Ecotoxicology and Environmental Safety 130 (2016) 29–36

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of ecologically relevant concentrations of cadmium in a freshwater fish

L.S. Pereira [a], J.L.C. Ribas [a], T. Vicari [b], S.B. Silva [e], J. Stival [e], A.P. Baldan [c], F.X. Valdez Domingos [d], M.T. Grassi [e], M.M. Cestari [b], H.C. Silva de Assis [a,*]

[a] Department of Pharmacology, Federal University of Parana, Brazil
[b] Department of Genetics, Federal University of Parana, Brazil
[c] School of Agricultural Sciences and Veterinary Medicine, Pontifical Catholic University of Parana, Brazil
[d] Nacional Institute of Amazon Research (INPA), Brazil
[e] Department of Chemistry, Federal University of Parana, Brazil

ARTICLE INFO

Article history:
Received 19 September 2015
Received in revised form
30 March 2016
Accepted 30 March 2016
Available online 7 April 2016

Keywords:
Metal
Oxidative stress
Rhamdia quelen
Biomarkers
Genotoxicity

ABSTRACT

Sub-chronic effects of ecologically relevant concentrations of cadmium (Cd) were evaluated in the catfish *Rhamdia quelen*. The fish were exposed to Cd (0, 0.1, 1, 10 and 100 μg L^{-1}) for 15 days. Bioconcentration was observed in the liver of fish exposed to 10 and 100 μg L^{-1} of cadmium. The liver glutathione S-transferase activity decreased at 0.1 and 1 μg L^{-1} and increased at 100 μg L^{-1} and lipoperoxidation increased in all tested concentrations. Fish exposed to 0.1, 1 and 100 μg L^{-1} Cd presented increase in hepatic lesion index. In the kidney, the catalase activity and LPO reduced in all exposed groups. The gluthatione peroxidase, etoxiresorufin-*O*-deethylase activities and metallothionein increased at the highest concentration of Cd, but the level of reduced glutathione decreased. The genotoxicity was observed at 0.1 and 100 μg L^{-1}. Neurotoxicity was not observed. The results showed that low concentrations (range of μg L^{-1}) of Cd caused hepato-, nephro- and hematological alterations in this freshwater fish species.

1. Introduction

Cadmium (Cd) is a non-essential metal for biological systems. It can produce adverse effects due to its high toxicity even in low concentrations and its potential of bioaccumulation in the food-chain (Ensafi et al., 2006). This metal can reach the environment from the natural weathering of minerals, forest fires, and volcanic emissions and by human activities such as mining, fuel combustion, disposal of metal-containing products, and application of phosphate fertilizer (Kaličanin, 2009). It has been detected in the waters of several countries, as Tunisia (Barhoumi et al., 2009), Belgium (Bervoets et al., 2009; Reynders et al., 2008), and Canada (Tompsett et al., 2014). In Brazil, concentrations above the permitted limits (1 and 10 μg L^{-1} of cadmium) by the National Council of the Environment (CONAMA, 2005) was found in freshwater (Barbosa et al., 2010; Rietzler et al., 2001).

Cadmium is not a redox active metal and does not participate in the Fenton reaction; however it is involved in the formation of reactive oxygen species (ROS). One of the possible mechanisms may be the indirect increase of ROS, through the displacement of ions such as iron and copper, which are redox active metals and could cause increased ROS via the Fenton reaction (Filipič, 2012). Therefore, the cadmium can accumulate in tissues and increase the production of the ROS. These can react with biological molecules such as proteins or lipids, causing lipid peroxidation (LPO), protein carbonylation (PCO), antioxidant and biotransformation systems alterations (Cao et al., 2010; Cao et al., 2012; Liu et al., 2011; Pretto et al., 2011; Roméo et al., 2000), and histopathological lesions.

Besides the antioxidant enzymes, other environmental contamination biomarkers can be used as tool to evaluate the organism response to pollutants, such as acetylcholinesterase activity for neurotoxic effects. This enzyme is responsible for the degradation of the neurotransmitter acetylcholine in the synaptic cleft. Its inhibition is strongly associated with exposure to organophosphorus and carbamate insecticides (Cunha et al., 2007; Guiloski et al., 2013; Mela et al., 2013), but other environmental pollutants as metals can also alter its activity (Pretto et al., 2010).

The potential genotoxicity of the metals can be evaluated by the piscine micronuclei and also by nuclear morphological alterations (NMA) (Vicari et al., 2012). Some studies also related the

* Correspondence to: Federal University of Parana, P.O. Box 19031, 81531-990 Curitiba, Parana, Brazil.
E-mail address: helassis@ufpr.br (H.C. Silva de Assis).

http://dx.doi.org/10.1016/j.ecoenv.2016.03.046
0147-6513/

effects of metals and other contaminants with alterations in blood cells as leukocytes, thrombocytes and erythrocytes (Carvalho and Fernandes, 2006). The hematological parameters are biomarkers to assess the health of an organism (Oost et al., 2003; Tavares-Dias et al., 2002) and was also evaluated in this study.

The silver catfish (*Rhamdia quelen*, Quoy and Gaimard) was used as experimental model. It is a omnivorous fish, endemic from South America and presents economic importance in the Southern Brazil (Barcellos et al., 2001). The responses of this species after environmental pollutants exposure have been studied for some authors (Becker et al., 2009; Mela et al., 2013; Miron et al., 2005), however, there are few studies evaluating their responses to Cd (Pretto et al., 2011, 2010), mainly in low concentration how was evaluated in this study.

Since the Cd has been finding in aquatic environment the aim of this study was evaluate biochemical, hematological, histopathological and genotoxic biomarkers, and Cd bioconcentration, in *R. quelen* exposed to different concentrations of this metal.

2. Materials and methods

2.1. Animals and experimental design

Fifty adult males of *R. quelen* (weight 118.03 ± 58.60 g and length 22.67 ± 4.08 cm) were obtained from an Aquaculture Research Laboratory. The fish were acclimated in 100 L tanks in the laboratory conditions (filtered water, aeration constant, temperature 26 ± 2 °C, photoperiod of 12 h) for 30 days. They were fed once a day during all experiment (commercial fish pellets 35% crude protein, Primor, Brazil). After the acclimation period, the fish were exposed, in 100 L tanks with 10 animals per tank, to different Cd concentrations (0, 0.1, 1, 10 and 100 $\mu g\ L^{-1}$). The concentrations of 1 and 10 $\mu g\ L^{-1}$ correspond to the limits allowed for freshwater in Brazil (CONAMA, 2005). The other two concentrations are 10 fold lower and 10 fold higher than the allowed concentrations. One third of the water was replaced daily with the initial Cd concentration for each tank. All procedures and protocols were approved by the Institutional Ethical Committee for Animal Use, under certificate number 524.

During the exposure the water samples (1 L) were collected in polypropylene vials for chemical analysis, and stored (4 °C) until analysis. After 15 days of exposure, fish were anesthetized with benzocaine 1%. The blood was taken from caudal vein puncture and fish were euthanized by spinal cord section. Total and liver weights were measured to calculate the hepatosomatic index (HSI). The brain, a fragment of the muscle, liver and posterior kidney were sampling for biomarker analyses.

2.2. Water analyses

The water physic-chemical parameters such as pH, alkalinity, dissolved oxygen, total ammonia, toxic ammonia, dissolved organic carbon (DOC) and temperature were measured.

Cadmium concentrations in water were determined in a Graphite Furnace Atomic Absorption Spectrophotometer (AA 6800, Shimadzu, Japan) using a pyrolytic graphite tube, with a heating time of 57 s (drying at 150° C, pyrolysis at 500° C, atomization at 2200° C, and cleaning at 2400° C), current of 8 mA, wavelength at 228.8 nm, slit width of 1.0 nm. Background correction was carried out with a D_2 lamp. Before the determination, water samples were digested according to the procedure described by Sodré et al. (2004).

2.3. Cadmium bioconcentration

Pools of liver and muscle were digested in nitric acid (65%) and hydrogen peroxide (30%) for analysis of the cadmium bioconcentration. The method used was the ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) and the analyses were carried out by Center of Food Research and Processing at Federal University of Parana (CEPPA-UFPR).

2.4. Biochemical biomarkers

The liver (70 mg) and the posterior kidney (70 mg) were homogenized at 1:5 (w/v) in Tris–HCl/ saccharose buffer (20 mM/ 500 mM) at pH 8.6, centrifuged at $15.000 \times g$ for 30 min, at 4 °C, and the supernatant was used for determination of metallothionein (MT) concentration at 412 nm, according to Viarengo et al. (1997). The other biochemical analysis were performed with 100 mg of tissue homogenized at 1:10 (w/v) in phosphate buffer (0.1M) at pH 7.0 and centrifuged at $15.000 \times g$ for 30 min, at 4 °C. The supernatant was used for measurement of some biochemical biomarkers. The catalase (CAT) activity was assayed according to Aebi (1984), at 240 nm, with 20 mM H_2O_2 solution; the superoxide dismutase (SOD) activity was measured at 440 nm using the method described by Gao et al. (1998), where 1 U SOD represents 50% inhibition of pyrogallol autoxidation. The glutathione peroxidase (GPx) activity was measured at 340 nm as described by Hafeman et al. (1974), with 5 mM H_2O_2, 2 mM GSH, 0.2 mM NADPH and 1 U mL^{-1} of glutathione reductase; the glutathione S-transferases (GST) activity was assayed according to Keen et al. (1976), with 3 mM GSH, at 340 nm. The ethoxyresorufin-*O*-deethylase (EROD) activity was assayed by fluorimetry at 530 nm of excitation and 590 nm of emission, as described by Burke and Mayer (1974) with modifications, using 2.6 μM of 7-etoxiresorufin and 2.6 mM of NADPH.

The GSH concentration was measured at 415 nm according to Sedlak and Lindsay (1968); the lipid peroxidation (LPO) levels was assayed at 570 nm, using the ferrous oxidation – xylenol (FOX) assay (Jiang et al., 1992); and the protein carbonilation (PCO) was measured at 360 nm by derivatization of the protein carbonyl groups with 2,4-dinitrophenol hydrazine to yield dinitrophenyl hydrazones (Levine et al., 1994).

The brain and the muscle (100 mg) were homogenized at 1:10 (w/v) in phosphate buffer (0.1 M) at pH 7.5, and centrifuged at $12.000 \times g$ for 20 min, at 4 °C. The supernatant was used for the measurement of the acetylcholinesterase (AChE) activity at 405 nm, according to Ellman et al. (1961), modified by Silva de Assis (1998), with 9 mM ATC (acetylthiocholine) and 0.75 mM DTNB.

The Bradford's method (Bradford, 1976), with bovine serum albumin as the standard, was used for determination of the protein concentration at 595 nm in all tissues.

2.5. Histopathological biomarker

A fragment of liver was fixed in ALFAC solution (ethanol 80%; formaldehyde 40% and glacial acetic acid) for 16 h, dehydrated in a graded series of ethanol and embedded in Paraplast Plus resin (Sigma®). Sections of the 5 μm were stained in Hematoxilin/Eosin and observed in a photomicroscope. The liver lesion index was determined according to Bernet et al. (1999), using the formula:

$$\text{Lesion index} = \sum (S \times IF),$$

Where S = score value attributed according to the extension of each observed lesion, and IF = importance factor.