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Ecotoxicity of the antihistaminic drug cetirizine to *Ruditapes philippinarum* clams

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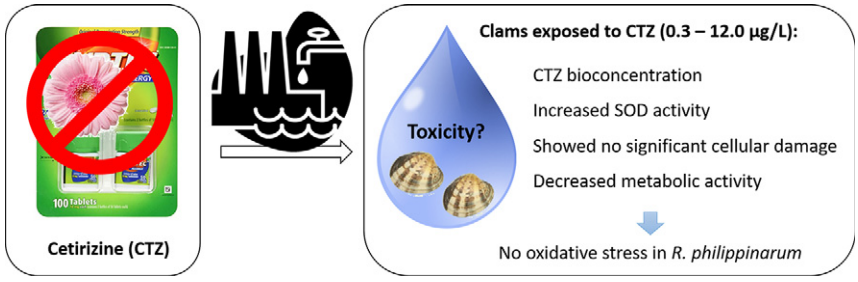
HIGHLIGHTS

- The antihistamine cetirizine (CTZ) was bioconcentrated in clams.
- The activity of superoxide dismutase was induced.
- Absence of significant cellular damage exerted by CTZ in clams
- CTZ decreased the metabolic capacity of clams.
- No oxidative stress induced by CTZ in the clams

GRAPHICAL ABSTRACT



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ABSTRACT

Cetirizine (CTZ) is an antihistaminic drug present in the aquatic environment, with limited information on its toxicity to organisms inhabiting this system. This study intended to evaluate the effects of CTZ on oxidative stress and energy metabolism biomarkers in the edible clam *Ruditapes philippinarum* after a 28 days exposure to environmentally relevant CTZ concentrations (0.0, 0.3, 3.0, 6.0 and 12.0 µg/L). The results obtained showed that CTZ was accumulated by clams reaching maximum concentrations (up to ~22 ng/g FW) at the highest CTZ exposure concentrations (6.0 and 12.0 µg/L). The bioconcentration factor (average maximum values of ~5) decreased at 12.0 µg/L reflecting a reduction in clams uptake or increase of excretion capacity at this condition. The present study revealed that, in general, clams decreased the metabolic potential after exposure to CTZ (decrease in electron transport system activity), a response that led to the maintenance of glycogen content in organisms exposed to CTZ in comparison to control values. Our findings also showed that, CTZ did not exert significant levels of oxidative injury to clams. However, comparing the control with the highest exposure concentrations (6.0 and 12.0 µg/L) a significant increase of the antioxidant enzyme superoxide activity (~53 and ~44%) was observed in clams exposed to CTZ. Moreover, a tendency to increase lipid peroxidation (~14 and ~9%) and carbonyl groups on proteins (~11 and ~3%) was observed in clams exposed to CTZ (6.0 and 12.0 µg/L) compared to control condition. Overall the present study suggests that toxic impacts may be induced in *R. philippinarum* if exposed for longer periods or higher CTZ concentrations.

1. Introduction

The worldwide contamination of aquatic systems by pharmaceutical drugs with toxic effects to inhabiting organisms is a well-known

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environmental issue (Monteiro and Boxall, 2010). Antihistamines are a group of pharmaceuticals commonly used for the treatment of allergic reactions and have been found in the aquatic environment due to global consumption and generally poor degradation in wastewater treatment plants (WWTPs) (Golovko et al., 2014; Kosonen and Kronberg, 2009). The highest concentrations of antihistamines in water bodies are commonly observed in spring, the season of increased concentrations of aerial pollen derived from flowering plants, which is associated to a higher consumption of these drugs. Cetirizine (CTZ), a second-generation antihistaminic drug, is a potent histamine H1 receptor antagonist with antiallergic properties. CTZ (the active substance of Zyrtek and Reactin among other pharmaceuticals) is used to treat chronic idiopathic urticaria, perennial and seasonal allergic rhinitis, allergic asthma, physical urticaria, and atopic dermatitis (DrugBank, 2017). When consumed 70% of the CTZ dose (maximum of 20 mg/tablet/day) is excreted unchanged by renal mechanisms, approximately 10% in the faeces and 8 to 10% is metabolized by the P450 cytochrome oxidase pathway (Campoli-Richards et al., 1990; DrugBank, 2017; Wood et al., 1987).

CTZ has been detected in water bodies worldwide (Bahlmann et al., 2012, 2009; Bebianno et al., 2016; Calisto et al., 2011; Fick et al., 2009; Kosonen and Kronberg, 2009; Larsson et al., 2009, 2007, Nödler et al., 2014, 2011). Kosonen and Kronberg (2009) identified the presence of antihistamines including CTZ in influents and effluents of a sewage treatment plant from Turku (Finland) with concentrations up to 220 ng/L and in recipient river waters with concentrations between 4 and 8 ng/L. These authors found that during the treatment applied in the WWTPs, the concentration of CTZ dropped about 16% of the influent level. Fick et al. (2009) found very high CTZ concentrations in an effluent of a WWTP in Patancheru (India) at 2.1 mg/L, but upstream and downstream samples showed lower CTZ concentrations, varying between 9 and 530 μg/L. CTZ was also determined in wells in India, with concentrations ranging from 550 ng/L to 28 μg/L. These authors referred that some of the tested wells were still used as drinking water sources. Bahlmann et al. (2012) reported maximum CTZ concentrations of 0.49 and 0.51 μg/L in wastewaters from, respectively, influents and effluents of Berlin (Germany) WWTPs. In surface waters, also from Berlin, CTZ was detected with concentrations up to 0.72 μg/L. Nödler et al. (2014) found CTZ in the Venice lagoon (Italy), in the San Francisco Bay (USA) and in the Baltic Sea (Germany) with maximum concentrations of 2.7, 6.3 and 13 ng/L, respectively. Recently Bebianno et al. (2016) found CTZ (up to 1.28 μg/L) in an effluent from a psychiatric hospital in Montpon (France) containing a mixture of 25 pharmaceutical drugs.

Despite the presence of CTZ in the environment, few studies evaluated the possibility of toxic risks induced by this drug to aquatic organisms, being limited to the works performed with bacteria (Bergheim et al., 2014; Borowska et al., 2016), gastropod haemocytes and larvae (Letullier et al., 2014; Rittschof et al., 2003), bivalves adults (Teixeira et al., 2017) and plathelminthes adults (Li, 2013). Rittschof et al. (2003) reported that CTZ (evaluated as Zyrtec) inhibited the larval settlement of the barnacle *Amphibalanus amphitrite* at 40 ng/mL. Recently, Al-Aidaroos et al. (2017) also reported that CTZ, at sublethal concentrations ($1/5^{th}$ LC50 – 1.18 mg/L), inhibited barnacle (*Amphibalanus amphitrite*) larval development, decreased larval settlement and respiration rate, revealing the involvement of histamine in larval settlement and metamorphosis processes. Bergheim et al. (2014) studied the *in vitro* toxicity and the bio- and photopersistence of different pharmaceutical drugs, including CTZ. These authors found that CTZ (up to 6 μg/spot) was more toxic than its photoproducts in the *Vibrio fischeri* (bacteria) bioluminescence assay. However, an opposite response was observed with *Pseudomonas putida* (bacteria) growth inhibition test, with a lower percentage of inhibition for the non-irradiated drug (~10%, at 8 mg/L) comparing to the irradiated CTZ (40–60%, also at 8 mg/L). Borowska et al. (2016) tested the efficiency of wastewater ozonation for the reduction of nitrogen-containing pharmaceuticals and observed that CTZ ozonation products elicited biological effects on bacteria (*Aliivibrio fischeri*) bioluminescence with a 10 and 50% effect concentration at 0.4 and 4 mg/L, respectively. Letullier et al. (2014) evaluated the *in vitro* effects of four pharmaceutical drugs including CTZ (0.5–500 μg/L) in haemocytes of the abalone *Haliotis tuberculata*, revealing that the drug affected lysosomal integrity, although not posing danger. Recently Teixeira et al. (2017) demonstrated that *Mytilus galloprovincialis* mussels exposed to CTZ (0.3–12.0 μg/L) increased their energy reserves and maintained metabolic potential in comparison to non-contaminated organisms. These authors also demonstrated that, although exposed mussels increased their antioxidant defenses they were not able to prevent cellular damage.

Nevertheless, besides these studies, scarce information is available regarding the impacts of CTZ in aquatic organisms induced at the cellular level, namely on biochemical alterations (Teixeira et al., 2017), which are valuable markers to provide "early-warning signals" of impairments on species performance (Colin et al., 2016). Thus, the present study aimed to evaluate the effects of CTZ on oxidative stress biomarkers and energy metabolism in the edible clam *Ruditapes philippinarum*, by exposing the organisms for 28 days to CTZ at environmentally relevant concentrations.

2. Materials and methods

2.1. Study area and test organism

The clam *R. philippinarum* (Adams & Reeve, 1850), commonly known as the Manila clam, was selected to study the ecotoxicological impact of CTZ. Clams were collected in February 2016 in the Mira channel, located in the Ria de Aveiro (Portugal) that, according to Castro et al. (2006) and Cerqueira and Pio (1999), is the least impacted channel of this ecosystem due to the less dense human settlements and to low industrial and harbour activity, which are located further North in the lagoon. The evaluation of drug contamination in the Ria de Aveiro is still scarce (Calisto et al., 2011; Paíga et al., 2013; Silva et al., 2014, 2013) but studies conducted by Calisto et al. (2011) determined the concentration of pharmaceutical drugs (the antiepileptic carbamazepine and the antihistamine CTZ) in this aquatic system, identifying CTZ in one (Costa Nova) of eleven sampling areas, with concentrations of 0.04 μg/L. The drug was also found in the wastewater influents/effluents of the Aveiro WWTPs with an average concentration of 0.3 μg/L. *R. philippinarum* is an exotic species, introduced in the Ria de Aveiro in 2010 (Maia and Gaspar, 2014), which is used in biomonitoring studies with different types of contaminants worldwide (Casatta et al., 2016; Moschino et al., 2011; Oaten et al., 2017; Piló et al., 2017; Sacchi et al., 2013; Won et al., 2016). For laboratory experiments, *R. philippinarum* individuals with similar size (mean length: 4.2 ± 0.3 cm; mean width: 3.0 ± 0.2 cm, condition index: 5.2 ± 0.2%) were selected to minimize the effect of body size on biochemical response and CTZ uptake. The organisms were acclimated to laboratory conditions during 7 days in seawater (salinity 25 g/L), under continuous aeration, temperature 18 ± 1 °C and 12:12 h (light/dark) photoperiod. During the acclimation period clams were not fed.

2.2. Experimental conditions

After acclimation, clams were chronically (28 days) exposed to CTZ. The organisms were distributed by four CTZ exposure concentrations (0.3, 3.0, 6.0 and 12.0 μg/L) plus the control condition (0.0 μg/L). Each exposure concentration was prepared by spiking the medium (3 L), using a defined volume (according to the exposure concentration) of a 10 mg/L CTZ stock solution (prepared in NaCl 25 g/L). For each concentration, 3 replicates were performed, each one with 3 individuals, corresponding to a total of 9 organisms ($3 \times 3 = 9$) per condition. Organisms were placed in containers filled with 3 L of artificial seawater (salinity 25 g/L) and submitted to continuous aeration, temperature 18 ± 1 °C and a 12:12 h (light/dark) photoperiod. For each condition, blanks (two replicates) were performed to evaluate the losses of CTZ (bio-