Aquatic Toxicology 88 (2008) 250–256

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Biochemistry, cytogenetics and bioaccumulation in silver catfish (*Rhamdia quelen*) exposed to different thorium concentrations

Lizelia M. Correa[a], Daiani Kochhann[a], Alexssandro G. Becker[a], Maria A. Pavanato[a], Susana F. Llesuy[b], Vania L. Loro[c], Alice Raabe[c], Márcia F. Mesko[c,d], Érico M.M. Flores[c], Valderi L. Dressler[c], Bernardo Baldisserotto[a,*]

[a] *Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[b] *Departamento de Química Analitica y Fisicoquímica, Universidad de Buenos Aires, Buenos Aires, Argentina*
[c] *Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[d] *Pólo em Cruz Alta, Universidade Estadual do Rio Grande do Sul, 98025-810 Cruz Alta, RS, Brazil*

ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 29 April 2008
Accepted 1 May 2008

Keywords:
Fish
Oxidative stress
Micronuclei
Ecotoxicology
Bioaccumulation

ABSTRACT

The objective of this study was to evaluate the effect of thorium (Th) bioaccumulation on the metabolism of silver catfish (*Rhamdia quelen*) through biochemical parameters of the muscle (glycogen, glucose, lactate, protein, and ammonia). In addition, lipidic peroxidation levels (TBARS), catalase (CAT) and glutathione-*S*-transferase (GST) in the gills and in hepatic and muscular tissues were also analyzed. Cytogenetic parameters were studied through the evaluation of nuclear abnormalities in red blood cells. Silver catfish juveniles were exposed to different waterborne Th levels (in $\mu g\,L^{-1}$): 0 (control), 25.3 ± 3.2, 69.2 ± 2.73, 209.5 ± 17.6, and 608.7 ± 61.1 for 15 days. The organs that accumulated the highest Th levels were the gills and skin. The increase of waterborne Th concentration corresponded to a progressive increase of Th levels in the gills, liver, skin and kidneys, with the highest accumulation in the gills and skin. Metabolic intermediates in the muscle were altered by Th exposure, but no clear relationship was found. CAT and GST activities in the hepatic and muscular tissues of this species suggest that the enzymatic activities can be stimulated at the lowest Th levels and inhibited at the higher levels (mainly in $608.7\ \mu g\,L^{-1}$). The results of the cytogenetic assay contribute to this hypothesis because the higher toxicity in blood samples was found in juveniles exposed to 69.2 and $209.5\ \mu g\,L^{-1}$ Th.

1. Introduction

Thorium (Th) is a natural element present in the aquatic environment. It is used in several anthropogenic activities, and it has the potential for use as a nuclear fuel (Poston, 1982). Thorium occurs predominantly as a tetravalent cation, and it is a trace constituent in phosphates, simple and multiples oxides, and silicates. It is the main element in thorianite (ThO_2) and thorite ($ThSiO_4$), among other minerals (Ivanovich and Harmon, 1982). Thorium is almost insoluble in water, but it may be adsorbed in particulate and suspended material within water (Langmuir and Herman, 1980). The level of adsorption ranges from 0.003 to $1.72\ \mu g\,L^{-1}$ for waterborne Th and 183 to $3445\ \mu g\,g^{-1}$ for Th in suspended solids (Tonetto and Bonotto, 2002). Thorium can also be found in abundance in lakes that originated from the mining of other metals, which are sometimes converted for use in aquaculture. Yusof et al. (2001) found concentrations up to $297\ \mu g\,L^{-1}$ of dissolved Th in lakes of tin mining.

Cellular biomarkers are important tools for evaluating fish exposure to toxic agents that can alter oxidative balance and the structure and function of vital organs and that can even result in death (Radi and Matkovics, 1988; DiGiulio et al., 1989; Mather-Mihaich and Di Giulio, 1991; Au et al., 1999; Ahmad et al., 2000). Oxidative stress is defined as an imbalanced state between pro-oxidants and antioxidant agents with a potential for producing deleterious effects that can damage macromolecules such as DNA, lipids, and proteins (Halliwell and Gutteridge, 1999). Enzymes and others compounds of non-enzymatic nature provide an antioxidant defense system, which protects against this oxidative stress. Catalase (CAT) and glutathione-*S*-transferase (GST) are two important antioxidant enzymes of this system (Storey, 1996; Trenzado et al., 2006). Among the tests used to investigate genotoxicity, the micronuclei (MN) test has been shown to be a sensitive indicator of chromosome damage and has been used with success (Al-Sabti et al., 1994; Al-Sabit and Metcalfe, 1995).

* Corresponding author. Tel.: +55 55 3220 9382; fax: +55 55 3220 8241.
E-mail address: bernardo@smail.ufsm.br (B. Baldisserotto).

0166-445X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2008.05.003

Currently, there is only one study focused on Th exposure in aquatic animals (rainbow trout, *Oncorhynchus mykiss*) in literature (Poston, 1982). Therefore, it is important to conduct additional studies regarding the influence of this element on aquatic organisms. The aim of this study is to evaluate the effect of 15 days of waterborne Th exposure on Th accumulation, metabolic and oxidative parameters in several organs, and cytogenetic parameters in the erythrocytes of the silver catfish, *Rhamdia quelen*.

2. Materials and methods

2.1. Experimental protocol

Silver catfish juveniles (6.41 ± 0.17 g and 8.78 ± 0.10 cm) were acquired from the fish culture sector at the Universidade Federal de Santa Maria (Brazil). Fish were kept in continuously aerated tanks (250 L) with dechlorinated well water and a natural photoperiod (12 h light and 12 h dark) for at least 2 weeks before experimental use. The temperature and pH of the water were kept at 22 ± 1.0 °C and 7.6 ± 0.2, respectively.

After the acclimation period, fish were transferred into 40 L aquaria (7 fish per tank) and exposed to different waterborne Th concentrations ($\mu g\,L^{-1}$): 0 (below detection limit, 0.05 $\mu g\,L^{-1}$, control), 25.3 ± 3.2, 69.2 ± 2.73, 209.5 ± 17.6, and 608.7 ± 61.1 (three replicates per treatment) for 15 days. Waterborne Th was adjusted to the appropriate levels using $Th(NO_3)_4{\cdot}5H_2O$ (BDH Chemical Ltda, England Analar®, purity > 99%, compound of low radiotoxicity according to the dealer). Water was air saturated through constant aeration in a static system. Fish were fed to satiety once a day with commercial fish pellets (Supra 42% crude protein, Alisul Alimentos S.A., Carazinho, Brazil). Thorium levels in the food were below detection limits ($0.08\ ng\,g^{-1}$). Uneaten food and feces were siphoned daily, and at least 20% of the water in the aquaria was replaced by water with previously adjusted pH and waterborne Th levels.

2.2. Tissue collection

After the experimental period, the fish were placed in recipients with water and ice for 5 min for anesthetizing. Blood was then collected from the caudal vein using heparinized syringes. Fish were then killed by spinal section, and the tissues (bile, gills, liver, muscle, brain, skin and kidney) were removed, weighed separately, and immediately frozen in liquid argon. The tissues were then stocked in a −70 °C freezer for subsequent analysis of metabolites and enzymatic activity or in a −20 °C freezer for posterior digestion with concentrated nitric acid (HNO_3, 65%, Merck). Th concentration in digested samples was determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan DRCII PerkinElmer SCIEX (USA)), using conditions recommended by the manufacturer. Aqueous calibration standards were prepared by sequential dilution of a stock solution of Th ($10\ \mu g\,L^{-1}$, Spex CertiPrep, Metuchen, USA). Standards were prepared daily using 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, and 2.00 $\mu g\,L^{-1}$ Th. Sample digests were diluted when necessary. Accuracy was evaluated by analysis of two biological certified reference materials: IRMM BCR 668 (mussel tissue, $10.7 \pm 1.2\ \mu g\,g^{-1}$) and NIST SRM 1566b (oyster tissue, $0.0367 \pm 0.0043\ \mu g\,g^{-1}$). Recovery tests were also performed for sample digests and water samples. After every ten measurements, two standard Th solutions were analyzed to check the slope of the calibration curve. If slope difference was higher than 5%, the calibration curve was prepared again using all standards. The precision of ICP-MS measurements was considered acceptable up to 2%.

2.3. Biochemical parameters

Liver and muscle glycogen levels were determined according to Bidinotto et al. (1998) after KOH and ethanol addition for hydrolysis and precipitation of glycogen. For protein analysis, tissues were warmed at 100 °C with KOH and centrifuged at $1000 \times g$ for 10 min. The supernatant was used to determine the total protein level according to Lowry et al. (1951). For lactate and glucose level determination, tissue samples were homogenized by adding 20% trichloroacetic acid using a motor-driven Teflon® pestle and centrifuged at $1000 \times g$ for 10 min for flocculation of the proteins. The completely deproteinated supernatant was used for lactate determination using the method described by Harrower and Brown (1972), and glucose was measured according to Duboie et al. (1956).

2.4. Parameters of oxidative stress

The gills, liver and muscle tissues were homogenized in 1.15% (w/v) KCl solution containing 1 mM PMSF. The homogenates were centrifuged at $600 \times g$ for 10 min to eliminate nuclei and cell debris, and the supernatant fraction obtained was frozen at −70 °C for further measurements. The supernatants were used for analysis of CAT, GST and lipid peroxidation (LPO) levels. Catalase activity was determined by using the method described by Boveris and Chance (1973), in which the disappearance of H_2O_2 is followed spectrophotometrically at 240 nm. The results were reported as $pmol\,mg^{-1}$ protein.

Glutathione-*S*-transferase activity towards CDNB (1-chloro-2,4-dinitrobenzene) was determined spectrophotometrically at 340 nm using the method described in Habig et al. (1974). Activity was calculated from the changes in absorbance at 340 nm using the extinction coefficient of $9.6\ mmol^{-1}\ cm^{-1}$. One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of 1 μmol of CDNB with GSH per minute at 25 °C.

Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) using the method described by Buege and Aust (1978). In this method, absorbance measurements at 535 nm were used to measure the reaction between thiobarbituric acid and the LPO products, resulting in the formation of a chromogen (Schiff's base). The results were reported as $nmol\,mg^{-1}$ protein. The protein content of the homogenate was measured using the method described in Lowry et al. (1951) using bovine serum albumin as the standard.

2.5. Cytogenetic parameters

The frequency of micronuclei in the erythrocytes was evaluated according to the criteria described by Countryman and Heddle (1976) and Fenech (1993). Immediately after sampling, a drop of blood was smeared on clean slides (two per fish), which were dried at room temperature and after 24 h were fixed in 100% methanol for 10 min. Afterwards, they were stained with 4% Giemsa solution for 10 min, air-dried, and then prepared for permanent use. Cytological analysis was done under an optical microscope (1000× objective). A total of 2000 erythrocyte cells were examined per fish in coded slides. The presence of other nuclear abnormalities in erythrocytes (NAE) was also analyzed.

2.6. Water parameters

Water alkalinity ($33.9 \pm 0.5\ mg\,L^{-1}\ CaCO_3$) was determined weekly by the sulfuric acid (H_2SO_4) method (Greenberg et al., 1976). Measurements of dissolved oxygen ($6.24 \pm 0.05\ mg\,L^{-1}$) (YSI model Y5512 oxygen meter) and water pH (7.60 ± 0.01) (Quimix 400A pH