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Research Paper

Global transcriptomic analysis of zebrafish in response to embryonic exposure to three antidepressants, amitriptyline, fluoxetine and mianserin

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ABSTRACT

Antidepressants are among the most commonly detected pharmaceuticals in aqueous systems, and, as emerging organic pollutants, may exert negative effects on non-target aquatic organisms. Previously, it has been revealed that antidepressant exposure significantly inhibits the growth and development of fish during their early developmental stages. Thus, in the present study, we aimed to identify and compare the underlying mechanisms of action of different antidepressants at the transcriptional level using zebrafish (*Danio rerio*) embryos. Through high-throughput RNA sequencing (RNA-Seq) data analysis, 32, 34, and 130 differentially expressed genes (DEGs) were obtained from zebrafish larvae after 120 h of embryonic exposure to sublethal concentrations of amitriptyline, fluoxetine, and mianserin, respectively. The expression profiles of the identified DEGs showed similar trends in response to the three antidepressant treatments, suggesting consistent toxic effects of low concentrations of these three drugs on the regulation of gene expression in fish. Several metabolic and signaling pathways, including glycolysis/gluconeogenesis and the insulin pathway, were affected in the exposed fish larvae. The expression profiles of selected DEGs were then verified by the qRT-PCR method, which indicated significant positive correlations with the RNA-Seq results. Next, we determined the concentration-dependent expression patterns of 6 selected DEGs in fish larvae exposed to three antidepressants at a series of environmentally relevant concentrations. The results revealed a significant concentration-dependent reduction in the levels of *dual-specificity phosphatase 5* (*dusp5*) mRNA, as well as a non-concentration-dependent gene expression inhibition of *prostaglandin D2 synthase b* (*ptgdsb*); the circadian rhythm-related genes, i.e. those encoding nuclear receptor subfamily 1, group D, member 1 (*nr1d1*) and period 2 (*per2*); and genes encoding early growth response factors (*egr1* and *egr4*), in the antidepressant-treated fish larvae. In summary, to our knowledge, our findings demonstrate, for the first time, that the three different categories of antidepressants have common effects on the gene expression involved in multiple biological processes and signaling pathways during the early development of fish and thus provide information for characterizing the adverse outcome pathways and on the ecological risk assessment of these pharmaceutical pollutants in the aquatic environment.

1. Introduction

Human and veterinary pharmaceuticals, a class of emerging organic contaminants, were first detected in sewage water effluents in the 1970s (Hignite and Azarnoff, 1977). Pharmaceuticals are designed to cure disease and improve human health; however, because of random discharge of excess or expired drugs into the environment, especially into waste water, and inefficient removal of most pharmaceutical residues by waste-water treatment plants (WWTPs), low concentrations of these pharmaceuticals and their decomposed products remain widely distributed in aquatic systems (Cunningham et al., 2006). Over the last two decades, a large number of studies have demonstrated the presence of pharmaceutical residues in various aquatic environmental media worldwide, including surface water and sediments, sewage water, and even drinking water in the USA (Benotti et al., 2009) and some European (Carballa et al., 2004; Gracia-Lor et al., 2012) and Asian

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countries (Kim et al., 2007; Wen et al., 2014). Although the environmental concentrations of pharmaceuticals in water are usually low, at ng/L levels, their active ingredients can still act on the physiological systems of non-target aquatic organisms and affect their health and development, thus posing a great ecological risk to the aquatic environment (Fent et al., 2006; Santos et al., 2010).

Antidepressants are the most commonly prescribed drugs for the treatment of anxiety, panic disorder, and depression globally. They are also the most commonly detected drugs in the aquatic environment (Fong and Ford, 2014). There are four categories of antidepressants used for the clinical treatment of depression: tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), tetracyclic antidepressants (TeCAs), and monoamine oxidase inhibitors (MAOIs). Amitriptyline is the most common clinically used TCA worldwide. The highest reported environmental concentration of amitriptyline is 3.7 ng/L, in the surface river water in Shanghai, China (Wu et al., 2015); however, its concentration is as high as 365 ng/L in sewage water in Saudi Arabia (Alidina et al., 2014). Furthermore, its concentration ranges from 6.0 to 207 ng/L in the effluents of WWTPs in American and European countries (Kasprzyk-Hordern et al., 2008; Kostich et al., 2014; Lajeunesse et al., 2012; Togola and Budzinski, 2008). Fluoxetine, the active pharmaceutical ingredient in Prozac, is a typical SSRI used for the treatment of depression (Gonzalez-Rey and Bebianno, 2013). The concentrations of fluoxetine in effluents from WWTPs in Europe are much higher than those in Asian countries (Alidina et al., 2014; Bueno et al., 2007; Ryu et al., 2011; Salgado et al., 2011), with the highest reported concentration being 929 ng/L (Bueno et al., 2007). Mianserin is a TeCA. This class of antidepressants is widely used owing to their lower toxicities than that of TCAs (Chou et al., 2007). However, there are only a few studies on the occurrence of mianserin in the environment, which has been reported to be found in ng/L levels in sewage-contaminated river water and at concentrations of up to 0.9 ng/L in tap water in Poland (Giebultowicz and Nalecz-Jawecki, 2014). Furthermore, previous studies (Bringolf et al., 2010; Schultz et al., 2010) have shown that the concentrations of fluoxetine in aquatic organisms are much higher than those in their living waters or sediments. Chu and Metcalfe (2007) reported that fluoxetine concentrations ranged from 0.14 to 1.02 μg/kg in three fishes and suggested that the antidepressants are absorbed by aquatic organisms and accumulated in their tissues.

According to previous studies, exposure to antidepressants could affect the reproductive capacity, survival, growth, and predator and learning behaviors of aquatic organisms (Eisenreich and Szalda-Petree, 2015; Lister et al., 2009; Mennigen et al., 2010; Painter et al., 2009; Schultz et al., 2011; Yang et al., 2014). However, the mechanisms of action of antidepressants are still elusive. The effects of SSRIs such as fluoxetine on non-target species are most likely mediated by 5-hydroxytryptamine (serotonin; a neurotransmitter), and its receptor (Hedlund, 2009), which is associated with the modulation of neuronal mechanisms. Gonzalez-Rey and Bebianno (2013) reported that short-term exposure to a very low concentration (75 ng/L) of fluoxetine can induce acetylcholinesterase activity and arouse a neurotoxic response in mussels. Pittman and Hylton (2015) also reported neuronal alterations in zebrafish upon fluoxetine exposure. Moreover, several studies have reported adverse effects of antidepressants on the immune system. Amitriptyline, for instance, modulates the antioxidant capacity, induces oxidative stress, and changes the immune-related physiological and chemical parameters and gene expression in zebrafish (Yang et al., 2014) and rodents (Mika et al., 2015; Vismari et al., 2012), similar to the reported effects of fluoxetine on the immune system of mussels (Gonzalez-Rey and Bebianno, 2013). In addition, a brain-specific cDNA microarray analysis of zebrafish has indicated that mianserin disrupts neuroendocrine-related gene expression (e.g., of genes encoding aromatase and estrogen receptors), suggesting the modulation of neuroendocrine processes by mianserin (van der Ven et al., 2006).

To better understand the underlying mechanisms of different categories of antidepressant-induced toxicities in non-target fish populations, we performed global transcriptome sequencing (RNA-Seq) using the embryos of zebrafish (*Danio rerio*) at early developmental stages, followed by treatment with amitriptyline, fluoxetine, and mianserin. The responsive genes demonstrating similar expression trends were then further verified in fish embryos exposed to these chemicals at a series of environmentally relevant concentrations. The present study aims to identify and compare the mechanisms of action of three different types of antidepressants at relatively low environmentally relevant concentrations and to further explore their universal and individual mechanisms of effect in fish.

2. Materials and methods

2.1. Chemicals

Amitriptyline hydrochloride (CAS number 549-18-8; 98 + %) and mianserin hydrochloride (CAS number 21535-47-7; 98 + %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluoxetine hydrochloride (CAS number 56296-78-7; 98 + %) was purchased from CNW Technologies GmbH (Germany). These compounds were dissolved in Milli-Q water, separately, to obtain 10 g/L stock solutions and were stored at 4 °C until use. All other chemicals used were of analytical grade and purchased from Sigma-Aldrich or Sangon (Shanghai, China).

2.2. Zebrafish embryos

Wild-type (Tuebingen) zebrafish were maintained in flow-through aquarium systems on a 14 h light:10 h dark photoperiod at 28 ± 0.5 °C. They were fed with flake food and live brine shrimp (*Artemia nauplii*) twice daily. Adult male and female fish were placed together in breeding tanks in the evening before embryos were required. The embryos were collected the next morning after spawning and were examined under a stereo microscope to remove unfertilized or unhealthy embryos. Embryos that had reached the blastula stage were considered normal and were used for the following exposure experiments at 4 h post fertilization (hpf). The embryos were washed three times to remove debris and were then incubated at 28 ± 0.5 °C. The embryo media (1 × E3 medium with 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.3 mM $MgSO_4$) or exposure solutions were replaced completely every 24 h. The concentrations of the three antidepressants in water had no significant changes during the exposure, on the basis of the results of liquid chromatography-tandem mass spectrometry (LC–MS/MS) (Table 1) and our previous study (Yang et al., 2014).

2.3. Experimental design

2.3.1. Selection of the exposure concentrations of antidepressants

Owing to the availability of a large amount of mammalian safety data for pharmaceuticals, biological “read-across” approaches have been employed to predict the potential effects of pharmaceuticals on

Table 1
The measured concentrations of fluoxetine and mianserin in the exposure experiment by liquid chromatography-tandem mass spectrometry (LC–MS/MS) after 12 h- or 24 h-exposure.

Norminal concentrations	Fluoxetine		Mianserin	
	12 h (n = 3)	24 h (n = 3)	0 h (n = 3)	24 h (n = 3)
Control	ND[a]	ND	ND	ND
0.1 (μg/L)	0.11 ± 0.01	0.10 ± 0.02	0.14 ± 0.01	0.13 ± 0.01
1 (μg/L)	1.02 ± 0.05	1.00 ± 0.04	2.25 ± 0.09	2.03 ± 0.20
10 (μg/L)	9.74 ± 0.53	9.83 ± 0.03	16.91 ± 0.87	15.74 ± 0.89

[a] Not detected.

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