Chemosphere 88 (2012) 377–383

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos

Houjuan Xing [a,b], Shu Li [a], Zhilei Wang [b], Xuejiao Gao [a], Shiwen Xu [a,*], Xiaolong Wang [c,*]

[a] College of Veterinary Medicine, Northeast Agricultural University, 59 Mucai Street, Harbin 150030, China
[b] Animal Health Supervision Institute of Heilongjiang Province, 243 Haping Road, Xiangfang District, Harbin 150069, China
[c] Wildlife Resource College, Northeast Forestry University, Ministry of Education, 26 Hexing Road, Xiangfang District, Harbin 150040, China

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 22 January 2012
Accepted 19 February 2012
Available online 19 March 2012

Keywords:
Oxidative stress
Histopathology changes
Chlorpyrifos
Atrazine
Cyprinus carpio L.

ABSTRACT

We investigated oxidative stress response and histopathological changes in the brain and kidney of the common carp after a 40-d exposure to CPF and ATR, alone or in combination, and a 20-d recovery. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content were measured using standard assays. Our results indicated that exposure to ATR, CPF or a combination of the two for 40 d induced significant changes in antioxidant enzyme (SOD, CAT and GSH-Px) activities and MDA content in the brain and kidney of the common carp. Pathological changes included tissue damage that was more severe with increased of exposure dose. To our knowledge, this is the first report to study oxidative stress and histopathological effects caused by subchronic exposure to ATR, CPF and ATR/CPF combination on common carp. The information presented in this study may be helpful to understanding the mechanisms of ATR-, CPF- and ATR/CPF combination-induced oxidative stress in fish.

1. Introduction

Many freshwater ecosystems are contaminated with industrial, domestic and agricultural chemicals, such as herbicides and insecticides, which are ubiquitous and can spread regionally and globally (Jin et al., 2010b). Atrazine (ATR) and chlorpyrifos (CPF) are the most common pesticides in the freshwater ecosystems of the world (Whyatt et al., 2007; Dong et al., 2009). Field surveys performed in many countries have showed that ATR and CPF are common contaminants of surface and ground water (McConnell et al., 1997; Miller et al., 1999; Banks et al., 2005; Du Preez et al., 2005; Gojmerac et al., 2006). In fact, agriculture water and irrigation water are the main sources of these contaminants to the aquatic environment, where they subsequently deteriorate water quality. Chemical pollution in the environment with pesticides has been increasing due to their extensive usage in agriculture (Trasande et al., 2011). Alterations in the chemical composition of natural aquatic environments may affect the freshwater fauna, particularly fish. Because human consumption of fish, the effects of pesticides on fish have important significance in the evaluation of adverse effects of pesticides to human health (Begum and Vijayaraghavan, 1996). Recent studies have indicated that the pesticide toxicity in fish may be related to an increased production of reactive oxygen species (ROSs), leading to oxidative damage. ROSs are products of the electron transport chain, enzymes, and redox cycling and their production may be enhanced by xenobiotics (Oruç, 2010). Oxidative stress occurs when ROSs overwhelm the cellular defences and damage proteins, membranes, and DNA (Kelly et al., 1998). Oxidative stress is defined as a disruption of the pro-antioxidant balance, leading to potential damage. In recent years, increasing emphasis is on the use of biomarkers as a tool for monitoring both environmental quality and adaptation of organisms (Oruc et al., 2004). The antioxidant defence system has been extensively studied because of the potential to use oxyradical-mediated responses as biochemical biomarkers (Van der Oost et al., 2003).

The main antioxidative enzymes for the detoxification of ROSs in all organisms are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The reactions of these enzymes with oxyradicals have been studied in fish (Avci et al., 2005). It has been shown that the antioxidants from fish may be useful biomarkers of exposure to aquatic pollutants (Ahmad et al., 2000). Pesticides may cause oxidative stress that leads to the generation of free radicals and alterations in antioxidants or free oxygen radical scavenging enzyme systems (Almeida et al., 1997). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity (Khrer, 1993).

The effects of pesticides on fish may have diagnostic significance in the evaluation of adverse effects of pesticides to human health because fish have an important role in the food chain. We

* Corresponding authors. Tel./fax: +86 451 55190407, +86 451 82190617.
E-mail addresses: shiwenxu@neau.edu.cn (S. Xu), wxlz1969@yahoo.com.cn (X. Wang).

0045-6535/$ - see front matter Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2012.02.049

focused on the common carp (*Cyprinus carpio* L.) because they are an ideal indexical organism for the study of aquatic systems because they have a wide distribution, they are bottom-dwelling fish whose feeding habits expose them to many different types of environmental contaminants, they are easily captured, and they are important for human consumption. Many studies have described the adverse effects of ATR and CPF on fish (Steinberg et al., 1995; Hussein et al., 1996; Wiegand et al., 2001; De Silva and Samayawardhena, 2005; Kavitha and Venkateswara Rao, 2008). However, in the present study, we investigated the histopathological characteristics and antioxidant responses by assaying for CAT, SOD, GSH-Px and ROSs in the brain and kidney of common carp that received intraperitoneal administration of ATR and CPF. These results will enable us to further understand the hepatic damage and antioxidation mechanisms in fish.

2. Materials and methods

2.1. Fish

Fish model was developed as described in our previous article (Xing et al., 2011). The common carp (mean body length, 12.5 ± 1.29 cm; mean body weight, 190 ± 10 g) used in this study were purchased from an aquarium specialising in freshwater fish species and maintained in laboratory tanks (90 × 55 × 45 cm) with continuous aeration. The fish were acclimated to experimental conditions for 15 d using dechlorinated tap water ($CaCO_3$: 230 ± 3.16 mg L^{-1}, Ca: 42.5 ± 1.2 mg L^{-1}; dissolved oxygen concentration 7.21 ± 0.17 mg L^{-1}; pH 7.4 ± 0.2). The water temperature was adjusted to 20 ± 1 °C and the photoperiod was 12 h of light and 12 h of dark. Commercial food was given once a day until satiation. No mortality was observed either in control animals or in any of the treatment groups.

2.2. Chemicals

ATR (purity 98.0%) and CPF (purity 99.5%) were purchased from Sigma–Aldrich Chemical Co. (USA). Stock solutions of ATR and CPF were prepared in analytical grade acetone (purity 99%), and all working solutions were made from this stock solution. The concentration of acetone was kept at <0.05% in all pesticide solutions used.

2.3. Experimental design

2.3.1. Exposure test

The fish were divided into eleven groups as follows: three ATR treatment groups (4.28, 42.8 and 428 μg L^{-1}), three CPF treatment groups (1.16, 11.6 and 116 μg L^{-1}), three ATR/CPF combination treatment groups (1.13, 11.3 and 113 μg L^{-1}), one solvent control (acetone) group, and one water control group. The binary mixtures were composed of a 1:1 mass ratio of ATR and CPF. The fish were exposed under semi-static conditions for 40 d, with water and pesticide completely replaced once every 2 d by transferring fish to freshly prepared pesticide solutions. Other conditions of fish acclimated were consistent with the previous description (2.1). The exposure treatment is detailed in Table 1.

At the end of the exposure, fish were sacrificed by decapitation and bled. Then the liver, kidney, gill and brain were excised immediately on an ice-cold plate washed in physiological saline solution. The tissues were divided into two portions: one for protein and antioxidant enzyme analysis and a second was fixed in Bouin's solution for histological examination.

Table 1
Treatments of fish for 40 d exposure at sub-lethal concentrations of atrazine, chlorpyrifos and their mixture and treatments of exposed fish for 20 d recovery.

Batches	Toxicant	Treatments of pesticides	Fish number of exposure treatments	Fish number of recovery treatments
1	Control	Pesticide-free water	20	10
2	Solvent control	Add acetone in pesticide-free water	20	10
3	Atrazine	4.28 μg L^{-1}	20	10
4		42.8 μg L^{-1}	20	10
5		428 μg L^{-1}	20	10
6	Chlorpyrifos	1.16 μg L^{-1}	20	10
7		11.6 μg L^{-1}	20	10
8		116 μg L^{-1}	20	10
9	Mixture of atrazine and chlorpyrifos	1.13 μg L^{-1}	20	10
10		11.3 μg L^{-1}	20	10
11		113 μg L^{-1}	20	10

2.3.2. Recovery test

Ten fish from each exposure group were kept as a set in fresh, pesticide-free water for 20 d in large 200 L glass aquaria with filters and continuous aeration. The conditions during the recovery experiment were the same as those described above (Table 1). At the end of the recovery period (60 d), the animal treatment and tissue isolation methods employed were the same as those described above.

2.4. Antioxidant enzyme analyses

2.4.1. Preparation of post-mitochondrial supernatant

The tissues were homogenised (1:10 w/v) with a glass–Teflon homogeniser (Heidolph S01 10R2RO) in 50 mM potassium phosphate buffer (pH 7.0; containing 0.5 mM EDTA). The homogenate was centrifuged at 12 000*g* for 30 min at 4 °C to obtain the post-mitochondrial supernatant for measuring malondialdehyde (MDA) levels and antioxidant enzyme activities.

2.4.2. Determination of antioxidant enzyme activities and MDA levels

Total SOD activity was determined by the method of Marklund and Marklund (1974), which involved the autoxidation of pyrogallol. SOD activity was assessed spectrophotometrically at 420 nm and expressed as the amount of enzyme per mg of protein.

The CAT activity was determined according to the method of Beutler (1984), which involves a mixture of 1 M Tris–HCl, 5 mM EDTA (pH 8.0), 10 mM H_2O_2 and H_2O, and the rate of H_2O_2 consumption at 37 °C was measured at 230 nm and then used for the quantitative determination of CAT activity. An extinction coefficient for H_2O_2 at 230 nm was 22 and used to calculate the activity of the enzyme.

GSH-Px activity, which catalyses the oxidation of GSH to GSSG by H_2O_2, was determined according to the methods by Beutler (1984). The rate of GSSG formation was measured by following a decrease in absorbance of a reaction mixture containing NADPH and glutathionereductase at 37 °C and 340 nm, as NADPH is converted to NADP. *t*-Butyl hydro-peroxide was used as a substrate.

MDA content was measured after incubation at 95 °C with thiobarbituric acid in aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm (Ohkawa et al., 1979). Specific activity was defined as the unit of activity per milligram protein. Protein content was determined according to the method by Lowry et al. (1951) using bovine serum albumin as the standard.