Neurotoxicology and Teratology 62 (2017) 27–33

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Acute effects of amitriptyline on adult zebrafish: Potential relevance to antidepressant drug screening and modeling human toxidromes

Konstantin A. Demin[a,1], Tatiana O. Kolesnikova[a,1], Sergey L. Khatsko[a], Darya A. Meshalkina[b,1], Evgeniya V. Efimova[b], Yuri Yu. Morzherin[a], Allan V. Kalueff[c,d,e,f,*]

[a] Ural Federal University, Ekaterinburg 620002, Russia
[b] Institute of Translational Biomedicine (ITBM), St. Petersburg State University, St. Petersburg 199034, Russia
[c] School of Pharmaceutical Sciences, Southwest University, Chongqing 400700, China
[d] Institutes of Chemical Technologies and Biological Sciences, Ural Federal University, Ekaterinburg 620002, Russia
[e] Laboratory of Biological Psychiatry, ITBM, St. Petersburg State University, St. Petersburg 199034, Russia
[f] ZENEREI Research Center, Slidell, LA 70458, USA

ARTICLE INFO

Keywords:
Zebrafish
Amitriptyline
Aquatic screening
Serotonin toxicity
Serotonin syndrome

ABSTRACT

The need to develop novel antidepressants is an emerging problem in biomedicine. An aquatic vertebrate species, the zebrafish (*Danio rerio*) may serve as a useful in-vivo screen for CNS drugs, and displays high sensitivity to a wide range of antidepressants. Amitriptyline is a commonly used tricyclic antidepressant which acts primarily as a serotonin and noradrenaline reuptake inhibitor. Here, we characterize drug-induced behavioral and neurochemical responses in adult zebrafish following their acute exposure to amitriptyline. Overall, the drug at 1 and 5 mg/L significantly increased time spent in top and shortened the latency to enter it, thereby paralleling recent reports on zebrafish 'serotonin toxicity-like behavior' caused by various serotonergic agents. The 10 mg/L dose of the drug also significantly decreased top entries and maximal velocity and evoked overt ataxia, likely due to emerging non-specific toxic effects. Amitriptyline at 5 and 10 mg/L also dose-dependently increased serotonin turnover, but not noradrenaline levels, in zebrafish whole-brain samples. Overall, zebrafish high sensitivity to acute effects of amitriptyline can help improve our understanding of psychopharmacological profiles of this compound and the related CNS drugs, and contributes further to the development of aquatic experimental models of human toxidromes.

1. Introduction

Amitriptyline is a commonly used tricyclic antidepressant (TCA) that acts as an inhibitor of serotonin and noradrenaline reuptake (Sindrup and Jensen, 1999; Tatsumi et al., 1997). As a common TCA, amitriptyline is widely prescribed to treat depression, anxiety and other conditions, including neuralgia, diabetic neuropathy and anorexia (Bansal et al., 2009; Halmi et al., 1986; Moore et al., 2012; Sindrup et al., 1990; Vainio et al., 1995; Vrethem et al., 1997; Watson et al., 1998; Zychowska et al., 2013). Amitriptyline is metabolized by liver cytochrome P450s into an active metabolite, nortriptyline, which also has TCA-like activity (Lehmann et al., 1982). Amitriptyline is excreted with urine either unchanged or as its active (nortriptyline) and inactive metabolites (Baker et al., 2014; Kasprzyk-Hordern et al., 2008). In addition to serotonin/noradrenaline reuptake inhibition, both amitriptyline and nortriptyline act as serotonin-, histamine- and muscarinic acetylcholine receptors antagonists, as well as sigma-1 and neurotrophic tyrosine kinase A/B receptors agonists (Ellis and West, 2011; Jang et al., 2009; Nguyen et al., 2001; Owens et al., 1997; Rauser et al., 2001; Sriram, 2010; Werling et al., 2007).

The effects and mechanism(s) of action of antidepressant drugs remains poorly understood, necessitating novel models for their screening in-vivo and the discovery of new efficient drug targets. Although it is rarely used as a first-line antidepressant (due to toxicity, risks of overdose and common 'TCA-like' side effects), amitriptyline continues to be widely used globally because of its high efficiency and low cost (Barbui and Hotopf, 2001). In addition to clinical importance, amitriptyline is also relevant to environmental biomedicine. For example, the growing clinical use of antidepressants (including amitriptyline and other TCAs) also raises bioenvironmental concerns (Hughes et al., 2013), especially in large cities, where amitriptyline (excreted with patients' urine) is even found in drinking water (Togola and Budzinski,

* Corresponding author at: School of Pharmaceutical Sciences, Southwest University, Chongqing 400700, China.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).
[1] These authors have equally contributed to this study.

http://dx.doi.org/10.1016/j.ntt.2017.04.002
Received 7 December 2016; Received in revised form 31 March 2017; Accepted 18 April 2017
Available online 22 April 2017

2008). Collectively, this calls for further extensive research on modeling biological effects of amitriptyline on human health.

The zebrafish (*Danio rerio*) is rapidly becoming a promising model organism for both toxicological and neuropharmacological in-vivo studies (Kalueff et al., 2014). Due its cost-efficiency, easy housing, rapid reproduction, and high genetic and physiological homology to humans, this fish have become a popular tool in toxicological screening and studying brain disorders (Kalueff et al., 2014; Maximino et al., 2013a, 2013b; Neelkantan et al., 2013; Sackerman et al., 2010; Wong et al., 2010). Since zebrafish central monoaminergic systems are also robustly expressed (Maximino et al., 2013a, 2013b), this organism is particularly useful for antidepressant screening in-vivo (Stewart et al., 2013). The present study aimed to examine the value of zebrafish-based aquatic screens for TCAs and associated toxicity by assessing acute behavioral and neurochemical effects of amitriptyline in adult zebrafish.

2. Methods

2.1. Animals and housing

Adult mature (5–7 months) male and female wild type short-fin zebrafish (approximately 50:50 male:female ratio) were obtained from a local commercial distributor (Zootrade Inc., Ekaterinburg, Russia) and housed in groups of 15 fish per 20-L tank, filled with filtered system water maintained at 22–25 °C. Illumination (950–960 lx) was provided by 36 ceiling-mounted 18-Wt fluorescent light tubes with a 10/14 light/dark cycle according to the standards of zebrafish care (Westerfield, 2000). All fish used in this study were experimentally naive and fed twice daily with Tetramin-Pro (Terta GMBH, Osnabruck, Germany). Animal experiments were approved by IACUC of Ural Federal University, and fully adhered to National and Institutional guidelines and regulations.

2.2. Behavioral testing and pharmacological manipulations

Behavioral testing was performed between 11.00 and 17.00 h using tanks with water adjusted to the holding room temperature, to assess zebrafish behavior in the novel tank test. Prior to testing, fish were pre-exposed in a 0.5-L plastic beaker for 20 min to either drug-treated or drug-free vehicle, 0.1% solution of dimethyl sulfoxide (DMSO, Tathimpharmpreparaty Inc., Kazan, Russia) known to be devoid of own behavioral effects in zebrafish, and is commonly used in zebrafish drug studies (Goldsmith, 2004). For treatment, fish were randomly divided in 4 groups (n = 15): drug-free control, 1 mg/L, 5 mg/L and 10 mg/L amitriptyline. Doses were chosen based on our pilot studies with this drug. The standard 20-min pre-treatment time was chosen here based on our prior experience with various CNS drugs (Riehl et al., 2011). Fish were then exposed to the novel tank test, assessing their anxiety and locomotion (Levin et al., 2007; Stewart et al., 2011b; Stewart et al., 2011c). The tank consisted of a 1.5-L rectangle tank (15 cm height × 25 cm length × 7 cm width; Aquatic Habitats, Apopka, FL, USA) filled with water filled up 14 cm height and divided into two equal virtual horizontal portions by a line marking the outside walls. Trials were recorded by web-camera for further analyses. Zebrafish behavior was then processed by trained observer blinded to the treatments, which used RealTimer (Open Science, Krasnogorsk, Russia) to manually score different behavioral endpoints (intra-rater reliability > 0.85), such as the number and duration (s) of freezing bouts, number of complete, circle moves, erratic movements and erratic bottom movements. Freezing was defined as a total absence of movement, except for the gills and eyes, for > 2 s. Others parameters, including maximal velocity (cm/s), average velocity (cm/s), distance traveled (cm), top entries, time spent in top, the latency to top entry (s), were collected using idTracker (Perez-Escudero et al., 2014). Furthermore, the highest dose (10 mg/L) group phenotype was analyzed for behavioral toxicity using the two-point scale (0 – absent, 1 – present), also measuring the duration (s) and the number of specific ataxia movements, such as swimming on a side, upside down or vertically. In all our analyses, the fish belonged to the same baseline population (same holding tank), and were allocated randomly to the tested groups.

2.3. Neurochemical analyses

In a separate cohort of adult zebrafish (24 fish per group), we applied the high-throughput liquid chromatography (HPLC) to examine neurochemical alterations evoked by acute amitriptyline exposure. Briefly, zebrafish were exposed to 5 or 10 mg/L of amitriptylin or to vehicle for 20 min, and then quickly euthanized by iced water exposure. Zebrafish brains were immediately extracted on ice with subsequent cryoconservation in liquid nitrogen. At the day of analyses, the brains were submerged in 5 μL of 0.1 M perchloric acid with 10 ng/mL DHBA (as internal standard) per mg of tissue, and sonicated on ice. The brain extracts were next centrifuged for 15 min at 12000 *g* on + 4 °C, and filtered through the hydrophilic PVDF membrane with 0.22-μm pores (Millex-GV, Merk Millipore, Darmstadt, Germany). Extracts from 24 whole brains were pooled (3 brains per sample), resulting in 8 pooled samples per each treatment group (n = 8). Detection of noradrenaline, serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in whole-brain samples was performed by HPLC with electrochemical detection (HPLC-ECD) on HTEC-510 chromatography system on the reverse phase C-18 CA-5ODS column (Eicom, San Diego, USA) using the standard chemicals of HPLC grade obtained from Sigma-Aldrich (St. Louis, MO, USA). The mobile phase was 0.1 M phosphate buffer (pH = 4.5), 1.85 M sodium octanosulphonate, 0.17 mM EDTA and 18% methanol. The retention times for noradrenaline, serotonin and 5-HIAA in the present experimental setting were 4.64, 22.39 and 8.60 min, respectively. The amount of each neurochemical was calibrated using a standard curve plotted for 3 different concentrations of the standards' solutions (10, 25 and 100 ng/mL), and expressed as pg/mg brain tissue. The level of HPLC column noise recorded (< 0.05 mV) afforded clear detection of neurotransmitter signals (> 50 mV) in the present study. The Limit of Blank (LOB) values for neurochemicals in this HPLC assay were 1928.5 au for noradrenaline, 5870.0 au for serotonin, and 12,514.7 au for 5-HIAA. The Limit of Detection (LOD) values were 0.61 pg/mg brain tissue for noradrenaline, 2.11 pg/mg for serotonin, and 4.09 pg/mg for 5-HIAA, respectively. The Limit of Quantification (LOQ) values in this study were 2.04 pg/mg for noradrenaline, 7.03 pg/mg for serotonin, and 13.62 pg/mg for 5-HIAA. In addition, whereas synaptically released serotonin is quickly metabolized, the 5-HIAA/serotonin ratio was computed for each sample (as a commonly used serotonin turnover index in the brain), to comprehensively assess amitriptyline-evoked alterations in serotonergic neurotransmission.

2.4. Statistical analyses

Behavioral and HPLC data were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc test, and expressed as mean ± SEM (n = 15 per group). For behavioral toxicity analyses, due to the lack of toxicity endpoints in control fish (mean values = 0, with zero variance), behavioral responses to 10 mg/L amitriptyline were expressed as the numbers of animals (per 15-animal group) showing general signs of toxicity/ataxia and its specific subtypes, such as swimming on a side, upside down, and vertically. The Chi-square test with Yates correction was then applied to these categorical data, to compare the occurrence of ataxia-like behaviors in control and the 10 mg/L amitriptyline groups. All experimenters were blinded to the treatments. All animals tested were included in final analyses without attrition or exclusion, and all planned analyses were reported here. Statistical significances was set at $P < 0.05$ in all tests.