Ecotoxicology and Environmental Safety 114 (2015) 204–211

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effects of trophic exposure to dexamethasone and diclofenac in freshwater fish

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ARTICLE INFO

Article history:
Received 18 August 2014
Received in revised form
4 November 2014
Accepted 25 November 2014
Available online 2 February 2015

Keywords:
NSAIDs
Glucocorticoids
Steroids
Oxidative stress
Gonads

ABSTRACT

Steroidal and non-steroidalanti-inflammatories are pharmaceutical prescribed in human medicine and have the potential to contaminate water and sediments via inputs from sewage treatment plants. Their impacts on humans and ecosystems are emerging issues in environmental health. The aim of the present work was to evaluate the effects of diclofenac and dexamethasone in male fish *Hoplias malabaricus* after trophic exposure. Fish were fed twice every week with *Astyanax* sp. submitted to intraperitoneal inoculation with diclofenac (0; 0.2; 2.0 or 20.0 μg/kg) or dexamethasone (0; 0.03; 0.3 or 3.0 μg/kg). After 12 doses, blood was collected for testosterone dosage. The gonad and liver were collected to calculate gonadosomatic (GSI) and hepatosomatic index (HSI). Antioxidants enzymes activity and biotransformation were also evaluated in liver and gonads. In liver, diclofenac caused oxidative stress with increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and lipoperoxidation (LPO). The GST activity was reduced by diclofenac in liver. Trophic exposure of *H. malabaricus* to dexamethasone caused an increase in antioxidant system (GPx, CAT, GST, and GSH) and LPO in liver. However, it reduced antioxidant system (GPX and GST activities and GSH) in gonads. Both diclofenac and dexamethasone reduced the levels of testosterone, causing impairment to reproduction. Diclofenac reduced HSI at the 0.2 μg/kg, but not GSI. Our results suggest that the anti-inflammatory drugs diclofenac and dexamethasone caused oxidative stress and reduced testosterone levels that can have a negative impact in aquatic organisms.

1. Introduction

Pharmaceuticals are considered a new class of pollutants and negative effects on terrestrial and aquatic environments have been attributed recently to their presence in the ecosystems. Studies pointed out the occurrence of endocrine disruptors in discharged effluents as a cause for sexual disturbance in fish of water streams (Kümmerer, 2008).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently detected pharmaceuticals in treatment plants and surface waters worldwide, due to their volume of consumption and incomplete removal during the wastewater treatment processes (Islas-Flores et al., 2013). Diclofenac is a NSAIDs generally used as an analgesic to reduce pain and to treat inflammatory disorders. The consumed volumes of diclofenac are estimated to be 940 t per year in the world (Zhang et al., 2008). The concentration of diclofenac usually found in the aquatic environment is approximately 2 μg/L (Santos et al., 2010).

Pharmaceutical residues may be transported through food chains and can be harmful to others species. In a study of trout exposed to 1 μg/L diclofenac it was detected that the residues of the drug is in the rate of 72.8 ± 23.3 ng/g muscle (Schwaiger et al., 2004).

NSAIDs exposure can cause disruption of endocrine system by alteration of aromatase activity which might subsequently influence sex hormone balance (Ji et al., 2013). Other studies have reported cytological and histological effects (Schwaiger et al., 2004; Triebskorn et al., 2004; Hoeger et al., 2005; Mehinto et al., 2010) and gene expression changes after diclofenac exposure on fish (Cuklev et al., 2012).

Glucocorticoids are used in human and veterinary medicine mainly because of their anti-inflammatory and immunosuppressive properties. Glucocorticoids regulate the hypothalamic–pituitary–adrenal (HPA) axis through feedback effects on the synthesis and secretion of hypothalamic and pituitary hormones (Patchev et al., 1995). It has been estimated that 27,000 human prescriptions of dexamethasone were dispensed only in the United States in 2004 (Kostich and Lazorchak, 2008). The most

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http://dx.doi.org/10.1016/j.ecoenv.2014.11.020
0147-6513/

potent glucocorticoid cortisone derivate is dexamethasone and relatively high levels have been detected in sewage effluent (Herrero et al., 2013). Sanchez et al. (2011) detected a high concentration of dexamethasone (10 μg/L) in river water collected downstream from a French pharmaceutical factory. However, the concentration of dexamethasone usually found in sewage effluents is approximately 0.3 μg/L (Chang et al., 2007).

Various fish species also have been exposed to dexamethasone experimentally to observe physiological questions such as stress responses (Pierce et al., 2010). Few studies about the effects of dexamethasone as an aquatic contaminant are found, mainly about its potential as endocrine disruptor to aquatic organisms. A review by Milla et al. (2009) demonstrated that, for fish males, an elevation in cortisol decreased 11-ketotestosterone production resulting in smaller gonads and poor sperm quality. This information led to the hypothesis that synthetic glucocorticoids, such as dexamethasone, may have detrimental effects on teleost fish reproduction. Therefore are few studies of oxidative stress on fish caused by pharmaceuticals (Praskova et al., 2014).

Oxidative stress is also considered an important factor affecting reproductive performance (Costantini et al., 2011). Lipid peroxidation is also associated with oxidative stress resulting in the oxidation of polyunsatured lipids (Quinn et al., 2011). Pharmaceuticals in the aquatic environment can alter CYP450 activity in fish and modify enzymatic pathways mediated by CYP450 and cause physiological effects and toxicity (Burkina et al., 2013).

Fish can serve as sensitive bioindicators for exposure to aquatic pollutants (Van der Oost et al., 2003). The experimental model used in this study was *Hoplias malabaricus* (Characiformes, Erythrinidae), a carnivorous fish with large distribution in tropical rivers and lakes and very consumption by human populations (Ferreira et al., 2003). This fish has the advantage that it is easily fed under laboratory and has been explored in several biological studies (Monteiro et al., 2013; Silva de Assis et al., 2013).

Since the both anti-inflammatories drugs discussed above are found in aquatic environment, the aim of the present work was to evaluate the effects related to oxidative stress (superoxide dismutase, catalase, glutathione peroxidase activities, reduced glutathione and lipoperoxidation levels) and biotransformation parameters in liver and gonads and also endocrine disruption of diclofenac and dexamethasone in male fish *H. malabaricus* after trophic exposure.

2. Material and methods

The present work was approved by the Animal Experimentation Ethics Committee of Federal University of Parana (http://www.bio.ufpr.br/portal/comissao-de-etica-para-o-uso-de-animais/), under number 453 and all protocols were realized in accordance with International Guidelines for Animal Use.

2.1. Chemicals

Dexamethasone (D1756) and diclofenac sodium salt (D6899) were obtained from Sigma Aldrich. All chemicals and reagents were purchased from Sigma-Aldrich Chemical Corporation (USA) and Merck.

2.2. Experimental design

Adult *H. malabaricus* males weighing 171.4 ± 15.7 g (length: 25.3 ± 4.4 cm) were purchased from Santa Candida Commercial Farm, Santa Cruz da Conceição, São Paulo, Brazil. Fish were kept at 24 ± 1 °C in glass aquaria (120 L capacity) for 30 days in filtered and dechlorinated tap water on a simulated natural photoperiod (12 hours dark:12 hours light). After acclimation to laboratory conditions, fish were randomly divided into seven groups ($n=10$

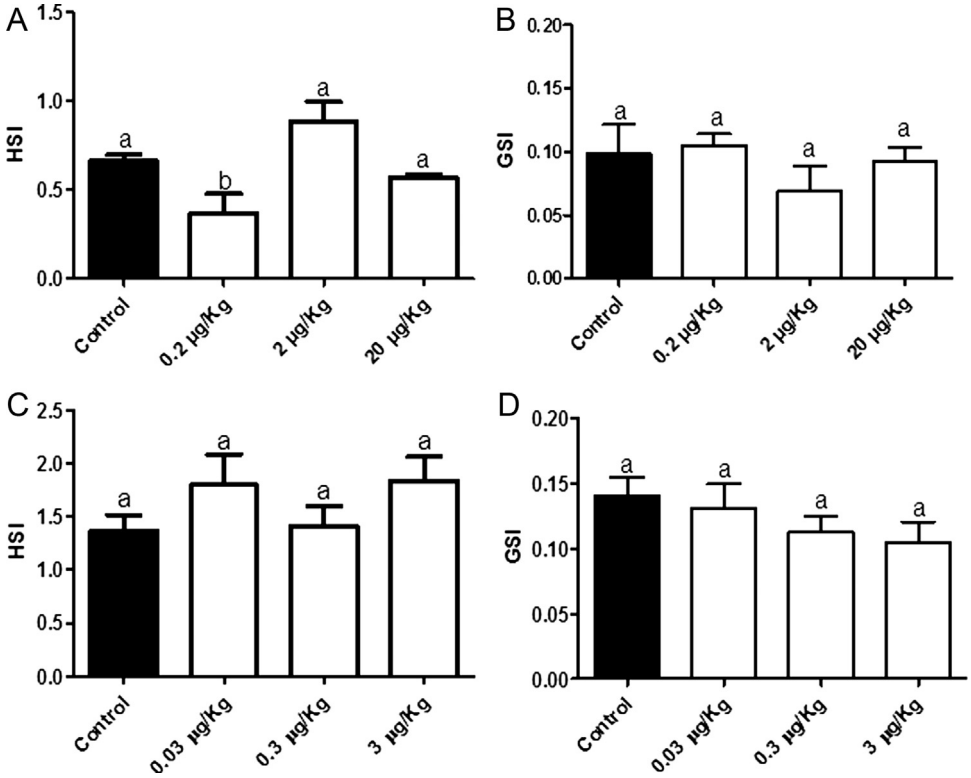

Fig. 1. Hepatosomatic (A) and gonadosomatic (B) index of *Hoplias malabaricus* after diclofenac exposure; gepatosomatic (C) and gonadosomatic (D) index of *Hoplias malabaricus* after dexamethasone exposure. Values are expressed as mean ± standard error of mean. b—indicates statistically significant differences ($p<0.05$) compared to control group; ANOVA, Bonferroni. $N=10$.