Transformation Products of Fluoxetine Formed by Photodegradation in Water and Biodegradation in Zebrafish Embryos

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Abstract

The present study investigates the biotic and abiotic formation of transformation products (TPs) of fluoxetine (FLX). In a sunlight simulator, direct (ultra-pure water) and indirect (surface water) photolysis of FLX were investigated. 26 TPs were detected, which were formed by O-dealkylation, hydroxylation, CF3-substitution and N-acylation with aldehydes and carboxylic acids. In zebrafish embryos the bioconcentration factor of FLX was found to be 200, and about 1% of FLX taken up by the embryos was transformed to the well-known TP norfluoxetine (NFLX). Seven metabolites known from photodegradation as well as three new metabolites formed by N-hydroxylation, N-methylation and attachment of an amine group were identified in zebrafish embryos.

1. Introduction

Fluoxetine (FLX; trade name Prozac) is a frequently used antidepressant of the class of selective serotonin re-uptake inhibitors (SSRI) and ranges among the 20 most prescribed pharmaceuticals in USA. 10 % of the consumed drug are excreted unchanged and about 20 % as norfluoxetine (NFLX), the major human metabolite. FLX and NFLX have been detected in samples from surface water, sediments and aquatic biota and described as acute toxic substances for aquatic organisms (Nałecz-Jawecki 2007). Various studies on FLX degradation by means of abiotic oxidation processes revealed TPs formed by hydroxylation and O-dealkylation (cleavage of the C−O bond) (Lam et al. 2005). TPs containing acidic functional groups (ionization in ESI(-) mode) were insufficiently considered. Furthermore, formed metabolites apart from NFLX have been described vary rarely in the literature. Gulde et al. (2016) identified N-acylation as additional biotransformation process in activated sludge.

Further studies of biotic and abiotic reaction pathways are required since TPs might also have effects on aquatic life. The aim of the present study was therefore to identify further environmentally relevant transformation products (TPs) of FLX.

2. Materials and Methods

LC-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS; Agilent 6550 QTOF) was used to identify TPs of FLX from laboratory experiments. Direct and indirect photodegradation of FLX with simulated sunlight was performed with a sunlight test chamber (UVACUBE 400 from Hoenle UV Technology, Gräfling, Germany) equipped with a SOL 500 RF2 solar simulator and H2 glass filter glass. TPs were investigated in both pure (pH 6,8 and 10) and surface water (pH 8). Biotic TPs were identified from zebrafish (Danio rerio) embryos exposed to FLX. At 48 h postfertilization (hpf), ultrapure water was replaced by FLX test solution (5 mg/L) and metabolites were identified at 96 hpf. Analysis of fluoxetine and its metabolites in fish was conducted on three replicates consisting of five pooled embryos each. Extraction was done with 5 pooled embryos spiked with a mixture of 300 µl ACN and 200 µl of 12 µg/l FLX-D5 aqueous solution. Identification of TPs was based on accurate mass measurements, assignment of chemical formula and interpretation of accurate mass fragmentation patterns using information of typical losses and adduct formation during FLX transformation. A more detailed description of the method is given in Tisler et al. (2019).

3. Results and Discussion

Higher pH favors the neutral species of FLX and the neutral/anionic species of primary TPs and, therefore, photodegradation. NFLX proved to be a minor TP in photolysis (≥2% of degraded FLX). In addition, 27 TPs could be tentatively identified from photodegradation experiments (Tisler et al. 2019). TP formation occurred mainly by four different mechanisms: 1) Twelve TPs were identified by cleavage of the phenolether bond (O-dealkylation) which primarily formed 3-(methylamino)-1-phenyl-1-propanol (TP 166) and 4-(trifluoromethyl)phenol) (TFMP); 2) two hydroxylated TPs of the benzyl moiety were identified in negative and positive ionization mode; 3) CF3 substitution to benzoic aldehyde/acid formed seven TPs; 4) eleven TPs were identified by N-acylation. The adduct formation at the amine group is a well-known transformation mechanism in biological processes, but rarely described in abiotic processes. N-acylation of FLX in the photolysis with aldehydes and carboxylic acids is shown in Figure 1. Most of these TPs showed a characteristic in-source fragment during electrospray ionization (IF) which is formed by cleavage of the trifluoromethyl phenol moiety. The presence of 31 mg/L NO_3^- in the surface water could be the reason for higher abundance of most of the TPs in surface water compared to pure water, due to production of hydroxyl radicals. These results demonstrate their relevance in environmental processes.

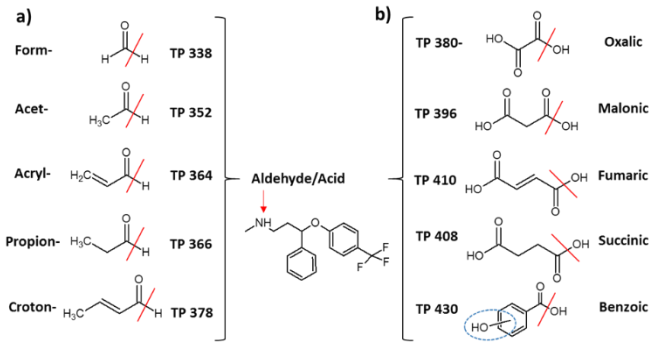

Figure 1: Formation of FLX TPs due to N-Acylation reactions with various aldehydes and carboxylic acids

In zebrafish embryos, about 1 % of FLX taken up by the embryos was transformed to the well-known metabolite NFLX. The bioconcentration factor of FLX was found to be 110. Seven metabolites known from photodegradation (TPs 338, 364, 354, 410, TFMP and the two hydroxyl-TPs) and three new metabolites (TP 326c,409, 324) have been identified (Tisler et al. 2019). In addition to the hydroxyl-FLX TPs 326a and 326b, a hydroxylamine derivative of FLX (TP 326c) has been identified. TP 409 can be an N-acylation product of FLX with the amino acid L-valine (Mishra et al. 2017). TP 324 was assumed to be formed by methylation of FLX. Generally, in photochemical processes low-molecular weight TPs have been produced due to the loss of structural moieties of FLX. In biotic processes in Zebrafish however, adduct formation was the dominant reaction pathway (e.g. N-acetylation). The study highlights the importance of considering a broad range of TPs of FLX in fresh water systems to understand the environmental fate and ecotoxicological effects of FLX.

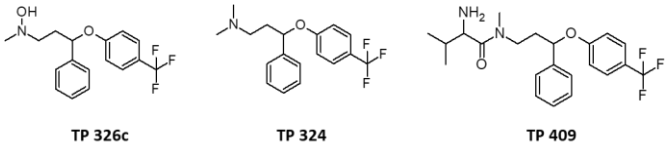

Figure 2: Proposed structures of metabolites, which were formed by zebrafish embryos but not detected in photolysis
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