Marine Environmental Research 121 (2016) 31–39

Contents lists available at ScienceDirect

Marine Environmental Research

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

Ecotoxicological potential of non-steroidal anti-inflammatory drugs (NSAIDs) in marine organisms: Bioavailability, biomarkers and natural occurrence in *Mytilus galloprovincialis*

M. Mezzelani [a], S. Gorbi [a], Z. Da Ros [a], D. Fattorini [a], G. d'Errico [a], M. Milan [b], L. Bargelloni [b], F. Regoli [a, *]

[a] Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche, Ancona, Italy
[b] Dipartimento di Biomedicina Comparata e Alimentazione (BCA), Universita di Padova, Italy

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
17 March 2016
Accepted 20 March 2016
Available online 22 March 2016

Keywords:
Emerging contaminants
Pharmaceuticals
Non steroidal anti-inflammatory drugs
Mytilus galloprovincialis
Bioaccumulation
Biomarkers
Adriatic sea

ABSTRACT

Pharmaceuticals represent a major environmental concern since the knowledge on their occurrence, distribution and ecotoxicological potential is still limited particularly in coastal areas. In this study, bioaccumulation and cellular effects of various non steroidal anti-inflammatory drugs (NSAIDs) were investigated in mussels *Mytilus galloprovincialis* to reveal whether common molecules belonging to the same therapeutic class might cause different effects on non target organisms. Organisms exposed to environmental concentrations of acetaminophen (AMP), diclofenac (DIC), ibuprofen (IBU), ketoprofen (KET) and nimesulide (NIM) revealed a significant accumulation of DIC, IBU and NIM, while AMP and KET were always below detection limit. Nonetheless, for all tested NSAIDs, measurement of a large panel of ecotoxicological biomarkers highlighted impairment of immunological parameters, onset of genotoxicity and modulation of lipid metabolism, oxidative and neurotoxic effects. Laboratory results were integrated with a field study which provided the first evidence on the occurrence of DIC, IBU and NIM in tissues of wild mussels sampled during summer months from an unpolluted, touristic area of Central Adriatic Sea. Overall results demonstrated *M. galloprovincialis* as a good sentinel species for monitoring presence and ecotoxicological hazard of pharmaceuticals in the Mediterranean.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The presence of pharmaceutical compounds in aquatic ecosystems has became a topic of growing concern in the last decade (Fent et al., 2006; Boxall et al., 2012). The occurrence of such emerging contaminants in the aquatic environment originates from the large consumption in human and veterinary medicine, agriculture and aquaculture (Boxall et al., 2012) and the limited removal of these molecules by many wastewater treatment plants (WWTPs, Santos et al., 2010).

Compounds used as contraceptives, beta-blockers, antiepileptic, anti-inflammatory, antidepressants or antibiotics have been documented at concentrations ranging from a few ng/L to μg/L in surface and ground waters around the world (Santos et al., 2010; Al Aukidy et al., 2012). Designed to be biologically active at low concentrations, environmental pharmaceuticals might be potentially dangerous for chronically exposed, non-target organisms (Fent et al., 2006; Boxall et al., 2012). This new awareness was reflected in some international actions: the European Medicine Agency (EMEA) issued in 2006 the Guideline on Environmental Risk Assessment Of Medicinal Products for Human Use, aimed to evaluate the risks of pharmaceuticals in ecosystems; the European Parliament extended in September 2010 the legislation on pharmaco-vigilance (Directive 2001/83 and Regulation 726/2004) to the environment (ecopharmacovigilance); more recently, European Commission added 17α-ethinyl estradiol, 17β-estradiol and diclofenac to the Watch List of the daughter Water Framework Directive (2013/39/EU). Overall, the urgent need is recognized for prioritizing more than 4000 substances in terms of their occurrence in natural ecosystems, bioavailability for non target aquatic organisms, mechanisms of uptake and biotransformation, mode of

* Corresponding author. Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche, via Brecce Bianche, 60131, Ancona, Italy.
E-mail address: f.regoli@univpm.it (F. Regoli).

http://dx.doi.org/10.1016/j.marenvres.2016.03.005
0141-1136/© 2016 Elsevier Ltd. All rights reserved.

action at subcellular level, onset of biologically adverse effects and ecotoxicological relevance (Boxall et al., 2012).

Non steroidal anti-inflammatory drugs (NSAIDs) are one of the most relevant therapeutic class, largely used worldwide for their analgesic, antipyretic and anti-inflammatory properties. They act as non-selective inhibitors of cyclooxygenase isoforms, COX-1 and COX-2, involved in the synthesis of different prostaglandins from arachidonic acid (Santos et al., 2010). Their annual consumption in Italy is estimated higher than 100 ton (OsMed, 2013), and they represent 15% of drugs detected in monitoring surveys worldwide (Santos et al., 2010). Despite NSAIDs residues are ubiquitously present in surface waters, groundwaters and coastal areas (Pal et al., 2010; Santos et al., 2010; Lolić et al., 2015), our knowledge on their bioaccumulation and ecotoxicological potential is still limited, particularly for marine organisms. In this context, bivalves are universally considered as useful bioindicator species due to their worldwide distribution, sedentary and filter-feeding habit, easy sampling, elevated tolerance to environmental conditions and marked capability to accumulate organic and inorganic chemicals. Moreover, the possibility to measure several biochemical, cellular and physiological biomarkers, make bivalves suitable organisms for investigating the effects of chemical pollutants (Regoli et al., 2014).

Haemocytes of the freshwater mussels *Dreissena polymorpha* revealed DNA fragmentation and enhanced percentage of apoptotic cells when exposed to various doses of acetaminophen (30–450 μg/L), diclofenac (60–250 μg/L) and ibuprofen (450–909 μg/L) (Parolini et al., 2009). More limited variations of the same parameters were reported after *in vivo* exposure of *D. polymorpha* to lower concentrations of acetaminophen (0.75–1.51 μg/L) and ibuprofen (2–8 μg/L), along with modulation of antioxidant enzymes (catalase, glutathione peroxidases and glutathione S-transferases) and a decrement of lysosomal membrane stability (Parolini et al., 2010, 2011).

The marine clam, *Ruditapes philippinarum*, exhibited significant immunological alterations after 7 days of exposure to ibuprofen (500 and 1000 μg/L) (Matozzo et al., 2012). The same species, exposed for 7 days to 100 μg/L ibuprofen, revealed transcriptional changes for several genes involved in arachidonic acid metabolisms, apoptosis, peroxisomal proliferator-activated receptors, nuclear factor-kappa B and xenobiotic metabolisms (Milan et al., 2013); these effects were paralleled by a statistically significant inhibition of superoxide dismutase, acetylcholinesterase and lysozyme activities (Milan et al., 2013). Byssus strength and energy available for growth and reproduction decreased in *Mytilus edulis* exposed to 100 and 1000 μg/L of diclofenac for 7 and 14 days (Ericson et al., 2010). In the Mediterranean mussel, *Mytilus galloprovincialis*, ibuprofen and diclofenac (250 ng/L) determined, after two weeks, a transitory induction of antioxidant enzymes (superoxide dismutase, catalase, glutathione S-transferases and glutathione reductase) and increase of lipid peroxidation in digestive gland; ibuprofen also enhanced the levels of gonad vitellogenin-like proteins in males, suggesting a potential role as endocrine disruptor of this molecule for marine mussels (Gonzalez-Rey and Bebianno, 2011, 2012, 2014).

The aim of this study was to provide new insights on ecotoxicity of pharmaceuticals by comparing the bioaccumulation and the responsiveness of *M. galloprovincialis* toward 5 different NSAIDs with similar characteristics in terms of mode of action, posology and therapeutic indication for human beings. Mussels were exposed to Acetaminophen (AMP), Diclofenac (DIC), Ibuprofen (IBU), Ketoprofen (KET) or Nimesulide (NIM), and chemical analyses on bioaccumulation of such NSAIDs in mussel tissues were integrated with a multi-biomarker approach, based on a wide array of molecular and subcellular responses reflecting early warning signals of biological disturbance, modulation of specific cellular pathways, onset of various typologies of cellular damages and toxicity. Selected biomarkers included lysosomal and immunological responses (Neutral red retention time NRRT, granulocytes-hyalinocytes ratio, phagocytosis capacity), lipid peroxidation (lipofuscin and neutral lipids), peroxisomal proliferation (Acyl CoA oxidase ACOX), neurotransmission system (AChE), levels of antioxidant defenses (CAT, GST, GR, GPx, and levels of glutathione GSH), total oxyradical scavenging capacity (TOSC) and genotoxic effects (DNA integrity and micronuclei MN). Results on biomarker responses were elaborated within a recently developed, quantitative model (Sediqualsoft) which integrates and differently weight large data-sets of biomarker variations, providing synthetic indices of hazard based on number, typology, biological relevance and magnitude of observed effects (Piva et al., 2011; Benedetti et al., 2012; Regoli et al., 2014).

Results on bioaccumulation under laboratory conditions were also compared with values detected for the first time in a wild mussels population sampled during summer months from an Adriatic touristic location.

Obtained results were expected to clarify the potential role of *M. galloprovincialis* as suitable sentinel organism to evaluate bioavailability and potentially adverse effects of NSAIDs in marine ecosystems. The comparison between different NSAIDs could also reveal whether molecules belonging to the same therapeutic class could cause different effects on non target organisms, thus providing useful insights for a preliminary prioritization on ecological sustainability among tested molecules.

2. Materials and methods

2.1. Chemicals

Acetaminophen, AMP (CAS 103-90-2), Diclofenac, DIC (CAS 15307-86-5), Ibuprofen, IBU (CAS 15687-27-1), Ketoprofen, KET (CAS 22071-15-4) and Nimesulide, NIM (CAS 51803-78-2) were obtained from Sigma Aldrich (Milan, Italy); these chemicals were used for both the exposure treatments and for analytical purposes.

2.2. Mussel exposure

Mussels *M. galloprovincialis* (5 ± 1 cm shell length) were obtained from a local farm (Ancona, Adriatic Sea); 360 mussels were randomly distributed into six 20 L aquarium (60 mussels per tank) and acclimatized for 10 days to laboratory conditions with aerated seawater, at 18 ± 1 °C, salinity 37, pH 8.0 ± 0.5 and oxygen saturation >94%. Due to their low solubility in water, stock solutions of AMP, DIC, IBU, KET and NIM were prepared in methanol and stored at room temperature for the duration of the experiment. Working solutions were prepared daily by diluting the stock solutions in seawater.

The experimental design included five tanks with organisms exposed to 25 μg/L of AMP, DIC, IBU, KET and NIM respectively and a solvent control tank (CTRL) where methanol was added at the same concentration used in the NSAIDs treatments (0.003%). The chosen exposure concentration is higher than those typically found in marine field conditions (from a few units to hundreds of ng/L; Gros et al., 2012; Lolić et al., 2015), but still environmentally realistic for particularly challenged sites (up to tens of μg/L; Togola and Budzinski, 2008). Water was changed daily, redosing concentrations of molecules and solvent; no mortality was observed during the experiment. After 14 days, 30 specimens for each treatment were dissected for chemical analyses, whole tissues were pooled in 5 samples (each containing tissues of 6 organisms) and stored at −20 °C. The remaining 30 specimens for each treatment were used for biological analyses preparing 10 replicates of

Download English Version:

https://daneshyari.com/en/article/4550566

Download Persian Version:

https://daneshyari.com/article/4550566

Daneshyari.com