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Freshwater molluscs from volcanic areas as model organisms to assess adaptation to metal chronic pollution

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Abstract

Cellular biomarkers of exposure and biological effects were measured in digestive gland of snails (*Physa acuta*) sampled in sites with and without active volcanism in São Miguel Island (Azores). Metal content in digestive cell lysosomes was determined by image analysis after autometallography (AMG) as volume density of autometallographed black silver deposits (Vv_{BSD}). Lysosomal structural changes (lysosomal volume, surface and numerical densities – Vv_{LYS}, Sv_{LYS} and Nv_{LYS}–, and surface-to-volume ratio – S/V_{LYS}–) were quantified by image analysis, after demonstration of β-glucuronidase activity, on digestive gland cryotome sections. Additional chemical analyses (atomic absorption spectrophotometry) were done in the digestive gland of snails. The highest metal concentrations were found in snails from the active volcanic site, which agreed with high intralysomal Vv_{BSD}. Digestive cell lysosomes in snails inhabiting sites with active volcanism resembled a typical stress situation (enlarged and less numerous lysosomes). In conclusion, the biomarkers used in this work can be applied to detect changes in metal bioavailability due to chronic exposure to metals (volcanism), in combination with chemical analyses.

Keywords: Volcanism; Exposure biomarkers; Biomarkers of effect; Snails

1. Introduction

Chemical analyses of tissues of sentinel species have been worldwide used to determine the bioavailable fraction of metals in aquatic environments. However, the accuracy of these analyses has been widely discussed because of the existence of a great number of variables affecting the estimation of metal bioavailability in terms of metal concentrations in soft tissues of molluscs (Fischer, 1986; Soto et al., 1995; Boening, 1999; Marigómez et al., 2002; Rainbow, 2002).

A biomarker approach based on cellular responses to pollutants in sentinel molluscs can be useful in biomonitoring programmes (Soto and Marigómez, 1997a,b). Changes occurring at cell or tissue levels are less affected by abiotic or biotic factors (i.e. salinity, temperature, season, changes in weight,...) and provide realistic and feasible indication of the fraction of bioavailable metals present in the environment and its biological effects (Marigómez et al., 2002).

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The digestive gland of molluscs is known to be the major target site for metal accumulation and detoxification (Herwig et al., 1989; Soto and Marigómez, 1997b; Marigómez et al., 2002). A main target cell compartment involved in metal metabolism and sequestration of other xenobiotics is the endolysosomal system of digestive cells (Marigómez et al., 1995; Dimitriadis and Papadaki, 2004).

Two well-established biomarkers were selected for the present study. Intralysosomal accumulation of metals revealed by autometallography (AMG) in digestive cells was used as biomarker of metal exposure. Changes in the structure of digestive cell lysosomes were measured as effect biomarker. AMG has been used in combination with image analysis to localize and quantify metals in cell compartments of invertebrate tissues as volume density of autometallographed black silver deposits (Vv_{BSD}), which has been proposed as a biomarker of metal exposure (Soto et al., 1996a,b, 2002; Soto and Marigómez, 1997a,b; Da Ros et al., 2000; Marigómez et al., 2002; Dimitriadis and Papadaki, 2004). AMG is a sensitive histochemical technique that only requires very few atoms of a given metal in the tissue to catalyse the deposition of metallic silver around them (Danscher, 1984).

Lysosomes are cell organelles with a high content of acid hydrolases devoted to the intracellular digestion of endogenous and exogenous compounds (Cajaraville et al., 1995a,b). Lysosomes have a crucial role in the detoxification of toxic substances, and for that reason changes in lysosomal structure have been used as general marker of pollutant induced stress in a number of field and laboratory studies using molluscs as sentinel organisms (Lowe et al., 1981; Moore, 1988; Cajaraville et al., 1991, 1995a,b; Etxeberria et al., 1994; Marigómez et al., 1996; Domouhtsidou and Dimitriadis, 2001, 2004; Marigómez and Baybay-Villacorta, 2003; Koukouzika and Dimitriadis, 2005).

It has been shown that, in aquatic organisms, contaminants cause a significant increase in lysosomal size that eventually can be accompanied by increases in lysosomes number (Lowe et al., 1981; Cajaraville et al., 1991, 1995a,b). Investigations on lysosomal responses in digestive cells of freshwater molluscs are more limited (Giamberini and Pihan, 1997; Giamberini and Cajaraville, 2005; Guerlet et al., 2006). Up to now most of the studies dealt with controlled laboratory exposures to metals or with field studies in areas with strongly marked dissimilar metal availabilities due to industrialisation and release of untreated or partially treated sewage. Few works have been done taking into account natural metal sources with no anthropic origin.

The Azores archipelago is remote from industrial activities despite of the appearance of an expanding tourism. However, the volcanic origin and the unusual geological features of the archipelago may well enhance a continuous availability of trace metals to biota. The archipelago is located in the North Atlantic Ocean at the triple junction of Eurasian, African and North American plates characterised by a complex tectonic settlement, where the seismic and volcanic phenomena are common (Nunes et al., 1993). Therefore, the remaining volcanic activity in certain sites of São Miguel, which is one of the nine islands comprising the Azores archipelago, provides a good "field-laboratory" for investigating the capacity of freshwater snails *Physa acuta* to cope with continuous input of natural metal sources during generations. The main objective of the present investigation is to determine whether freshwater molluscs living in volcanic areas are able to assess adaptation to metal chronic pollution based in the use of selected biomarkers of exposure and effect.

2. Materials and methods

2.1. Experimental design

Different populations of freshwater snails *Physa acuta* were collected from two different sites (Fig. 1) exhibiting dissimilar volcanic profiles at São Miguel: Tanque do Monte (TM) (4.01 m.y.), a site of volcanic origin that has no volcanic activity since 2 million years ago, and Lagoa da Furnas (LF) (0.75 m.y.), a sampling station located inside a crater of a still active volcano showing several active hydrothermal points.

2.2. Histological processing

A portion of the digestive gland of 10 snails per station was fixed in Bouin's fluid at 4 °C for 24 h (Martoja and Martoja-Pierson, 1970), dehydrated in ethanol (70% for 2 h; 96% for 2 plus 2 h; 100% for 2 plus 2 h), cleared in methylbenzoate (overnight), rinsed in benzene (45 plus 45 min) and embedded in paraffin (at 60 °C for 4 h). Histological sections (7 μm) were cut in a Leitz 1512 microtome (Leica Microsystems, Wetzlar, Germany), mounted in albumin coated slides (Menzel-Glaser, Braunscheig, Germany), dried at 40 °C for 24 h, and stored at room temperature until staining.

A second portion of the digestive gland was cryoprotected in phosphate buffer (0.1 M, pH=7.4) plus sucrose (0.5%), embedded in Cryo-M-Bed, frozen in liquid nitrogen and stored at −40 °C. Frozen samples