Ecotoxicology and Environmental Safety 150 (2018) 144–151

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effects of the antidepressant, mianserin, on early development of fish embryos at low environmentally relevant concentrations

Ming Yang[a,*,1], Shuai Liu[a,1], Lei Hu[a,b], Jing Zhan[a], Penghui Lei[b], Minghong Wu[c,*]

[a] School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China
[b] School of Life Science, Shanghai University, Shanghai 200444, China
[c] Shanghai Applied Radiation Institute, Shanghai University, Shanghai 200444, China

ARTICLE INFO

Keywords:
Tetracyclic antidepressant
Mianserin
Zebrafish embryos
Water exposure
Dose response

ABSTRACT

Pharmaceuticals have been considered as emerging organic contaminants in the environment that might pose huge risk to the non-target aquatic organisms. Mianserin, a tetracyclic antidepressant, is present at low detectable concentrations in the aquatic environment; however, limited attention has been devoted to its potential adverse effects on the aquatic animals. In the present study, we first performed an acute toxicity test for mianserin exposure using zebrafish (*Danio rerio*) embryos during 4–124 h post fertilization (hpf). Time-dependent lethal concentrations of mianserin exposure on the zebrafish embryos were firstly determined at mg/L levels. Then, a series of sublethal concentrations of 0.01, 0.1, 1, 10, 100, and 1000 μg/L of mianserin were prepared for the short-term exposure of zebrafish embryos for 120 h. The results showed that mianserin exposure reduced the body length of zebrafish larvae, in addition to altering multiple physiological and biochemical parameters in the exposed embryos/larvae. A dose-dependent inhibition of the total antioxidant capacity and total cholinesterase activity was revealed in the exposed fish larvae upon increasing the concentrations of mianserin exposure. A U-shaped concentration-dependent response curve was observed for the adrenocorticotropic hormone; however, an inversed U-shaped response curve was obtained for the monoamine oxidase level in response to mianserin exposure. Activities of the total adenosine triphosphatase (T-ATPase), Na^+/K^+-ATPase, and Ca^{2+}/Mg^{2+}-ATPase were significantly increased in the fish larvae exposed to relatively high doses of mianserin; interestingly however, low dose of mianserin at 10 ng/L inhibited their Na^+/K^+-ATPase and T-ATPase activities. Additionally, the coordinated regulation of cyclic adenosine monophosphate and protein kinase A was observed in the mianserin-exposed fish larvae, implying a reserved signaling pathway involved in the fish response to the antidepressant. Therefore, our study demonstrated that mianserin exposure significantly affected the early development of fish embryos at environmentally relevant concentrations, and suggested that the risk of pharmaceutical contamination of the aquatic environment, even at low doses, should receive more attention.

1. Introduction

Owing to the growing consumption of medicines worldwide, many pharmaceuticals as well as their metabolites are excreted in feces, which end up in the aquatic environment through effluents from sewage disposal plants (Brooks et al., 2003; Kolpin et al., 2002; Richardson, 2008). Therefore, in the last few years, pharmaceuticals have been considered as emerging environmental pollutants owing to their ubiquity in the environment (Togola and Budzinski, 2008). Antidepressants are one of the most commonly prescribed pharmaceuticals in the United States (Schultz et al., 2010). The increasing prescription and consumption of antidepressants are related to the increase in the prevalence of psychiatric disorders (Silva et al., 2012). The highly frequent use and continual output of the medicines from wastewater disposal plants and other continuous exposures have resulted in continuous contamination of water, along with accumulating concentrations of antidepressants (Gros et al., 2007).

Mianserin, a tetracyclic antidepressant, is approved in various countries for the treatment of major depression, its chemical structure differs from that of tricyclic antidepressants. Because of the blockage of presynaptic α2-adrenoceptors, the antidepressant effect of this drug is associated with an increase in the noradrenergic neurotransmission

* Corresponding authors.
E-mail addresses: mingyang@shu.edu.cn (M. Yang), 601543197@qq.com (S. Liu), 514998210@qq.com (L. Hu), 292291247@qq.com (J. Zhan), 2292678223@qq.com (P. Lei), mhwu@staff.shu.edu.cn (M. Wu).
[1] These authors contributed equally.

https://doi.org/10.1016/j.ecoenv.2017.12.024
Received 11 January 2017; Received in revised form 3 December 2017; Accepted 9 December 2017

(Marshall, 1983). Clinically, mianserin is used for the treatment of both depression and sleep disorders, and is considered superior to the selective serotonin reuptake inhibitors (SSRIs) regarding suicidal risk during the treatment of depressive episodes (Cohen, 2006). Because of random discharge and excretion of drugs into the aquatic environment, studies have reported the occurrence of mianserin in water systems, up to 62.3 ng/L in wastewater treatment plants (Loos et al., 2013) and 0.9 ng/L in tap water (Giebułtowicz and Nałęcz-Jawecki, 2014). To the best of our knowledge, previous studies have mainly focused on SSRIs owing to their potential adverse effects in the aquatic organisms (Estévez-Calvar et al., 2016; Magni et al., 2016). It has been reported that SSRIs can destroy the physiological processes in fish leading to altered reproductive behaviors, abnormal embryonic development, and reduced reproductive capability (Calisto and Esteves, 2009; Mennigen et al., 2008; Park et al., 2012). However, the adverse effects of tetracyclic antidepressants, such as mianserin on the aquatic organisms have not been thoroughly studied (Fong and Ford, 2014; Ford and Fong, 2015). Most of the previous studies on mianserin have been performed in rodents and humans (Cross et al., 2016; Stasiuk et al., 2016), with the exception that Van der Ven et al. (2006a; 2006b) have demonstrated the endocrine disrupting potency of mianserin by gene expression analysis in the zebrafish exposed to mianserin, in addition to our previous study indicating that mianserin exposure could alter the mRNA expression of genes related to many biological processes in zebrafish embryos/larvae (Wu et al., 2017).

Huggett et al. (2003) have proposed a threshold discrimination method to predict the pharmacological interactions of an active pharmaceutical ingredient with aquatic species. This method, called “the fish plasma model” presumes a conserved molecular drug target in the wildlife species. Hence, the target will be affected when the plasma levels in the fish roughly approach the human therapeutic doses. In the present study, we first predicted the mianserin concentration in the fish plasma that is equal to its therapeutic concentration in humans based on the fish plasma model, and then, we prepared a series of concentration gradients of mianserin, including those that were below and above the predicted fish plasma effective concentration for water exposure. Different parameters were evaluated in zebrafish embryos after a short-term exposure to mianserin during their early developmental stages, including adrenocorticotropic hormone (ACTH) as an indicator of hormone levels, monoamine oxidase (MAO), total cholinesterase (TChE), total antioxidant capacity (T-AOC), total adenosine triphosphatase (T-ATPase), Na^+/K^+-ATPase, and Ca^{2+}/Mg^{2+}-ATPase as a biomarker, and cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) as indicators of the involved pathway. Some recent evidences have implicated that low doses of antidepressants could induce negative effects on non-target aquatic species (Estévez-Calvar et al., 2016; Fong and Ford, 2014; Huang et al., 2017). Therefore, our study testified that environmentally relevant low doses of the antidepressant mianserin caused physiological and biochemical effects in the zebrafish embryos/larvae, and provided further reference for the risk assessment of the pharmaceuticals in the environment.

2. Materials and methods

2.1. Zebrafish embryos

Wild-type AB zebrafish were obtained from Wuhan (China Zebrafish Resource, CZRC). Fish were fed twice with brine shrimps daily and maintained under daily cycles of 14 h light (250 lx):10 h dark at 28 ± 0.5 °C. Zebrafish embryos were obtained by natural spawning of adult zebrafish. Embryos were raised at 28 ± 0.5 °C and examined under a stereomicroscope to remove the unfertilized embryos after four hours of fertilization (hpf); the embryos that reached the blastula stage were considered to have developed normally and were selected for subsequent experiments. All procedures were performed in conformity with the National Institutes of Health (NIH) guidelines and received the approval from the Institutional Animal Care and Use Committee at Shanghai University of China.

2.2. Chemicals

Mianserin hydrochloride (CAS number 21535-47-7) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions (1 g/L) of mianserin were prepared using ultrapure water and stored at 4 °C. All other reagents were of analytical grade (Anpel Scientific Instrument Company, Shanghai, China).

2.3. Experimental design

Mortality was measured every 12 h for 120 h after the exposure of zebrafish embryos to diverse concentrations of mianserin as an acute toxicity test. Briefly, 30 embryos (4 hpf) were randomly distributed into 6-well plates and exposed to a diluted toxicant solution until 124 hpf (8 mL solution per well), at the nominal concentrations of 0, 1, 3, 4, 5, 7, and 10 mg/L, according to Yang et al. (2014). Experiments were carried out in triplicates for each concentration of the mianserin solution. Zebrafish embryos were then exposed to mianserin at a sublethal concentration, the embryos (4 hpf) were randomly distributed into 6-well plates containing 30 embryos per well with concentrations of 10 and 100 ng/L; 1, 10, and 100 μg/L; and 1 mg/L for 120 h. Experiments were conducted in triplicates per exposure. Hatchability was recorded every 2 h starting from the hatching of the first embryo until 72 hpf. The samples were kept in an incubator at 28 ± 0.5 °C under a 14 h light:10 h dark cycle. After exposure to mianserin, the zebrafish embryos/larvae were examined for several toxicological endpoints, including the hatching rate and body length. Then, the larvae from each mianserin-exposed well were pooled into one sample, snap-frozen in liquid nitrogen, and stored at − 80 °C for subsequent biochemical analyses.

2.4. LC–MS/MS analysis

After 24 h-exposure, the mianserin-exposed solutions were collected and centrifuged at 1000 × *g* for 5 min. Then, the actual exposure concentrations of mianserin in these supernatants were measured by liquid chromatography-tandem mass spectrometry (LC–MS/MS). Briefly, an Agilent 1260 series liquid chromatography coupled to 6460 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA) was used for mianserin detection. The chromatographic separation was achieved using a Poroshell 120 EC-C18 reversed-phase column (3 × 100 mm, 2.7 μm, Agilent); the column temperature was maintained at 40 °C. The mobile phases were eluent A (acetonitrile) and eluent B (water/ammonium formate). The flow rate was 0.4 mL/min, and the injection volume was set at 5 μL. The gradient was as follows: 5:95A/B held for 0.75 min, 70:30 A/B at 7.5 min and held for 1 min. The critical MS/MS parameters were as follows: drying gas temperature, 300 °C; drying gas flow, 6 L/min; nebulizer pressure, 45 psi; sheath gas temperature, 350 °C; sheath gas flow, 11 L/min; capillary voltage (+), 3500 V; and nozzle voltage (+), 1000 V. All MS/MS data for mianserin were obtained in the positive ion mode by multiple reaction monitoring of the transition, *m/z* 265.11 → *m/z* 208.

2.5. Sample treatment for biochemical assays

Frozen tissue samples were homogenized in 800 μL of Tris-HCl buffer (100 mM, pH 7.4) using a homogenizer from Tiangen Biotech (Beijing, China). The homogenates were centrifuged at 9168 × *g* for 15 min at 4 °C. Then, the supernatants were collected and aliquoted for subsequent biochemical assays. The protein concentration in each sample was determined using the Bradford assay using bovine serum albumin as a standard. The protein concentration in each sample was calculated accordingly and used to calibrate the measured value of each