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Toxicological responses of *Laeonereis acuta* (Polychaeta, Nereididae) after acute, subchronic and chronic exposure to cadmium

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A B S T R A C T

The objective of this study was to analyze the toxicological responses of the estuarine polychaete *Laeonereis acuta* after acute (96 h), subchronic (7 days) and chronic (14 days) exposure to cadmium (Cd). Concentrations of metallothioneins (MT), lipid peroxidation (LPO), total Cd and metal-rich granules (MRG) were evaluated. Seasonal variations of MT and LPO levels in the wild were also measured. Polychaetes were obtained in the Quequén estuary located southeast of Buenos Aires Province, Argentina. For the acute toxicity assay, individuals were exposed to 10; 30, 65; 310; 600; 1300; 2000; 4300; 8100; 16300 μgCd L^{-1}, which included levels of environmental relevance and median lethal concentrations (LC_{50}) for related species of polychaete. Based on 96 h LC_{50} values, polychaetes were exposed to sublethal doses of Cd. The concentrations for both subchronic and chronic assays were: 10; 30; 65; 310; 600; 1300; 2000; 4300 μgCd L^{-1}. The 96 h LC_{50} value was 8234.9 μg L^{-1}, which was within the values reported for other species of polychaete, indicating a high tolerance to Cd. MT induction was not observed for any time exposure. In additoin, LPO levels showed no differences with respect to control levels, which indicated an absence of oxidative damage caused by Cd. However, the total Cd and MRG-Cd concentrations in *L. acuta* in all tested treatments showed significant differences with respect to control levels. *L. acuta* were able to accumulate Cd in their tissues in the form of granules which are the main mechanism of Cd detoxification.

1. Introduction

Cadmium (Cd) is a persistent toxic metal that is ubiquitous in aquatic environments. Sources of Cd to aquatic habitats include mine drainage, wastewater from metal smelting, runoff of agricultural fertilizers, and atmospheric fallout from fossil fuel combustion and refuse incineration (Timbrell, 2001; Martelli et al., 2006). Among marine ecosystems, shallow coastlines and estuaries are most affected by human activities because they are exposed to toxic anthropogenic effluents transported by rivers from remote and nearby urban, industrial and agricultural areas (Kennish, 2002). Sediments in these environments can act as a sink and source of many contaminants including Cd (Förstner and Wittmann, 1983; Dekov et al., 1998). As a result, benthic organisms living in close contact with sediments are particularly exposed to chemical stress.

Polychaetes, the most abundant taxon in benthic communities, are considered a suitable model species for the study of sediment and estuarine pollution (Nusetti et al., 2001; Pérez et al., 2004; Ait Alla et al., 2006; Rhee et al., 2007). In particular, polychaetes are commonly used in ecotoxicological studies due to their abundance, easy capture (Díaz-Jaramillo et al., 2011; Suriya et al., 2012; Won et al., 2012) and assimilation of heavy metals from sediments through their skin and intestine (Durou et al., 2005; Sun and Zhou, 2007). Cd is known to be toxic at low concentrations (Zang and Bolger, 2014). Many studies have reported enhanced lipid peroxidation and DNA damage after Cd treatment (Stohs and Bagchi, 1995; Tandon et al., 2003; Badisa et al., 2007).

Organisms have several defense mechanisms to toxic metals, and these systems can provide suitable biomarkers for the assessment of environmental stress (Won et al., 2012). Biomarkers are biochemical or physiological indicators of either exposure to or effects of environmental contaminants at the suborganism or organism level (Shugart et al., 1992; Livingstone, 1993: Sarkar et al., 2006). For this reason, biomarkers are regarded as early warning signals whose detection can avoid adverse effects (Van der Oost et al., 2003).

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The induction of metallothioneins (MT) in aquatic organisms has been recognized as a potential biomarker of heavy metal toxicity and bioaccumulation (Berthet et al., 2005; Amiard et al., 2006; Perceval et al., 2006; Martín-Díaz et al., 2007; Monserrat et al., 2007; Machreki-Ajmi et al., 2008). MT constitute a family of low molecular weight, cysteine-rich and heat stable proteins (Viarengo et al., 2007). The thiol (–SH) groups in MT enable them to bind heavy metals and sequester them in the organism. They play important roles in the homeostasis of essential metals such as zinc -Zn- and copper -Cu-, as well as detoxification of non-essential metals (e.g., Cd and mercury –Hg-) (Viarengo, 1989; Roesijadi, 1996; Vašák, 2005). MT induction is also considered an indicator of antioxidant processes and free-radical scavenging (Viarengo et al., 2000, 2007). Another mechanism of metal detoxification found in most invertebrates, including annelids, is precipitation into insoluble granules (or metal-rich granules; MRG) (Ng et al., 2008; Eisler, 2010; Khan et al., 2010). These granules are generally found in epithelial cells. Heavy metal cations are removed from the cytoplasm and sequestered within the vacuolar membrane in an insoluble, detoxified form. Subsequent cellular exocytotic events may extrude the granules from the cell followed by organismic excretory mechanisms that deposit the metal back into the environment (Fernandez and Jones, 1989; Ahearn et al., 2004).

Lipid peroxidation (LPO) is considered an important biomarker of cell damage resulting from the interaction of free radicals with membrane lipids (Barata et al., 2005). It has been used extensively to assess the detrimental effects of various pollutants, such as polycyclic aromatic hydrocarbons (Díaz-Jaramillo et al., 2011), fullerene and nanosilver (Marques et al., 2013) and Cd, Cu, Zn, Hg (Geracitano et al., 2002, 2004a, 2004b; Sandrini et al., 2006, 2008; Díaz-Jaramillo et al., 2011, 2013).

The deposit feeding polychaete *Laeonereis acuta* (Polychaeta: Nereididae) is a common infaunal species that has a wide distribution from Connecticut (United States) to the Northern Gulf of Mexico and from Santos (Brazil) to Golfo Nuevo (Patagonia, Argentina) (de Jesús-Flores et al., 2016). L. *acuta* is considered as a key estuarine species due to their influence on sediment properties and local diversity of species that prey on it (Botto et al., 1998; Palomo and Iribarne, 2000). Additionally, this species has been used as a biomonitor of heavy metals (Ferreira-Cravo et al., 2007; Geracitano et al., 2002, 2004a; Sandrini et al., 2006), as well as a model organism in toxicological assays (Geracitano et al., 2002, 2004b; Sandrini et al., 2006, 2008; Ferreira-Cravo et al., 2009; Marques et al., 2013).

Numerous toxicological studies have been carried out in the species, but there are few with regards to Cd (Sandrini et al., 2006, 2008) even though it is an ubiquitous and highly toxic pollutant. The objective of this study was to analyze the toxicological responses of *Laeonereis acuta* after acute, subchronic and chronic exposure of a wide range of Cd concentrations including levels typical of environmental concentrations.

2. Materials and methods

2.1. Sampling site

Polychaetes were obtained from Quequén Grande river (38°33′06.2″S – 58°43′30.5″W), located in the southeast of Buenos Aires province in Argentina. Its water drains into the Atlantic Ocean through an estuary where the Necochea-Quequén harbour is located. Significant urban and industrial activities are concentrated in the area as well as recreational activities. Sediments are composed of sand (70%), coarse silt (10.94%), fine silt (1.56%), clay (17.5%), organic matter (5.0%) (Chiodi et al., 2007), and the waters are alkaline (pH > 8 year round) (Carmona et al., 2011). Previous studies reported low levels of inorganic and organic contaminants in surface sediments (Chiodi et al., 2007; González et al., 2012, 2013) that were below levels established for the protection of aquatic life (Canadian Quality Guidelines for the protection of Aquatic Life –CSQG-, CCME, 2002).

2.2. Animals

Polychaetes (size > 4 cm in length) were manually collected in the estuarine zone at low tide with a shovel (0–25 cm depth) and then transported to the laboratory. Temperature, pH, and salinity were measured in situ by multiparametric monitor (Trademark Horiba, U-10). Individuals were acclimated in sediment for 4 days, and then in the test vessels without sediment for 6 days. During acclimation and bioassays, water was renewed every 48 h, maintaining constant conditions of temperature (18 °C), pH (8), salinity (10) and photoperiod (12:12 h light/dark). Individuals were fed ad libitum with frozen *Artemia* spp after each water renewal.

Animals were maintained in accordance with guidelines of the Institutional Committee for Care and Use of Laboratory Animals (CICUAL, acronym in Spanish) of Mar del Plata University, based on the "Guide for the Care and Use of Laboratory Animals" (2010, 8th Edition, National Research Council, The National Academies Press, Washington DC) and Directive 2010/63/UE of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2.3. Seasonal variation in MT and LPO

Natural variation in biomarker levels must be understood before they can be used in the laboratory (Mouneyrac et al., 2000). Different biotic and abiotic factors, including reproductive state, age, sex, temperature, salinity, and season may change levels of biochemical biomarkers whatever the contamination of the environment (Amiard et al., 2006). The influence of these factors on MT and LPO levels in polychaetes have been reported (Geracitano et al., 2004a; Gillis et al., 2004; Ait Alla et al., 2006; Díaz-Jaramillo et al., 2011; Gomes et al., 2013). These natural variations may interfere with the estimation of levels induced by pollutants, so seasonal sampling was conducted.

To establish the profile of seasonal variation of MT and LPO levels, the collections were initiated in May 2014 and extended until May 2015. The seasons were defined as spring (September, October and November), summer (December, January and February), fall (March, April and May) and winter (June, July and August). Polychaetes were captured in each season, cleaned (remnants of sediment and mucus), immediately frozen in three pools (n = 15 each) and stored at −80 °C.

2.4. Reagents

The stock solution of Cd (613.2 mgCd L^{-1}) was prepared from cadmium chloride ($CdCl_2$ ≥ 99.99%, Sigma-Aldrich Chemical Corporation USA) and double distilled water (ddH_2O). Each Cd concentration was prepared using a dilution of the stock solution. The analytical Cd concentrations of each treatment were measured by inductively coupled plasma spectrophotometry with an optical resolution (ICP-OES) at the beginning of the experiment (detection limit = 6 μgCd L^{-1}). A cadmium standard of 1000 mgCd ($CdCl_2$ in ddH_2O, Titrisol Merck) was used to prepare the calibration curve.

2.5. Acute toxicity assay

The experimental conditions for the acute assay (96 h) were based on Reish (1980) with water renewal every 48 h. Polychaetes were exposed to the following nominal concentrations of Cd: 10; 30; 65; 310; 600; 1300; 2000; 4300; 8100; 16300 μgCd L^{-1}, which included levels of environmental relevance and median lethal concentrations (LC_{50}) for other related species of polychaetes (EPA, 2001). A total of 10 individuals were used for each treatment and controls. The polychaetes were checked daily, and dead individuals were counted and removed. The absence of response to gentle mechanical stimulus was the criterion for death. Median lethal concentration was determined using the