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Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium in white shrimp, *Palaemonetes argentinus*

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ABSTRACT

Cadmium (Cd) is one of the most common pollutants in the environment and induces a range of tissue changes or damages and organ dysfunction. The histopathological effects of Cd and lipid peroxidation (LPO) on hepatopancreas of the freshwater shrimp, *Palaemonetes argentinus*, were studied. Shrimp were obtained from two lagoons with contrasting environmental quality, De los Padres (LP, impacted site) and Nahuel Rucá (NR, reference site), and were exposed to 3.06 and 12.24 μg Cd L^{-1} for 3, 7, 10 and 15 days. The health status of both populations was also evaluated by histological analysis of control individuals. After exposure, shrimp were transferred to clean water for 28 days to evaluate the recuperation capacity of hepatopancreas. Control shrimp from NR exhibited a normal hepatopancreas structure; unlike control shrimp from LP which showed several alterations. These results were attributed to the different environmental quality of lagoons. The exposure to Cd resulted in several alterations in the histological structure of the hepatopancreas of both populations. The observed alterations included haemocytic and connective infiltrations in the intertubular space, erosioned microvilli, ripple of basal lamina, atrophied epithelium and necrosis, however, the latter was only observed in shrimp from LP. The exposure also caused an increase of LPO levels in both populations. *P. argentinus* was able to repair the hepatopancreas structure from the damage caused by Cd, evidenced by the histopathological results and LPO levels. Obtained results are indicating that the histological analysis of the hepatopancreas proved to be a highly sensitive method for evaluating water quality, in both environmental and laboratory conditions.

1. Introduction

The hepatopancreas is the major metabolic organ in decapod crustaceans, accomplishing intestinal, hepatic, and pancreatic functions (Saravana Bhavan and Geraldine, 2000). It is the main site of synthesis and secretion of digestive enzymes, absorption of nutrients, storage of metabolic reserves (lipid and glycogen), excretion of metabolic wastes (Al-Mohanna and Nott, 1989; Johnston et al., 1998) and biotransformation and detoxification of pollutants. The crustacean hepatopancreas is a sensitive organ and liable to injury by pollutants (Vogt, 1987; Bautista et al., 1994; Saravana Bhavan and Geraldine, 2000; Wu et al., 2008). It is essentially composed of branched tubules and of different types of epithelial cells (E-cells, R-cells, F-cells and B-cells) lining the tubules. It is possible to use the patterns of change in the cells of the organ as an index to determine the impact of contaminants (Hinton et al., 1973; Moore, 1985; Sousa, 2003). The histological diagnosis is a highly sensitive method showing the integral response of an organism to the impact of a toxicant under certain physiological, nutritional and environmental conditions (Vogt, 1987). Another advantage of histopathological diagnosis relates to its ability to effectively provide information on the health status of the organism (Costa et al., 2013). Many pollutants have been demonstrated to have hepatopancreatic toxicity, such as pesticides (Saravana Bhavan and Geraldine, 2000; Desouky et al., 2013; Walker et al., 2010) and heavy metals (Frías-Espericueta et al., 2008; Liu et al., 2013; Wu et al., 2008), resulting in histological alterations.

Cadmium (Cd) is a ubiquitous metal in the environment and is released into the atmosphere, water and soil from industry,

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agriculture and other human activities. In aquatic ecosystems, Cd has a high solubility in water and a very high capacity to bioaccumulate in many species. It is not essential to organisms, and indeed, it is known to be toxic at low exposure concentrations (Zang and Bolger, 2014). It has been demonstrated that Cd inhibits the repair of the DNA and causes lipid peroxidation (Badisa et al., 2007).

Lipid peroxidation (LPO) is considered as an important biomarker of cell damage as a result of the interaction of free radicals with membrane lipids (El-Beltagi and Mohamed, 2013). It has been used extensively to assess detrimental effects of several pollutants, such as polycyclic aromatic hydrocarbons (Lavarías et al., 2011), endosulfan, (Ballesteros et al., 2009), cadmium, copper and zinc (Khan et al., 2011).

Histological alterations with other biomarkers can be used as tools to evaluate the toxicity of environmentally relevant chemicals in both a predictive and a retrospective way (Odendaal and Reinecke, 2003).

The shrimps of the genus *Palaemonetes* (Crustacea: Decapoda: Caridea) are considered as important animal models to be employed in the evaluation of pollution effects (Buikema et al., 1980, Key et al., 2006). Among them, the white shrimp *Palaemonetes argentinus* is an abundant freshwater shrimp, widely distributed in different countries of South America (Morrone and Lopreto, 1995). Some publications have reported the sensitivity of *P. argentinus* to pollution in laboratory tests (Collins and Capello, 2006; Chiodi Boudet et al., 2013; Galanti et al., 2013) and proposed that this species might be used as a bioindicator to provide information on environmental quality (Montagna and Collins, 2007).

Histological changes as a result of the exposure to organic and inorganic pollutants has been described in shrimp species (Doughtie and Rao, 1984; Vogt, 1987; Saravana Bhavan and Geraldine, 2000; Kutlu et al., 2005; Wu et al., 2008), however, there are few related studies on Cd effects on the histological structure of hepatopancreas, even though it is a common pollutant (Wu et al., 2008; Liu et al., 2013).

Therefore, the objective of this study was to determine the histopathological alterations induced by Cd in hepatopancreas of the freshwater shrimp *P. argentinus* from both polluted and unpolluted lagoons. The health status of both populations was evaluated by analyzing control individuals. The LPO was measured as biochemical evidence of the hepatopancreatic cellular damage. Recovery capacity of hepatopancreas was also evaluated.

2. Materials and methods

2.1. Collection and maintenance of organisms

Shrimp were obtained from two shallow lagoons situated in the southeastern area of Buenos Aires Province, Argentina. One of these sites (Nahuel Rucá lagoon – NR – 37°37'S-57°25'W) is considered as an unpolluted environment (Chiodi Boudet et al., 2010), whereas the other lagoon (De los Padres lagoon – LP – 37°57'S, 57°44'W) was previously characterized as a polluted environment (Chiodi Boudet et al., 2008). Sediments of LP lagoon have high metal concentrations (0.7 $\mu g\ Cd\ g^{-1}$; 1 $\mu g\ Hg\ g^{-1}$; 72 $\mu g\ Cr\ g^{-1}$; 15.3 $\mu g\ As\ g^{-1}$, 119 $\mu g\ Zn\ g^{-1}$; Chiodi Boudet et al., 2008) that exceed levels considered as safe for the biota (Cd: 0.6 $\mu g\ g^{-1}$, Hg: 0.17 $\mu g\ g^{-1}$, Cr: 37.3 $\mu g\ g^{-1}$, As: 5.9 $\mu g\ g^{-1}$, Zn: 123 $\mu g\ g^{-1}$; CCME, 2002). In contrast, sediments of NR have low metal concentrations (0.15 $\mu g\ Cd\ g^{-1}$; 0.02 $\mu g\ Hg\ g^{-1}$; 28 $\mu g\ Zn\ g^{-1}$; Chiodi et al., 2010), making to this area a good reference site. Previous studies conducted in these two shrimp populations revealed different tolerance to Cd, as a consequence to the environmental quality (Chiodi Boudet et al., 2013).

Shrimp (adults of both sexes, at sexual rest) were collected with a hand net and immediately transferred to the laboratory. Acclimation was performed in aquaria (140 L) with gently aerated freshwater and 12:12 h light/dark photoperiod for 3 days, as recommended for genus *Palaemonetes* by Buikema et al. (1980). Water temperature was maintained at 17.0 ± 0.9 °C, pH at 8.30 ± 0.05 and water hardness was 235 mg $CaCO_3\ L^{-1}$. Shrimp were daily fed with flake food (Tetramin), and the content of Cd was $< 0.05\ \mu g\ Cd\ g^{-1}$. During acclimation, those groups that had more than 2% mortality were not used for the experiments following the criteria establish by Khan et al. (1988).

The animals were cared for 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.

2.2. Reagents

The stock solution of Cd (613 mg $Cd\ L^{-1}$) was prepared from cadmium chloride ($\geq 99.99\%$, Sigma-Aldrich Chemical Corporation, USA) and double distilled water (ddH_2O). The different Cd concentrations assayed were prepared using a dilution series of the stock solution. The analytical Cd concentrations of each treatment at the beginning of the experiment were measured by Anodic Stripping Voltammetry (ASV), applying a modification of the technique described by Andrade et al. (2006) with a detection limit $< 5\ \mu g\ Cd\ L^{-1}$. A commercial standard of Cd (1000 mg Cd, $CdCl_2$ in H_2O, Titrisol Merck) was used for calibration.

2.3. 15 Days exposure and depuration assay

For each experiment, shrimp were randomly divided into 16 groups (exposure + depuration) allocated to controls and treatments. Each experimental treatment consisted of 100 shrimps, which were transferred into 20 L experimental glass aquaria.

The exposure concentrations were selected based on previous studies and LC_{50} values for Cd of each shrimp population (LC_{50}-96 h: 24.50 and 12.26 $\mu g\ Cd\ L^{-1}$ for LP and NR population, respectively) (Chiodi et al., 2013). Therefore, the shrimp of LP population were exposed to 3.06 and 12.26 $\mu g\ Cd\ L^{-1}$ (corresponding to 1/8 and 1/2 of the 96 h LC_{50}) for 3, 5, 7 and 15d. The shrimp of NR population was only exposed to 3.06 $\mu g\ Cd\ L^{-1}$ (1/4 of the 96 h LC_{50}) due to the concentration 12.26 $\mu g\ Cd\ L^{-1}$ is lethal for 50% of exposed population at 96 h. The shrimp were fed in the exposure tanks before the renewal of the medium; retiring uneaten food immediately. After exposure, shrimp were transferred to clean water for 7, 14, 21 and 28 days of depuration. Each exposure and depuration treatment had its corresponding control. The exposure medium was renewed every 48 h during the course of experiments.

All other conditions were kept the same as those used for acclimation.

2.4. Histopathological observation

For the histopathological studies, 4 individuals in intermoult were selected from each treatment. The moult stage was determined by microscopic examination of the developmental stage of the exopodite setae of the uropods, following the criteria established by Díaz et al. (1998).

Each hepatopancreas was carefully dissected and immediately fixed in Davidson's solution (ethanol, formol, acetic acid and