Environmental Pollution 214 (2016) 456–463

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Long-term exposure of polychaetes to caffeine: Biochemical alterations induced in *Diopatra neapolitana* and *Arenicola marina*☆

Adília Pires [a], Ângela Almeida [a], Vânia Calisto [b], Rudolf J. Schneider [c], Valdemar I. Esteves [b], Frederick J. Wrona [d], Amadeu M.V.M. Soares [a], Etelvina Figueira [a], Rosa Freitas [a, *]

[a] Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal
[b] Department of Chemistry & CESAM, University of Aveiro, Aveiro, Portugal
[c] BAM Federal Institute for Materials Research and Testing, Richard-Willstaetter Str. 11, Berlin, Germany
[d] Department of Geography, University of Victoria, National Water Research Institute, STN CSC, Victoria, BC, Canada

ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form
4 April 2016
Accepted 8 April 2016
Available online 23 April 2016

Keywords:
Invertebrates
Pharmaceuticals
Oxidative stress biomarkers
Energy reserves

ABSTRACT

In the last decade studies have reported the presence of several pharmaceutical drugs in aquatic environments worldwide and an increasing effort has been done to understand the impacts induced on wildlife. Among the most abundant drugs in the environment is caffeine, which has been reported as an effective chemical anthropogenic marker. However, as for the majority of pharmaceuticals, scarce information is available on the adverse effects of caffeine on marine benthic organisms, namely polychaetes which are the most abundant group of organisms in several aquatic ecossystems. Thus, the present study aimed to evaluate the biochemical alterations induced by environmentally relevant concentrations of caffeine on the polychaete species *Diopatra neapolitana* and *Arenicola marina*. The results obtained demonstrated that after 28 days exposure oxidative stress was induced in both species, especially noticed in *A. marina*, resulting from the incapacity of antioxidant and biotransformation enzymes to prevent cells from lipid peroxidation. The present study further revealed that *D. neapolitana* used glycogen and proteins as energy to develop defense mechanisms while in *A. marina* these reserves were maintained independently on the exposure concentration, reinforcing the low capacity of this species to fight against oxidative stress.

1. Introduction

The use of pharmaceuticals has increased globally and recent advances in the development of sensitive analytical techniques have revealed that these chemicals occur in several aquatic environments. As a consequence, recent efforts have been done to determine the potential impacts of pharmaceuticals, resulting in a high number of studies reporting drugs effects on aquatic organisms (among others, Aguirre-Martínez et al., 2013a,b; 2015, 2016; Almeida et al., 2014, 2015; Antunes et al., 2013; Freitas et al., 2015a,b; Gonzalez-Rey and Bebianno, 2012; Maranho et al., 2015; Méndez et al., 2013; Minguez et al., 2014; Parolini et al., 2010; Schmidt et al., 2011; Solé et al., 2010). Nevertheless, most of these studies are based on short-term experiments and overwhelmingly focused on relatively high concentrations that are unlikely to be found in aquatic environments, underestimating the real impacts of these drugs in the environment. In fact, although pharmaceuticals are continuously entering the aquatic environment, they will be most likely to have chronic rather than acute toxic effects (e.g. Crane et al., 2006). In addition, most of the studies have been devoted to evaluate the effects in freshwater environments (Aguirre-Martínez et al., 2015; Brandão et al., 2014; Chen et al., 2014; Contardo-Jara et al., 2011; Gagné et al., 2006; Isidori et al., 2005; Kim et al., 2009; Parolini et al., 2010) although recent efforts have also been made to study the toxicity in marine and estuarine organisms (Aguirre-Martínez et al., 2013a,b, 2016; Almeida et al., 2014, 2015; Freitas et al., 2015a,b; Martín-Díaz et al., 2009; Milan et al., 2013).

Among pharmaceutical drugs, caffeine (3,7-dihydro-1,3,7-

☆ This paper has been recommended for acceptance by Harmon Sarah Michele.

* Corresponding author. Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
E-mail address: rosafreitas@ua.pt (R. Freitas).

http://dx.doi.org/10.1016/j.envpol.2016.04.031
0269-7491/

trimethyl-1h-purine-2,6-dione) is a known psychoactive drug with mnemonic effects credited to the non-specific antagonism of adenosine receptors (Fredholm et al., 1999; Cunha and Agostinho, 2010). Caffeine is found in a variety of beverages and food products and in many pharmaceutical products, being widely used by humans as stimulant (Moore et al., 2008). With an estimated global average consumption of 70 mg/person/day (Buerge et al., 2003) caffeine is one of the most commonly consumed alkaloids (among others, Bradley et al., 2007; Fent et al., 2006; Palo and Choudhury, 2006; Moore et al., 2008). Although caffeine shows very high (>95%) removal efficiency within wastewaters treatment plants (WWTPs) (Jacobs et al., 2012; Kosma et al., 2014), an estimated half-life in aquatic environment of about 1.5 days (Lam et al., 2004) and high solubility (Seiler et al., 2005), it has been identified as one of the most ubiquitous compounds in several water bodies with concentrations in the range of ng/L to low μg/L (Crane et al., 2006; Deo, 2014; Fent et al., 2006; Gagné et al., 2006; Heberer et al., 2002; Moore et al., 2008: Metcalfe et al., 2003). For example, it has been detected in WWTPs influents and effluents (Bahlmann et al., 2009), surface waters (Martinez Bueno et al., 2011), groundwater (Silva et al., 2014) and treated drinking water (Stackelberg et al., 2004) with concentrations ranging from 0.028 μg/L such as those recorded in San Francisco Bay water (Klosterhaus et al., 2013), to 293 μg/L such as those determined in a hospital sewer in Tromsø (Norway) (Weigel et al., 2004).

Pharmaceuticals are compounds that may cause the overproduction of reactive oxygen species (ROS) (Livingstone et al., 2001) leading to subsequent compensatory antioxidant responses and, ultimately, to oxidative stress, altering cells redox status. In fact, after an exposure to organic contaminants, antioxidant enzymes (e.g. superoxide dismutase and catalase) may prevent oxidative damage by eliminating ROS, allowing the organism to totally or partially overcome oxidative stress. Under the presence of contaminants, organisms may also increase the activity of glutathione S-transferase (GSTs), detoxifying reactive xenobiotic metabolites through conjugation reactions (Wright and Welbourn, 2002; Newman and Unger, 2003). When the antioxidant and biotransformation systems are not able to completely eliminate the excess of ROS, cell damage may occur, namely the peroxidation of membrane lipids. Glutathione (GSH) is a biologically important intracellular thiol. GSH acts as a free radical scavenger, preventing cells from oxidative damage but it also participates in the detoxification of hydrogen peroxide by various glutathione peroxidases, both forming oxidized glutathione (GSSG). Thus, the ratio of reduced to oxidized glutathione is an indicator of cellular health, frequently used to assess exposure of cells to oxidative stress (Regoli and Giuliani, 2014). When under stressful conditions, marine invertebrates may rapidly decrease their energy reserves (e.g. glycogen and proteins, Naimo and Monroe, 1999; Baker and Hornbach, 2000; Freitas et al., 2015d) which are diverted to fight against stress. Therefore, glycogen and proteins are useful indicators for detecting oxidative stress in invertebrates. Xanthine oxidase is one of the metabolization routes of caffeine, which generates highly reactive ROS in the process (Gagné et al., 2006). Thus, when pro-oxidant forces overwhelm antioxidant defenses, long-term exposure to caffeine could lead to oxidative stress and to lipid peroxidation (LPO). In vertebrates, caffeine has been studied for its biochemical and physiological effects, with some results indicating an antioxidant effect (Shi et al., 1991; Devasagayam et al., 1996; Nikolic et al., 2003) and others demonstrating a pro-oxidant effect (Dianzani et al., 1991; Olcina et al., 2007) or no effects (Olcina et al., 2006). Nevertheless, most of the available ecotoxicological data about caffeine are referred to studies conducted on freshwater organisms whereas its effects on marine species have been only seldom investigated. Recently the impacts of caffeine on marine species have been examined, revealing effects after short (e.g. on *Oncorhynchus mykiss* trout hepatocytes caffeine produced LPO at a threshold concentration of 14 μM after 48-h exposure, Gagné et al., 2006) and long-term (e.g. on *Carcinus maenas* crabs caffeine induced LPO and DNA damage after exposure to 0.1, 5.0, 15 and 50 μg/L for 28 days, Aguirre-Martínez et al., 2013b; destabilization of lysosomal membrane in the clam *Ruditapes philippinarum* exposed to 0.1, 5.0, 15 and 50 μg/L of caffeine during 35 days; Aguirre-Martínez et al., 2013a) experiments.

Polychaetes are amongst the most abundant invertebrates in estuarine environments (Rodrigues et al., 2011). Essentially due to their life-history characteristics (e.g., sedentary life-style; abundant in the environment, important role in trophic chains, long life cycles) polychaetes have been used in standardized implemented suits of toxicity tests to assess the impacts induced in aquatic organisms by anthropogenic (e.g. organic and inorganic contamination) and natural stresses (including salinity alterations) (see for review Dean, 2008; Lewis and Watson, 2012). However, few species of polychaetes have dominated the literature to date, in particular *Hediste diversicolor* (Müller, 1776). Several studies have already demonstrated the capacity of this species to act as a good sentinel and bioindicator, namely to organic and inorganic pollutants (Durou et al., 2007; Sun and Zhou, 2008; Solé et al., 2009; Freitas et al., 2012), decrease on seawater pH (Basallote et al., 2012) and, more recently, to pharmaceuticals (Maranho et al., 2014, 2015) and nanoparticles (Buffet et al., 2014; Thit et al., 2015). Although less frequently, *Arenicola marina* (Linnaeus, 1758) has also been used in monitoring and ecotoxicological studies, revealing to be a sensitive species to organic and inorganic pollutants (Casado-Martínez et al., 2008; Hannam et al., 2008; Ramos-Gómez et al., 2011a,b), pH decrease (Campbell et al., 2014) and recently, to microplastics (Browne et al., 2013). More recently, *Diopatra neapolitana* (Delle Chiaje, 1841) has shown the capacity to respond to metals and metalloids contamination (Freitas et al., 2012), organic matter enrichment (Carregosa et al., 2014), pharmaceuticals (Freitas et al., 2015a,c), and alterations in physical conditions related to climate change (pH decrease, salinity changes and temperature increase, Pires et al., 2015a; Freitas et al., 2015d,e).

Therefore, in the present study, the biochemical alterations induced by caffeine were assessed on *A. marina* and *D. neapolitana*, two polychaete species co-existing in the same aquatic systems but with different life strategies, which may greatly influence the responses of specimens towards contaminants: i) *D. neapolitana* builds tubes, while *A. marina* lives in a U-shaped burrow in sand; ii) *D. neapolitana*, is an omnivorous scavenger, feeding on animal and vegetable debris, while *A. marina* is a deposit feeder. To evaluate the impacts of caffeine on the biochemical performance of both species, different energy, enzymatic and non-enzymatic markers of oxidative stress were measured after a chronic exposure (28 days) to environmentally relevant concentrations of this drug.

2. Methodology

2.1. Sampling and experimental setup

To assess the toxicity of caffeine, laboratory experiments were conducted with *D. neapolitana* and *A. marina*, collected in the Mira channel, an area with low metal and pharmaceuticals concentrations at the Ria de Aveiro lagoon (Portugal) (Freitas et al., 2014; Calisto et al., 2011). Sampling was done in April to avoid the reproductive period of both species (Pires et al., 2012a; Watson et al., 2000).

These species have a worldwide spatial distribution, being ecologically and economically relevant in many coastal systems namely in the Atlantic Ocean and Mediterranean Sea. At the Ria de