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Endocrine effects after ozonation of tamoxifen

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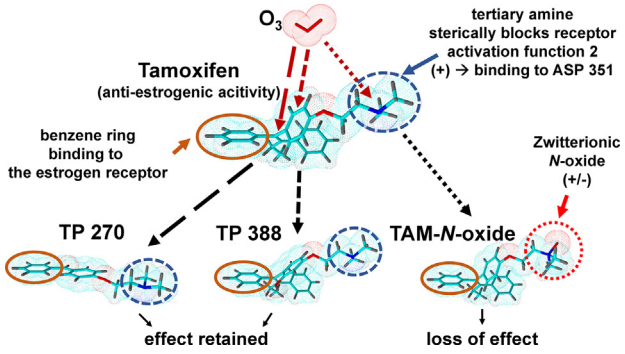
HIGHLIGHTS

- Anti-estrogenic activity and TPs after ozonation of tamoxifen were determined.
- Ozonation under neutral conditions removes the endocrine effect.
- Correlation between TP 270 and the anti-estrogenic activity was found.

GRAPHICAL ABSTRACT



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ABSTRACT

Ozonation is used as additional wastewater treatment option to remove recalcitrant micropollutants. It also removes the estrogenic activity found in wastewater but not always the anti-estrogenic activity. This can be explained by an incomplete removal of anti-estrogenic micropollutants or by formation of transformation products (TPs) which retain the activity. The present study investigates the degradation of the anti-estrogenic pharmaceutical tamoxifen in pure water, regarding TP formation and related anti-estrogenic effect using *Arxula adeninivorans* yeast estrogen screen (A-YES). In total, five transformation products were detected: three *N*-oxides and two further products (TP 270 and TP 388). For the transformation product TP 270 a correlation of the extent of formation with an increase of the anti-estrogenic activity was determined, demonstrating that transformation products from ozonation can be more active in a bioassay than the parent compounds. Our study shows also that the transformation of tamoxifen to *N*-oxides reduces the anti-estrogenic activity. The reactivity of amines towards ozone typically increases with pH, since only deprotonated amines react with ozone. Hence, removal of the endocrine activity by *N*-oxide formation may be disfavored at low pH.

1. Introduction

The determination of ecotoxicological effects, such as endocrine effects, is an important aspect for the evaluation of advanced wastewater treatment processes (Ternes et al., 2017). Endocrine effects can be caused by endocrine disruptive compounds (EDCs), a group of micropollutants affecting the hormonal system of organisms which

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can induce biological effects already at trace concentrations (Lee et al., 2007b). Estrogenic and androgenic EDCs, as well as their antagonists, are introduced into surface waters via wastewater treatment plant effluents since they are not completely removed during biological treatment (Gehrmann et al., 2016).

The implementation of ozone as additional treatment option for wastewater is broadly discussed and tested in large scale facilities to reduce the discharge of micropollutants into the environment (Hübner et al., 2015; Zucker et al., 2016). Estrogenic activity monitored by in vitro bioassays is considered an important evaluation parameter to assess the impact of micropollutants as well as their metabolites and transformation products (TPs) (Noguera-Oviedo and Aga, 2016; Schindler Wildhaber et al., 2015). However, after ozonation of wastewater residual estrogenic effects (Boehling et al., 2012) as well as their complete removal are reported (Altmann et al., 2012; Stalter et al., 2011). Anti-estrogenic activity on the other hand is reduced during biological treatment, but is not further reduced by ozonation (Gehrmann et al., 2016; Ternes et al., 2017).

Remaining agonistic effects can be caused by three different factors or a combination thereof: (I) the micropollutants are ozone resistant, (II) active TPs are formed that maintain the biological effect of the precursor (Hübner et al., 2015) or (III) compounds acting as antagonists are more efficiently removed, thus reducing a masking effect (Prasse et al., 2015). For anti-estrogenic or anti-androgenic micropollutants the same principle applies. This could for example be the case if 17α-ethinylestradiol (EE2) is present because it is well degraded by ozonation and no estrogenic TPs are formed (Huber et al., 2004).

To the authors' knowledge no investigations of (anti-) estrogenic effects of TPs after ozonation of tamoxifen (TAM) are reported yet. TAM is considered to be the prototype of nonsteroidal selective estrogen receptor modulators (SERMs) that show either an activating or deactivating estrogenic effect on different types of tissues (Feng and O'Malley, 2014). It is used in therapy to inhibit reoccurrence of estrogen receptor-positive breast cancer (Howell et al., 2004).

Only few studies reported environmental concentrations of TAM with usually 25 to 200 ng L^{-1} in wastewater treatment plant (WWTP) effluents and 25 to 50 ng L^{-1} in surface waters (Ashton et al., 2004; Coetsier et al., 2009; Ferrando-Climent et al., 2014; Verlicchi et al., 2012). In one study though, higher concentrations up to 369 ng L^{-1} TAM were reported in WWTP effluents and up to 212 ng L^{-1} in river Tyne (Roberts and Thomas, 2006). Hence, the predicted no effect concentration (PNEC) value of 200 ng L^{-1} (Ashton et al., 2004) can be exceeded in surface waters. TAM reacts at sufficient rates with ozone to be completely removed in wastewater matrices forming several TPs depending on the pH (Chen et al., 2008; Knoop et al., 2016). Some of these TPs showed a residual acute toxicity for aquatic organisms (DellaGreca et al., 2007; Ferrando-Climent et al., 2017).

This work focuses on (I) the removal of the anti-estrogenic activity of tamoxifen by ozonation at pH 3, 7, and 11, and (II) determination of the anti-estrogenic activity of formed TPs. A detailed study of kinetics and product formation at different pH values is out of the scope of this study but will be reported elsewhere.

2. Theory

2.1. Reaction of ozone with tamoxifen

In brief, five TPs can be formed by three pathways depending on the protonation of the tertiary amine (pK_a of corresponding acid = 9.5) (see Fig. 1) (Knoop et al., 2016). A reaction at the olefin group causes bond cleavage resulting in formation of TP 270. TP 388 forms upon hydroxylation of the aromatic ring. Both reactions are favored at acidic conditions (pH < 5) which suppresses the attack at the amine (cationic species largely prevails). At higher pH values ozone reacts faster with the tertiary amine than with the double bond or the aromatic ring resulting in *N*-oxide formation.

2.2. Effect induction at the estrogen receptor

The estrogen receptors alpha and beta (ERα and ERβ) have three major functional domains, the amino-terminal activation function-1 (AF-1) domain, the central DNA-binding domain (DBD) and the carboxyl-terminal ligand binding domain (LBD). Estrogenic compounds are bound to the hydrophobic cavity in the interior of the LBD. The binding of an estrogenic compound induces a conformational change of helix H12 that enables the activation function 2 (AF-2) and closes the entrance channel of the cavity thereby creating an interacting surface for coregulators to bind at the ER (Brzozowski et al., 1997; Pike et al., 1999). These coregulators essentially carry out the DNA-transcription activity of the ER (Feng and O'Malley, 2014). Antagonists have a basic moiety at a side chain, which on the one hand sterically blocks helix H12 and thus prevents to close the cavity. On the other hand the antagonist forms an ionic bond to aspartate 351 (ASP 351) of the ER protein, since the basic moiety is present as cationic species at pH 7. The ASP 351

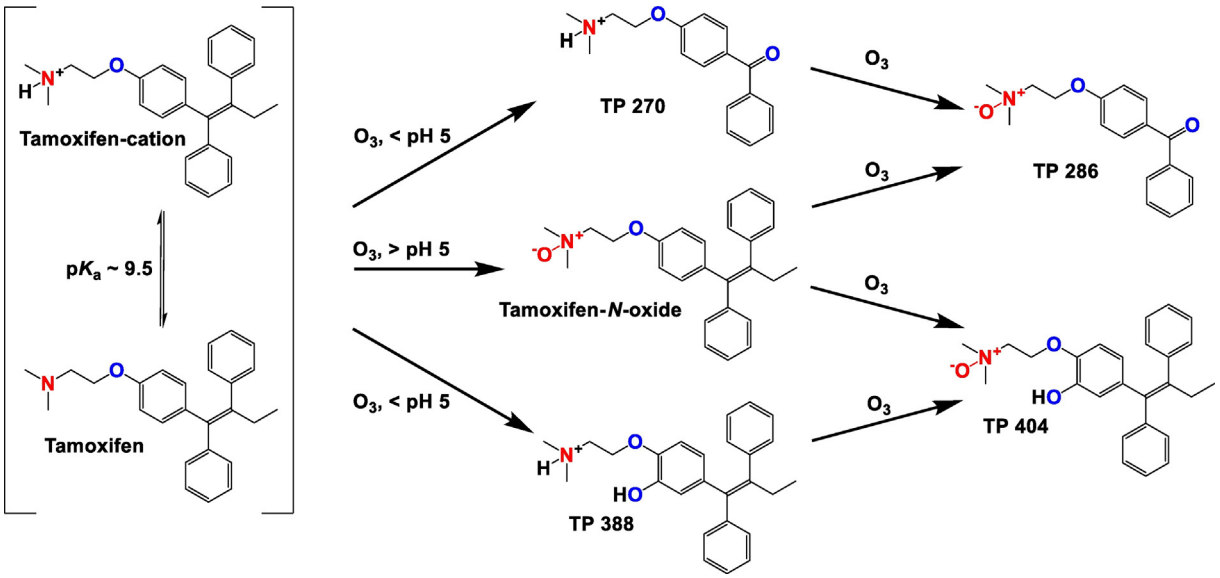

Fig. 1. Proposed TPs for the ozonation of tamoxifen according to Knoop et al. (2016).