Chemosphere 148 (2016) 21–31

Contents lists available at ScienceDirect

Chemosphere

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

Interactive effects of selected pharmaceutical mixtures on bioaccumulation and biochemical status in crucian carp (*Carassius auratus*)

Jiannan Ding, Guanghua Lu*, Yi Li

Key Laboratory for Integrated Regulation and Resources Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, PR China

HIGHLIGHTS

- Crucian carp was exposed to three pharmaceuticals alone and in binary combination.
- Addition of FLU increased ROX and PRP accumulation in liver of crucian carp.
- FLU inhibited metabolism response to ROX and PRP at catalytic and mRNA levels.
- ROX + FLU and PRP + FLU treatments induced higher oxidative stress than sole chemical.

ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form
5 January 2016
Accepted 6 January 2016
Available online 19 January 2016

Handling Editor: Jim Lazorchak

Keywords:
Pharmaceutical
Mixture
Interaction
Carassius auratus
Bioaccumulation
Biomarker

ABSTRACT

The aim of this study was to evaluate the interactive effects of fluoxetine (FLU), roxithromycin (ROX) and propranolol (PRP) on the bioaccumulation and biochemical responses in the crucian carp *Carassius auratus*. After 7 days of binary exposure (ROX + FLU and PRP + FLU), the addition of waterborne FLU at nominal concentrations of 4, 20 and 100 μg L^{-1} significantly increased the accumulation of ROX and PRP in fish livers in most cases, although elevated ROX and PRP bioaccumulation levels were not observed in muscles or gills. The inductive response of 7-ethoxyresorufin *O*-deethylase (EROD) to PRP and that of 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD) to ROX were inhibited by the co-administration of FLU at all tested concentrations. Correspondingly, marked inhibition of CYP1A and CYP3A mRNA expression levels was observed in the livers of fish co-treated with FLU + PRP and FLU + ROX relative to their PRP- and ROX-only counterparts, respectively. In addition, as reflected by superoxide dismutase (SOD) activity and malondialdehyde (MDA) content, co-exposure to ROX + FLU and PRP + FLU seemed to induce stronger antioxidant responses than single pharmaceutical exposure in fish livers. This work indicated that the interactive effects of pharmaceutical mixtures could lead to perturbations in the bioaccumulation and biochemical responses in fish.

1. Introduction

The aquatic environment is one of the most important sinks for human and veterinary pharmaceuticals, which mainly come from the discharge of sewage treatment plants (Daughton and Ternes, 1999). In sewage and surface waters, the occurrence of various pharmaceuticals, including antibiotics, anti-inflammatory drugs, beta-adrenergic receptor antagonist drugs (β-blockers), hormones and serotonin reuptake inhibitors (SSRIs), has been frequently reported in the ng L^{-1} to low μg L^{-1} range (Alder et al., 2010; Collado et al., 2014; Iglesias et al., 2014; Tang et al., 2015; Schultz et al., 2010; Velicu and Suri, 2009). In general, these commonly used pharmaceuticals belonging to different therapeutic classes are present in aquatic environment as mixtures, and may coexist with other chemical ingredients, such as insecticide (Kristofco et al., 2015). Consequently, aquatic species in their habitat may be suffering the effects of co-exposure to these chemicals. In a recent survey completed by 535 environmental scientists from 57 countries, the effects of pharmaceuticals in mixtures on non-target organisms has been considered one of the most important research

* Corresponding author.
E-mail address: ghlu@hhu.edu.cn (G. Lu).

http://dx.doi.org/10.1016/j.chemosphere.2016.01.017
0045-6535/

priorities regarding the risks of pharmaceuticals in the environment (Rudd et al., 2014).

Some previous studies have explored the effects of pharmaceutical mixtures on aquatic species (reviewed in Gonzalez-Rey et al., 2014; Vasquez et al., 2014). Most of these studies usually focus on low trophic level organisms such as algae (DeLorenzo and Fleming, 2008), crustaceans (Dietrich et al., 2010), and bivalves (Franzellitti et al., 2013, 2015; Gonzalez-Rey et al., 2014); however, our knowledge regarding the interactive effects of pharmaceutical mixtures on aquatic organisms occupying higher trophic levels, such as fish, remains limited. Recently, Galus et al. (2013) reported that chronic exposure of zebrafish (*Danio rerio*) to a quaternary mixture of pharmaceuticals (acetaminophen, carbamazepine, gemfibrozil and venlafaxine) could impact reproduction and induce histopathological changes, similar to those following single compound exposures. Li and Lin (2015) investigated acute toxicity in fish (*Cyprinus carpio*) caused by a mixture of 19 pharmaceuticals, including antibiotics, β-blockers, lipid regulators, psychiatric drugs and non-steroidal anti-inflammatory drugs, and demonstrated that the joint stress of a pharmaceutical mixture could induce a synergistic increase in mortality of fish compared to treatment with each pharmaceutical compound individually. In a study with goldfish (*Carassius auratus*), vitellogenesis, metabolism and serum steroid synthesis were distinctly increased by individual exposure to 17β-estradiol; when 17β-estradiol interacted with the fungicide ketoconazole, the vitellogenesis was further elevated, whereas the changes in metabolism and serum steroid synthesis were inhibited, revealing a complex joint outcome of pharmaceutical mixtures in fish (Yan et al., 2013).

Clearly, a complete understanding of the toxic effects of pharmaceutical mixtures on fish is an urgent need. A standard acute toxicity test by Li and Lin (2015) yielded a 96-h median lethal concentration (LC_{50}) of a nineteen-pharmaceutical mixture for fish (*C. carpio*) as 60.68 mg L^{-1}, which was much higher than environmentally relevant concentrations. However, Aguirre-Martínez et al. (2015) recently demonstrated that standard acute toxicity tests were not sensitive enough to evaluate the effects of pharmaceuticals on aquatic biota, as teratogenicity was observed on sea urchin (*Paracentrotus lividus*) after exposure to environmental concentrations of carbamazepine and ibuprofen at 0.00001 mg L^{-1}. Thus, it is necessary to apply more sensitive response endpoints and use at least a 2-tier approach when assessing the risk of pharmaceuticals in aquatic environments (Aguirre-Martínez et al., 2015). In this regard, the evaluation of a suite of biomarkers at the molecular level, including the activity of cytochrome P450 (CYP) enzymes [7-ethoxyresorufin *O*-deethylase (EROD) and 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD)], antioxidant enzymes [superoxide dismutase (SOD)], and by-products of lipid peroxidation (LPO) [malondialdehyde (MDA)], has been widely applied in pharmaceutical toxicity studies with fish (Bartram et al., 2012; Ding et al., 2015a; Liu et al., 2014; Li et al., 2011; Smith and Wilson, 2010). Further, underlying the variety of enzymatic activities, there are complex gene modulation processes acting as the regulatory system of xenobiotic exposure. Thus, the expression of associated genes is increasingly recognized as a biomarker for the effects of pharmaceuticals on fish (Corcoran et al., 2012; Thomas et al., 2012; Wassmur et al., 2010; Yan et al., 2013).

The present study was carried out on three pharmaceuticals belonging to different therapeutic classes: fluoxetine (FLU) from the SSRIs, roxithromycin (ROX) from the antibiotics, and propranolol (PRP) from the β-blockers. These pharmaceuticals are prevalent in the aquatic environment. The measured concentrations of FLU, ROX and PRP in environmental waters range from 0.004 to 3.6, 0.002 to 1.5, and 0.008–1.9 μg L^{-1}, respectively, with the highest concentrations occurring near water treatment plants (Table S1). In addition, all these pharmaceuticals are able to elicit adverse effects on fish (Ding et al., 2015a; Liu et al., 2014, 2015; Pelli and Connaughton, 2015; Silva et al., 2015).

PRP, a non-specific β-blocker, could induce CYP1A-associated EROD activity in several fish species such as rainbow trout (*Onchorynchus mykiss*) (Bartram et al., 2012; Laville et al., 2004) and crucian carp (*C. auratus*) (Ding et al., 2015a), while ROX, a macrolide antibiotic, seemed not significantly change EROD activity in liver of crucian carp (*C. auratus*) (Liu et al., 2014). But induction of CYP3A gene expression has been observed by ROX in rat (Lee et al., 2006). Zanger and Schwab (2013) also suggested that macrolide antibiotics, including ROX, are metabolized mainly by CYP3A enzyme. Moreover, both ROX and PRP have been reported to induce oxidative stress in crucian carp (*C. auratus*) (Ding et al., 2015a; Liu et al., 2014). Notably, wildlife monitoring data demonstrates that the two pharmaceutical compounds are able to accumulate to some degree in fish (Liu et al., 2015; Xie et al., 2015). Our previous trophic transfer studies, however, showed that the bioaccumulation of ROX and PRP in crucian carp (*C. auratus*) via dietary uptake might be negligible, as low bioaccumulation factors (BAFs) of 0.01–0.44 and 0.003–0.16, respectively, were obtained (Ding et al., 2015a, 2015b).

FLU is the most widely prescribed antidepressant drug of the SSRI class, and it is considered one of the pharmaceuticals with the highest toxicity on non-target organisms (Oakes et al., 2010). In fish, FLU shows great bioaccumulation potential (Nakamura et al., 2008; Paterson and Metcalfe, 2008). Mennigen et al. (2011) provided a strong case for neuroendocrine disruption in fish exposed to FLU, which would lead to changes in reproduction, food intake, stress and behavior. Also, linked to specific microRNA abundance, exposure to environmentally relevant concentrations of FLU was shown to disrupt key hepatic metabolic pathways and reduce overall fitness of zebrafish (*D. rerio*) (Craig et al., 2014). Moreover, FLU acts as a potent inhibitor of CYP-mediated reactions. Inhibition of CYP1s and CYP3A catalysed activities has been reported by FLU in *in vitro* studies of fish hepatocytes and liver microsomal fractions (Laville et al., 2004; Smith et al., 2012; Thibaut and Porte, 2008; Thibaut et al., 2006). On the other hand, FLU could cause oxidative stress in aquatic species, reflected by significant change in SOD activity and MDA formation (Chen et al., 2015; Gonzalez-Rey and Bebianno, 2013). Recently, Franzellitti et al. (2015) found that exposure to 0.3 ng L^{-1} FLU for 7 days obviously increased accumulation level of PRP in digestive gland of Mediterranean mussel (*Mytilus galloprovincialis*), highlighting the need for investigating interactive effects between FLU and other pharmaceuticals on bioaccumulation in aquatic species. This raises the question of whether FLU exposure can enhance the bioaccumulation of ROX and PRP in crucian carp (*C. auratus*) via dietary exposure.

Thus, the objective of this study was to investigate the bioaccumulation of FLU, ROX and PRP, alone and in binary combination, and their effects on the biochemical status of crucian carp (*C. auratus*), including metabolism and oxidative status, using the 2-tier approach, i.e., biomarkers at the molecular level (EROD, BFCOD, SOD and MDA) and the gene level (CYP1A mRNA and CYP3A mRNA). Moreover, multiple exposure regimens for the pharmaceuticals (aqueous exposure for FLU and dietary exposure for ROX and PRP) were applied in the present study.

2. Materials and methods

2.1. Chemicals and reagents

Roxithromycin (CAS 80214-83-1, purity>96.5%) was purchased from Labor Dr. Ehrenstorfer (Augsburg, Germany). Propranolol (propranolol hydrochloride, CAS 318-98-9, purity>98%, to be referred to as propranolol) and fluoxetine (fluoxetine