Science of the Total Environment 532 (2015) 31–39

Contents lists available at ScienceDirect

Science of the Total Environment

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

Biological fate and effects of propranolol in an experimental aquatic food chain

Jiannan Ding, Guanghua Lu *, Sheng Li, Yang Nie, Jianchao Liu

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

- Propranolol (PRP) was able to transfer along the experimental aquatic food chain.
- Crucian carp were able to metabolize PRP in a manner similar to mammals.
- PRP could cause biochemical perturbations in the liver of crucian carp.

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 8 May 2015
Accepted 1 June 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Propranolol
Algae
Daphnia
Crucian carp
Food chain transfer
Metabolism
Biomarker

ABSTRACT

The aim of this study was to evaluate the trophic transfer of the β-blocker propranolol (PRP) in an experimental aquatic food chain involving the green algae *Scenedesmus obliquus*, the water flea *Daphnia magna* and the crucian carp *Carassius auratus*, as well as the metabolism and effects of PRP in the liver of crucian carp. After a 48 h PRP aqueous exposure for algae, with a subsequent 48 h dietary exposure for daphnia and an 8 d dietary exposure for crucian carp, PRP was observed in each trophic level, despite significant bioaccumulation did not occur in daphnia and crucian carp. A portion of the absorbed PRP was metabolized by the crucian carp to *N*-desisopropylated propranolol, propranolol glucuronic acid, monohydroxylated propranolol, hydroxypropranolol glucuronide and dihydroxypropranolol glucuronide, which were similar to those in mammals. In addition, multiple biomarkers in the liver of crucian carp (7-ethoxyresorufin *O*-deethylase, EROD; 7-benzyloxyresorufin *O*-dealkylation, BROD; superoxide dismutase, SOD and malondialdehyde, MDA) were measured. BROD and MDA were not significantly affected by PRP, while EROD and SOD did change significantly during the 8 d dietary exposure. This work indicated that the trophic transfer of PRP, resulting in biochemical perturbations of fish biological systems, should be a concern for the assessment of the environmental risks to aquatic food chains.

1. Introduction

Beta-adrenergic receptor antagonist drugs, the so called 'β-blockers', are a class of human pharmaceuticals used to treat various cardiovascular disorders, such as angina, arrhythmias and hypertension. They can inhibit the action of adrenergic agonists by blocking the β-adrenergic receptors (β-ARs), ultimately reducing the heart rate and contractility (Owen et al., 2007). As with most pharmaceutical contaminants in aquatic environment, a range of ng L^{-1} to μg L^{-1} of β-blockers has been reported in sewage and surface waters in Europe (Alder et al., 2010; Bendz et al., 2005), the United States (Huggett et al., 2003) and recently in China (Yu et al., 2011), due to the large-scale use and incomplete removal during wastewater treatment (Maurer et al., 2007).

Propranolol (PRP), a non-specific β_1 and β_2-ARs blocker, as well as a serotonin (5-HT) receptor antagonist (Sprouse and Aghajanian, 1986), is one of the most frequently detected β-blockers in the aquatic environment. Although designed for human use, a growing body of evidence has suggested that PRP may cause adverse effects on various aquatic organisms. For algae, Maszkowska et al. (2014b) reported the half maximal effective concentration (EC_{50}) of PRP inhibiting growth of green algae (*Scenedesmus vacuolatus*) to be 24 mg L^{-1}. In aquatic invertebrate studies, the lowest observed effect concentration (LOEC) of PRP reducing heart rate of *Daphnia magna* was obtained at 55 μg L^{-1} (Dzialowski et al., 2006). As far as fish are concerned, researchers found that the heart rate, embryo-larval development (Finn et al., 2012), hatchability (Giltrow et al., 2009) and even gene expression (Lorenzi et al., 2012) could be affected by PRP. Moreover, the endocrine-disrupting potential of PRP on aquatic organisms was recently emphasized by Massarsky et al. (2011). All these findings highlight the need for a better understanding of the fate and toxicology of PRP in the aquatic environment.

Bioaccumulation through food chains is a key issue in the ultimate fate of some pollutants present in the aquatic environment,

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

http://dx.doi.org/10.1016/j.scitotenv.2015.06.002
0048-9697/

such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Madenjian et al., 1998; Yu et al., 2009). For pharmaceuticals, laboratory studies have confirmed that some antibiotics (e.g., oxytetracycline and tetracycline) and psychiatric drugs (e.g., carbamazepine) have the possibility of bioaccumulation and transfer through aquatic food chains (Boonsaner and Hawker, 2013; Kim et al., 2014; Vernouillet et al., 2010). Therefore, a growing interest has been given to the risk of pharmaceuticals posing for the aquatic trophic chains (Brodin et al., 2014; Chèvre, 2014; Zenker et al., 2014). In a field study investigating the bioaccumulation of various pharmaceuticals in the liver of crucian carp (*Carassius auratus*) caged in an effluent-receiving river, PRP has showed a higher bioaccumulation factor (BAF) (2782) than the other pharmaceutical substances such as roxithromycin (440), carbamazepine (917) and erythromycin (1482) (Liu et al., 2015a). Moreover, PRP is considered lipophilic ($\log K_{ow}$ = 2.43–3.65), highly resistant to hydrolysis (half-life of >1 year), bioavailable and mobile in the environment, suggesting its bioaccumulation potential in the water ecosystems (Bendz et al., 2005; Massarsky et al., 2011; Maszkowska et al., 2014a). Even so, to our knowledge, the behavior of PRP in aquatic food chains is still unexplored, making it difficult to predict the environmental fate of this compound.

Historically, ecotoxicological assessments for the β-blockers in the aquatic environment have focused on the parent drugs (Cleuvers, 2005; Ferrari et al., 2004). The guideline issued by the European Agency for the Evaluation of Medicinal Products (EMEA, 2006), however, suggests the information on the relevant metabolites should be incorporated in environmental risk assessments of human pharmaceuticals. The metabolism of PRP in mammals is well documented. In general, major metabolic pathways of PRP in mammals consist of side chain glucuronidation, aromatic ring hydroxylation and side chain *N*-desisopropylation (Beaudry et al., 1999; Masubuchi et al., 1994; Wu et al., 2001; Yoshimoto et al., 1995). Regarding fish, a recent study has shown that PRP exposure elevated 7-ethoxyresorufin *O*-deethylase (EROD) activity in the liver and gill of rainbow trout, suggesting the potential for metabolism of PRP in fish (Bartram et al., 2012). However, the direct evidence for metabolism of PRP in fish has yet to be provided.

The objective of this study was to investigate the bioaccumulation and trophic transfer of PRP in a model food chain involving the green alga *Scenedesmus obliquus*, the water flea *D. magna* and the crucian carp *C. auratus*. Additionally, the major metabolites of PRP in the liver of crucian carp were examined. Finally, in order to characterize the biological effects of PRP on fish in food chain conditions, multiple biomarkers at the molecular level including biotransformation enzymes [EROD and 7-benzyloxyresorufin *O*-dealkylation (BROD)], an antioxidant defense enzyme [superoxide dismutase (SOD)] and a byproduct of lipid peroxidation (LPO) [malondialdehyde (MDA)] in liver tissues were determined. We expect this study will be useful to improve the current environmental risk assessment procedures of β-blocker products.

2. Materials and methods

2.1. Chemicals and reagents

The test substance, propranolol (propranolol hydrochloride, CAS 318-98-9, purity >98%, to be referred to as propranolol), was obtained from J&K Scientific (Shanghai, China). Chemical stock solutions were prepared in methanol and stored at −20 °C, and the working solutions were diluted aliquots of the stock solutions. Nicotinamide adenine dinucleotide phosphate, ethoxyresorufin and benzyloxyresorufin were purchased from Sigma Chemical (St. Louis, MO, USA). Hydroxymethyl aminomethane (Tris) was purchased from Nanjing Sunshine Biotechnology Co., LTD. (Nanjing, China), and the purity was >99%. Bovine serum albumin (BSA) was purchased from Shanghai Huixing Biochemistry Reagent Co., Ltd. (Shanghai, China), and the purity was >98%. Acetonitrile and methanol (HPLC grade) were obtained from Merck Serono Co., Ltd. (Darmstadt, Germany). *n*-Hexane (HPLC grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water was purified using a Milli-Q integral water purification system (Millipore, Milford, MA, USA).

2.2. Test organisms

S. obliquus was obtained from Wuhan Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Strains of the chlorophyceae were cultivated in the media described by Lu et al. (2001), the formula details can be found in the Supplemental Material. Algae in the stationary phase were inoculated into 250-mL Erlenmeyer flasks in 200 mL of test culture, with an initial algal cell density of 10^6 cells mL^{-1}. Cultures were incubated in an environmental chamber at 22 ± 1 °C with a light/dark photoperiod of 16 h/8 h.

D. magna was obtained from the Chinese Center for Disease Control and Prevention (Beijing, China) and cultured continuously in artificial freshwater (AFW) as described in the guideline of Organization for Economic Cooperation and Development for the testing of chemicals (OECD, 2008). In brief, a total of 58.5 mg of $CaCl_2 \cdot 2H_2O$, 24.7 mg of $MgSO_4 \cdot 2H_2O$, 13.0 mg of $NaHCO_3$ and 1.2 mg of KCl was added into 1 L of deionized water. The culture media was renewed three times each week, and the daphnia was fed daily with *S. obliquus*. The culture was maintained at a constant temperature (22 ± 1 °C) with a light/dark cycle of 16 h/8 h.

C. auratus was obtained from the Nanjing Institute of Fishery Sciences (Nanjing, China). All fish were acclimatized for 2 weeks in de-chlorinated tap water (temperature 20 ± 1 °C; dissolved oxygen >6 mg L^{-1}; pH 7.2 ± 0.2; and $CaCO_3$ 116.7 ± 3.6 mg L^{-1}) before the experiments. The fish were fed every 48 h with uncontaminated daphnia until four days prior to the beginning of the experiment. Air stones were placed in each tank to maintain oxygen saturation in the water. Sewage and uneaten food were removed every other day by suction.

2.3. Trophic transfer experiment

Trophic transfer experiment consisted of a 48 h aqueous exposure for algae, a 48 h dietary exposure for daphnia and an 8 d dietary exposure for crucian carp. The algae were initially exposed to nominal PRP concentrations of 10, 100 and 1000 μg L^{-1}. The concentrations of PRP both in the exposure media and algae cells were determined at 0, 2, 4, 8, 12, 24, 36 and 48 h. At each sampling point, the PRP-exposed algae culture media were collected and centrifuged (1000 *g*, 10 min). The exposure media were decanted, and the algal pellets were resuspended in distilled water and centrifuged. This resuspend-centrifuge process was conducted twice. Pellets were then taken for PRP analysis or as food to the next trophic level.

In the trophic level of daphnia, approximately 100 adult daphnia (21–28 days old) were placed in a 500 mL glass beaker. The PRP-laden algal pellets were then added to each culture media as food source to give a final cell density of 10^5 cells mL^{-1}. The sampling time points were 0, 2, 4, 8, 12, 24, 36 and 48 h. At each sampling point, the daphnia media were sampled to determine the remaining concentration of the target compound, and the daphnia were harvested by a pipette, rinsed with fresh culture solution, then taken for PRP detection or introduced to the fish aquarium.

The dietary uptake tests of PRP via daphnia food by crucian carp were performed for 8 consecutive days. Initially, the fish were randomly assigned to each aquarium at a density of approximately 1.5 g/L fish/water. Then the contaminated daphnia (as described above) were fed everyday to the fish at a daily rate of 1% of body weight. In all cases daphnia were totally consumed within 10 min. At 1 h after each day's feeding, the water was renewed to ensure that the body burdens of PRP in fish were all contributed by food. After 1, 2, 4 and 8 d of the first feeding, five fish were sacrificed by cervical transection (two fish