Chronic Effects of Fluoxetine on Danio rerio

Subjects: Biology

Contributor: Daniela Correia, Melissa Faria, Miguel Oliveira

Fluoxetine is an antidepressant widely used to treat depressive and anxiety states. Due to its mode of action in the central nervous system (selective serotonin reuptake inhibitor (SSRI)), it becomes toxic to non-target organisms, leading to alterations detrimental to its survival.

Keywords: pharmaceuticals ; selective serotonin reuptake inhibitors ; chronic effects ; behavior ; biochemical biomarkers ; environmentally relevance

1. Introduction

Currently, mental health is a topic of concern, due to the increased stress levels of society. The COVID19 pandemic has increased the pressure on human health, with an increase in the occurrence of depression being reported [1]. Thus, an increase in the consumption of antidepressants is expected and will consequently lead to increased environmental levels and potential effects to biota present in an aquatic environment, the final destination of environmental pollutants. Fluoxetine, known to be a selective serotonin reuptake inhibitor (SSRI), is generally prescribed for the treatment of human depression, anxiety, compulsive behavior, and eating disorders [2][3][4][5][6][7]. This drug is known to act at the central nervous system, blocking the serotonin transport, leading to its accumulation in the synaptic cleft [4][5][8][9][10][11][12][13], allowing an attenuation of anxiety and depressive symptoms (anxiolytic effect) [12][14]. Serotonin (5-HT) is a neurotransmitter that has a fundamental role in regulating the development of the brain and spinal cord and, during this development, acts as an important neurotrophic factor in neuronal proliferation, differentiation, axonal growth, migration, and synaptogenesis [15][16]. Additionally, it has the ability to modulate parameters related to behavior such as locomotion, stress, appetite, reproduction, aggressiveness, and social interactions [2][7][13][16][17][18][19]. In the freshwater environment, concentrations of fluoxetine have been detected at levels ranging from 0.0004 to 3.645 µg/L in wastewater treatment plants (WWTP) [20][21][22][23][24][25][26][27][28][29][30][31], 0.0005 to 0.056 µg/L in surface waters and groundwaters [29][31][32][33][34][35][36][37] and, for drinking water, the levels vary between 0.0005 and 0.0008 µg/L [38][39]. Previous studies have demonstrated that fluoxetine can be toxic to fish, with exposure resulting in changes at different biological levels, from gene transcription, neurotransmission markers, enzymatic activities (e.g., oxidative stress, metabolism), hormone levels, reproductive processes, and accumulation in various tissues (e.g., brain and liver), resulting in a severe change in the histology of these organs [4][5][6][7][10][11][14][40][41][42][43][44][45][46][47][48]. In addition, this pharmaceutical can cause changes in behavior (e.g., locomotor activity, stress response, feeding, aggression, social and anti-predatory behavior) [4][5][6][7][8][9][11][15][16][45][49][50][51][52][53][54][55][56][57][58][59][60].

2. Chronic Effects of Fluoxetine on *Danio rerio*

Here evaluated different behavior endpoints associated with anxiety/stress response (locomotion, thigmotaxis, and exploratory behavior), sociability (social test), and biochemical changes associated with neurotransmission, biotransformation, and oxidative stress, after chronic exposure of juvenile zebrafish to the SSRI antidepressant fluoxetine. In general, the data showed that, in the low µg/L range, fluoxetine can cause mild biological alterations in the tested endpoints affecting fish locomotor activity which, in the long term, may compromise population fitness. The concentrations tested are considered environmentally relevant, and fluoxetine levels similar to those tested here, and even lower, have already been detected in the environment. For example, in Baiyangdian Lake, Pearl River, Songhua River, Yellow River, Huai River, Hai River, and Liao River, fluoxetine levels up to 0.101 µg/L were detected [61][62].

Alternating light and dark changes are often used to assess the stress response in zebrafish larvae, where, larvae respond to the shift from light to dark with a burst of activity [63][64]. However, this assessment is scarcely used in juvenile fish. After 12 days of exposure to fluoxetine, zebrafish juveniles altered swimming time during the dark period at the highest concentrations. Curiously, the total distance traveled during this period was unaffected by fluoxetine, which indicates a decrease in swimming velocity and, therefore, a lower effect on the stress reaction to this condition. After 21

days of exposure, the change in locomotor activity during the light period became more evident. In a study carried out by Zindler et al. (2020) [60], fluoxetine reduced the swimming distance of embryos in the dark and changed the maximum speed during light periods, after 96 h exposure. Thigmotaxis is seen as the tendency of an animal placed in a new environment, to remain close to the walls and avoid the center of the aquarium. In fish, the persistence of this activity in the outer zone can be considered a measure of anxiety [65][66][67]. Fluoxetine did not affect this behavior following 21 days of exposure.

Regarding the measurement of angles during the fish's path, the high amplitude angles (class 1) showed no effects of fluoxetine exposure, after 12 and 21 days of exposure. High amplitude angles are indicators of erratic swimming behavior (stress behavior) and the decrease in its frequency manifests the anxiolytic effect of fluoxetine on the stress behavior of exposed juveniles.

The novel tank test aims to assess the exploratory behavior in zebrafish, focusing on measures of "vertical" behavior patterns. This test explores the tendency of a fish to dive and stay at the bottom of a new environment (geotaxis), before exploring the upper areas. This test is used extensively to assess the effects of a wide variety of compounds, including pharmacological chemicals [65][68]. Drugs with antidepressant and anxiolytic properties have the ability to modify this behavior, consequently leading the fish to explore the new space sooner and spend less time in the bottom [69][70][71]. There was no evidence of effects over fish geotaxis behavior. Similar to this study, Marcon et al. (2016) [72] also failed to observe any changes in fish behavior to a novel environment following 7 days of exposure to 10 µg/L of fluoxetine. On the other hand, other studies, using embryos and adult zebrafish, and adult *Oryzias latipes* (Medaka), have reported effects of fluoxetine over their locomotion parameters in concentrations ranging from 0.01 µg/L to 10,000 µg/L, and exposure periods ranging from 3 min to 30 days. These studies indicate that fluoxetine decreases stress levels and increases fish exploratory behavior, with longer swimming periods at the top of the tank. In addition, there is also a decrease in freezing, lower latency to enter the top area, and an increase in the number of entries into the top area [5][9][14][15][16][49][51][56][57][58][73]. All these parameters indicate that fluoxetine reduces anxiety and stress levels in the fish brain system, resulting in more relaxed behavior.

The zebrafish is a social fish, living in shoals in its natural habitat [51][71][74][75][76]. This social interaction minimizes the risk of predation and if an organism is isolated, it can trigger anxiety-like behavior [77], reducing serotonin levels in the nervous system [78]. The change in social behavior can impact the reproduction and survival of the species. Chronic fluoxetine exposure did not alter fish social behavior. However, other studies, for example, Giacomini et al. (2016) [51] found that a 15-min exposure to 50 µg/L fluoxetine decreased social interaction in adult zebrafish, with animals spending less time near the shoal.

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