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Toxic effects of the antihistamine cetirizine in mussel *Mytilus galloprovincialis*

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ABSTRACT

Recent studies have become increasingly focused on the assessment of pharmaceuticals occurrence in aquatic ecosystems, however the potential toxicity to non-target organisms is still largely unknown. The antihistamine cetirizine is a commonly used pharmaceutical, already detected in surface waters of marine aquatic systems worldwide. In the present study *Mytilus galloprovincialis* mussels were exposed to a range of cetirizine concentrations (0.3, 3.0, 6.0 and 12.0 μg/L), resembling moderate to highly contaminated areas, over 28 days. The responses of different biochemical markers were evaluated in mussels whole soft tissue, and included energy-related parameters (glycogen content, GLY; protein content, PROT; electron transport system activity, ETS), and oxidative stress markers (superoxide dismutase activity, SOD; catalase activity, CAT; glutathione S-transferases activity, GSTs; lipid peroxidation levels, LPO; reduced (GSH) and oxidized (GSSG) glutathione content). The results obtained demonstrated that with the increase of exposure concentrations mussels tended to increase their energy reserves and maintain their metabolic potential, which was significantly higher only at the highest concentration. Our findings clearly revealed that cetirizine inhibited the activity of GSTs and although induced the activity of antioxidant enzymes (SOD and CAT) mussels were not able to prevent cellular damages observed through the increase of LPO associated to the increase of exposure concentrations. Thus, this study confirmed that cetirizine induces toxic effects in *Mytilus galloprovincialis*, which, considering their trophic relevance, wide use as bioindicator and wide spatial distribution of this species, can result in ecological and economic negative impacts at a large scale.

1. Introduction

The increasing consumption of pharmaceuticals by an exponentially growing human population has resulted in ubiquity of these compounds in the environment (e.g. Fent et al., 2006; Kümmerer, 2010; Nikolaou et al., 2007; Puckowski et al., 2016). Furthermore, due to their incomplete removal in Wastewater Treatment Plants (WWTPs), which may only reach 10% for some substances, pharmaceuticals are continuously introduced into aquatic environment (Voulvoulis et al., 2016). This fact, associated to their environmental persistence, may explain the detected concentrations of pharmaceuticals in the environment which range from ng L^{-1} to μg L^{-1} (for review see, Fent et al., 2006; Kümmerer, 2009; Santos et al., 2010). For these reasons, and because pharmaceuticals may preserve their biological activity in the environment (Huerta et al., 2012) with potential impacts to aquatic wildlife, over the last years increasing attention has been given to understand the impacts of these contaminants in aquatic ecosystems, namely on the inhabiting organisms (among others, Aguirre-Martínez et al., 2013; Almeida et al., 2014; Canesi et al., 2007; Freitas et al., 2016, 2015a,b; Martin-Diaz et al., 2009a,b; Pires et al., 2016; Quinn et al., 2011). Several studies have demonstrated that different pharmaceuticals accumulate and cause toxic effects

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in vertebrates (mainly fish, Abreu et al., 2016; Álvarez-Muñoz et al., 2015; Carlsson et al., 2013; Corcoran et al., 2010; Watanabe et al., 2016), and in invertebrates (mainly bivalves, Aguirre-Martínez et al., 2013; Almeida et al., 2015; Álvarez-Muñoz et al., 2015; Canesi et al., 2007; Contardo-Jara et al., 2011; Freitas et al., 2016; 2015a,b; Gonzalez-Rey and Bebianno, 2014; Martin-Diaz et al., 2009a,b; Pires et al., 2016; Quinn et al., 2011), when these organisms are exposed to pharmaceuticals under laboratory conditions or to wastewater effluent and surface waters receiving effluent discharges in the field.

The effects of pharmaceuticals exposure on freshwater and marine bivalves include impacts at cellular level with increased oxidative stress, embriotoxicity and immunotoxicity (Aguirre-Martínez et al., 2013; Binelli et al., 2009; Canesi et al., 2007; Contardo-Jara et al., 2011; Fabbri et al., 2014; Gagné et al., 2006; Martin-Diaz et al., 2009a,b; Matozzo et al., 2012; Munari et al., 2016; Parolini et al., 2011; Tsiaka et al., 2013). To assess the toxic impacts of pharmaceuticals a wide variety of bivalve species has been used, including mussels, which are useful bioindicator species that combine a wide distribution and long-life cycle with their filtration ability allowing bioaccumulation of contaminants from the surrounding environment (among others, Cajaraville et al., 2000; Gagné et al., 2007; Cravo et al., 2009; Ericson et al., 2010 in Gonzalez-Rey and Bebianno, 2014). Martin-Diaz et al. (2009a) showed a reduction in haemocyte lysosome membrane stability and an increase in oxidative stress in mussels (*Mytilus galloprovincialis*) exposed to the antiepileptic drug carbamazepine. Gonzalez-Rey and Bebianno (2014) assessed the effects of diclofenac (a non-steroidal anti-inflammatory drug) in mussel *M. galloprovincialis* and showed that this drug induced biochemical responses, including a significant induction of the activity of the enzymes superoxide dismutase and glutathione reductase in mussels gills in addition to high catalase activity and lipid peroxidation levels in their digestive gland. Recently Lacaze et al. (2015) showed that exposure to psychotropic drugs and antibiotics led to genotoxicity, immunotoxicity and cytotoxicity in mussel *M. edulis.*

To our knowledge, the effects of cetirizine in aquatic organisms, namely in bivalves and in particular in mussels, have not been evaluated before, despite the presence of this drug in different aquatic systems, which justifies the need for this study. The antihistamine cetirizine was detected in water bodies, namely in surface waters and influents/effluents of WWTPs with concentrations ranging from 4 ng/L to 1.4 mg/L, respectively (Bahlmann et al., 2012; Kosonen and Kronberg et al., 2009a,b; Larsson et al., 2007).

Taking into consideration that different studies demonstrated that regardless of their mode of action pharmaceuticals may induce oxidative stress in aquatic organisms, the present study aimed to evaluate cellular damages, defense mechanisms and the metabolic potential of *M. galloprovincialis* after exposure to cetirizine for 28 days. A range of cetirizine concentrations, resembling medium to highly contaminated areas was used.

2. Material and methods

2.1. Experimental conditions

For the present study the mussel *Mytilus galloprovincialis* was selected as it represents one of the most widely used bioindicator species of environmental pollution (among others, Martin-Diaz et al., 2009a). Furthermore, since this species is consumed worldwide, contamination by pharmaceuticals may put at risk not only human health but also the economic status of both producers and harvesters.

M. galloprovincialis individuals were collected at the Ílhavo channel in the Ria de Aveiro, a shallow lagoon in northwest Portugal. Previous studies already reported the presence of pharmaceutical residues in this aquatic system, which were limited to wastewater treatment plants effluents and in one surface water sample at the Mira channel (Costa Nova region) with cetirizine concentrations varying between 0.04 and 0.6 μgL^{-1} (Calisto et al., 2011).

For this study, mussels with similar fresh weight (20.7 ± 1.3 g) were selected in order to consider all the individuals as replicates. In order to reduce the possible contamination levels inherent to their natural environment (including metals), specimens were depurated and acclimated to laboratory conditions during 2 weeks in several glass aquaria, with artificial seawater (salinity 25) set up by mixing artificial sea salt (Tropic Marin Reef Mix) with reverse osmosis water, under continuous aeration, at 18 ± 1 °C, a photoperiod thereabout 12:12 h (light/dark) and fed twice per week with Algamac protein plus (150 000 cells/L/animal) purchased to Aquafauna Bio-Marine, Inc. During this period water was changed twice per week (before feeding the animals).

After this period mussels were exposed during 28 days to different conditions: 0.0 (Control, CTL), 0.3, 3.0, 6.0 and 12.0 μg/L of cetirizine. For each condition 3 containers (plastic 5 L vessels) were used, each one with 5 individuals (5 organisms x 3 containers x 5 conditions). During exposure period the medium was renewed every week and the individuals were fed three times per week with Algamac protein plus (150 000 cells/L/animal) purchased to Aquafauna Bio-Marine, Inc. Weekly, after water renewal, cetirizine concentrations were reestablished. During exposure, experimental conditions were maintained: 3 L of medium per container, salinity 25, continuous aeration, temperature 18 ± 1 °C and a 12:12 h photoperiod. Dead organisms were removed from the containers when identified. Organisms were considered dead when their shells gaped and failed to shut again after external stimulus. Weekly water samples were collected immediately before and after the water renewal to evaluate cetirizine concentrations over time. Water samples (ca 2 mL) were collected, using a pipette, and preserved in the freezer at −20 °C until cetirizine quantification.

After exposure (28 days) individuals were collected and immediately frozen with liquid nitrogen and preserved at −80 °C. Before laboratory analyses, frozen organisms were removed from their shells and the soft tissue pulverized in a mill with liquid nitrogen, for cetirizine quantification and biochemical markers measurement. For each organism, pulverized tissue was distributed in 0.5 g aliquots.

In parallel to the exposure assay a quality control experiment was conducted to evaluate cetirizine losses (due to degradation or adsorption onto containers) along the 28 days exposure period. For this, containers with the same exposure conditions were prepared without mussels. Water samples were taken weekly immediately before and after water renewals. Water samples (ca 2 mL) were collect using a pipette, and preserved in the freezer at −20 °C until cetirizine quantification.

2.2. Laboratory measurements

2.2.1. Cetirizine quantification

2.2.1.1. Reagents. The polyclonal antibody against mouse (IgG F(c) domain, from goat, lot 20 185) and the anti-cetirizine monoclonal antibody (mouse IgG1, clone B3212M, lot 5 K32007) were purchased from Acris Antibodies (Germany) and BIODESIGN International (Meridian Life Science Inc., USA), respectively. The tracer was produced and characterized as described in Bahlmann et al. (2009). 3,3′,5,5′-Tetramethylbenzidine (≥99%, CAS number: 54827-17-7); tetrabutylammonium borohydride (>97%, CAS number: 33725-74-5); sodium phosphate dibasic dihydrate (>99%, CAS Number