metal level tested, the inhibition was still high in hepatopancreas. It is worth noting that all levels of metal exposure selected in this work are of environmental relevance and water quality concern. Since ALA-D activity may be considered a specific biomarker of lead exposure (Stegema *et* n *al.* 1992) enzyme determinations in tissues from albin *B. glabrata* should be included in field monitoring programs as a useful and sensitive bioassay to assess the content of lead contamination in aquatic systems.

Acknowledgements. This work was partially supported by Grant Ex-183 to Dr. E. Wider from University of Buenos Aires.

REFERENCES

Batlle A M del C, Magnin P H, Wider E A (1981) Porfirinas y Porfirias, etiopatogenia, clínica y tratamiento. Ed. Universitaria de Buenos Aires.

Batlle A M dl1 C, Fukuda H, Parera V E, Wider E, Stella A M (1987) In Inherited porphyrias, lead intoxication is a toxogenic disorder. Int J Biochem 19: 717-720.

Bakalli R I, Pesti G M, Ragland W L, Konjuska V, Novak R (1995) δ-aminolevulinic acid dehydratase: a sensitive indicator of lead exposure in broiler chicks (*Gallus domesticus*). Bull Environ Contam Toxicol 55: 833-839.

Bustos N, Stella A M, Wider E , Batlle A M de1 C (1980) Studies on erythrocyte aminolevulinate dehydratase.1. Its purification and possible therapeutic applications. Int J Biochem 12: 265-275.

Fried B, Rao K S, Sherma J (1992) Fatty acid composition o *Biomphalaria glabrata* (gastropoda: Planorbidae) fed hen's egg yolk versus leaf lettuce. Comp Biochem Physiol 101A: 351-352.

Haux C, Forlin L (1988) Biochemical methods for detecting e f contaminants on fish. Ambio 17: 376-380.

Hoffman D, Christian Franson J, Pattee O, Bunck C, Murray H (1985) Biochemical and hematological effects of lead ingestion in nestling american kestrels (*Falco sparverius*). Comp Biochem Physiol 80C: 431-439.

Jackim E (1974) Enzyme responses to metals in fish. In: Vernberg F J, Vernbeng W B (eds) Pollution and Physiology of Marine Organisms. Academic Press, Inc, New York, pp 59-65.

Lowr y O H, Rosebrough N J, Farr A L, Randall R J (1951) Protein determination with the Folin phenol reagents. J biol Chem 193: 265-275.

Mayer F L, Versteeg D J, McKee M J, Folmar L C, Graney R L, McCume D C, Rattner B A (1992) Physiological and nonspecific biomarkers. In: Huggett R J, Kimerle R A, Mehrie P M Jr, Bergman, H L (eds) Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress Lewis Publishers, pp 5-86.

Münzinger A (1987) Biomphalaria glabrata (say), a suitable organism for a biotest.

Environ Technol Lett 8: 141-148.
Schmitt S N, Wildhaber M L, Hunn J B, Tieger M N, Steadman B L (1993) Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocyte δ-aminolevulinic acid dehydratase activity in fish blood. Arch Environ Contam Toxicol 25: 464-475.
Stegeman J J, Brouwer M, Di Gulio R T, Forlin L, Fowler B A, Sanders B M, Van Veld P A (1992) Enzyme and protein synthesis as indicators of contaminant exposure and effect. In: Huggett R J, Kimerle R A, Mehrie P M Jr, Bergman, H L (eds) Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress Lewis Publishers, pp 235-336.
Verrengia Guerrero N R (1995) Contaminantes metálicos en el Rio de la Plata: Monitoreo del sistema acuático y estudio de algunos efectos tóxicos en moluscos bivalvos por medio de bioensayos. Doctoral Thesis. School of Exact and Natural Sciences, Buenos Aires University.
Weinstein D A, Birk E M (1989). The effects of chemicals on the structure of terrestrial organisms. In: Levin S A, Harwell M A, Kelly J R, Kimball K D (eds) Ecotoxicology: Problems and Approaches. Springer-Verlag, New York, Inc., pp 181-212.