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Acute exposure to sunscreen containing titanium induces an adaptive response and oxidative stress in *Mytillus galloprovincialis*

Antoni Sureda[a,b,*], Xavier Capó[a], Carla Busquets-Cortés[a], Silvia Tejada[c]

[a] Research Group in Community Nutrition and Oxidative Stress, Department of Fundamental Biology and Health Sciences, University of Balearic Islands, Ctra. Valldemossa, km 7,5, Ed. Guillem Colom, 07122 Balearic Islands, Spain

[b] CIBEROBN (Fisiopatología de la Obesidad y la Nutrición CB12/03/30038), Instituto de Salud Carlos III, Balearic Islands, Spain

[c] Laboratory of Neurophysiology, Department of Biology, University of Balearic Islands, Ctra. Valldemossa, km 7,5, Ed. Guillem Colom, 07122 Balearic Islands, Spain

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ABSTRACT

The use of sunscreens to protect against ultraviolet radiation exposure progressively increases as result of a greater awareness of the people and the greater arrival of tourists. The components of these creams can end up in the waters affecting coastal species. Mediterranean mussels (*Mytillus galloprovincialis*) were subjected to an acute exposure of a sunscreen with TiO_2 in their composition during 24 h. The low and medium concentrations used in the assays contained a concentration of TiO_2 in the range of values found in coastal waters of the Balearic Islands. Titanium and metallothionein concentrations were progressively increasing in gills with the sunscreen concentration in a dose-dependent manner. The activities of the antioxidant enzymes and the detoxification glutathione *s*-transferase evidenced a hormetic shape response with increased activities at lower sunscreen concentrations, a response that was abolished at the highest concentration. In accordance with these enzyme activities, the levels of malondialdehyde, as a marker of lipid peroxidation, were significantly elevated by the higher sunscreen concentrations. Acetylcholinesterase activity maintained control activities except for the highest sunscreen concentration, where a significant decrease was evidenced. In conclusion, the treatment of mussels with a sunscreen containing TiO_2 in the range of Balearic coastal waters induces an adaptive response that is overcome by the highest concentration. Follow-up biomonitoring studies are necessary to control the concentration of sunscreen compound in coastal waters such as titanium since they can induce oxidative stress to affected organisms.

1. Introduction

Excessive exposure to ultraviolet (UV) radiation, mainly during childhood, has been identified as the major cause associated with the development of skin cancer in the future (Greinert et al., 2015). The only way to prevent the development of skin cancer is the protection against UV. In this way, the use of sunscreen is highly recommended to prevent skin cancer, sunburn, photoaging, and skin wrinkles (Nahar et al., 2014). Sunscreen cosmetics can contain in their composition two types of protective components: organic compounds that absorb the UV radiation, and/or inorganic filters that physically reflect UV radiation (Shetty et al., 2015). The main physical agents that can be found in sunscreens are zinc (Turja et al., 2014) and titanium (Ti) oxides (Bouillon, 2000). Titanium dioxide (TiO_2) is able to reflect and scatter UV radiation (Bouillon, 2000; Smijs and Pavel, 2011), but it can also act as a photocatalytic agent which could produce many radical oxygen species (ROS), such as hydroxyl radicals and superoxide anion (Brezova et al., 2005). For this reason, TiO_2 is particularly dangerous because the generated ROS could cause oxidative damage in marine organisms (Girardello et al., 2016). Therefore, it is increasingly common the prohibition of using sunscreens in national parks for conservation purposes (Danovaro et al., 2008). Furthermore, sunscreens contain many other organic compounds with potential dangerous effects to the environment such as benzophenones, balicylates, cinnamates, camphor derivatives, or triazines that act as UV filters (Díaz-Cruz et al., 2008).

Coastal tourism is the main economic source in Balearic Islands (Western Mediterranean). The biggest island of this archipelago is Mallorca with a population of one million of habitants, although during the summer this area receives about 10 millions of tourists traditionally attracted by sun, sand and sea products (Garín-Muñoz and Montero-Martín, 2007; Tovar-Sanchez et al., 2013). This high tourist number causes a high pressure in environment such as pollution and

* Correspondence to: Research Group on Community Nutrition and Oxidative Stress, Department of Fundamental Biology and Health Sciences, University of Balearic Islands, 07122 Palma de Mallorca, Spain.
E-mail address: tosugo@hotmail.com (A. Sureda).

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overexploitation of resources, particularly of hydric resources. A greater number of tourists means greater pressure on the environment and an increased use of sunscreens that will inevitably lead to their release into the aquatic environment, which may constitute a threat to marine organisms. Accordingly, a recent study has demonstrated the presence of concentrations among 12.1–37.6 μg/L of TiO_2 in marine coastal waters of Balearic Islands (Tovar-Sanchez et al., 2013).

Benthic organisms and, especially bivalves are extensively used as sentinel species and bioindicators of pollution since they possess a multitude of useful characteristics for this purpose. Mussels are sedentary, filter large amounts of water, accumulate many types of contaminants in their tissues and are quite tolerant to chemical pollution (Box et al., 2007; Burgos-Aceves and Faggio, 2017; Catsiki and Florou, 2006; Matozzo et al., 2016; Pagano et al., 2016, 2017; Santovito et al., 2005; Savorelli et al., 2017; Torre et al., 2013). All together make mussels a particular good model for monitoring environmental state in coastal ecosystems (Box et al., 2007; Sureda et al., 2011). Accumulation of pollutants in organisms causes cellular damage, being oxidative stress the principal reason of this injury (Aliko et al., 2015; Moyson et al., 2016; Sehonova et al., 2017a, 2017b). A stressful situation leads to an overproduction of ROS that can damage cellular components through lipid, protein and DNA oxidation (Bartoskova et al., 2013; Box et al., 2007; Chromcova et al., 2015; Faggio et al., 2016; Messina et al., 2014; Sureda et al., 2011). In order to avoid the ROS damage, organisms present a complex system of antioxidants and detoxifying mechanisms that could prevent ROS production, detoxify toxins and/or pollutants and repair or remove the damaging molecules (Espinosa-Diez et al., 2015). Among these antioxidant and detoxifying elements antioxidant enzymes can be found, such as Catalase (CAT) which is able to eliminate oxygen peroxide, superoxide dismutases (SOD) that eliminate superoxide anion, glutathione reductase (Grd) and glutathione peroxidase (GPx) which play a central role in the regeneration of glutathione based system. Other important enzyme is glutathione-*S*-transferases (GST) which play an important role in detoxifying processes (Cunha et al., 2005). Acetylcholinesterase (AChE) is commonly used as a biomarker of exposure to neurotoxic substances in bivalves inasmuch as its activity decreased in case of neurotoxic damage. Other non-enzymatic systems act so as to protect the organism in front of the toxic effects of metals; these systems are metallothioneins (MTs). MTs are low molecular weight, high sulfhydryl content proteins that exhibit strong affinity for metals, and for this reason they can avoid the toxic effects of metals in part (Company et al., 2010); indeed, it has been observed that a high concentration of metals increased MTs production (Chandurvelan et al., 2013).

The aim of the current work was to evaluate the acute effects of TiO_2-containing sunscreen on *Mytillus galloprovincialis* biomarkers of oxidative stress and MTs levels using sunscreen concentrations calculated from the TiO_2 levels described in Mallorca Island coastal waters.

2. Material and methods

2.1. Experimental design

Mediterranean mussels (*M. galloprovincialis*) were selected due to their wide distribution in Mediterranean Sea, sessile lifestyle and ability to accumulate a lot of contaminants in their tissues. Mussels purchased from a local mussel farm were acclimated for 24 h in aerated seawater tanks at constant temperature (22 °C), and at the end of this acclimation period 48 mussels with a similar size were selected (mean shell height of 7.09 ± 0.09 cm and a mean width of 3.25 ± 0.05 cm). For the experimental treatment, a commercial sunscreen containing 1.4% TiO_2 was selected for the present study. Sunscreen concentrations were determined taking into account that seawater in the Balearic Islands presents TiO_2 concentrations between 6.9–37.6 μg/L (Tovar-Sanchez et al., 2013). Mussels were divided in 4 experimental groups (N = 12) and were spread in 4 different 10 L aerated tanks: a control group in a tank containing only 10 L of seawater, a second group containing 10 L of seawater plus 0.2 g of sunscreen (that represents a concentration of 2.8 μg/L of TiO_2), a third tank containing 10 L of seawater plus 2 g of sunscreen (representing a concentration of 28 μg/L of TiO_2), and the fourth tank containing 10 L of seawater plus 20 g of sunscreen (that represents a concentration of 280 μg/L of TiO_2). Each different treatment was maintained and evaluated after 24 h of exposure. Gills from 12 mussels were processed for biochemical analysis; of the total of twelve samples, three subsamples collected at random were selected for the determination of titanium. No mortality was observed in any of the different experimental conditions. All procedures were performed following the guidelines of the Local Ethics Committee of the University of the Balearic Islands (Spain).

2.2. Titanium determination

Gill samples were removed from 3 mussels for each treatment, lyophilized for 24 h and weighed. Lyophilized gills were digested using a 1:1 HNO3:HClO4 mixture in a dry bath incubator during 2 h at 180 °C and subsequently diluted with MilliQ™ water to a final volume of 10 ml. Titanium presence in samples was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin-Elmer SL, Optima 5300DV spectrometer). Data are expressed as μg Ti / g dry weight. Each sample was analyzed in triplicate.

2.3. Preparation of gills for biochemical analysis

Gills from 12 specimens for each treatment were dissected and immediately homogenized in ten volumes (w/v) of 100 mM Tris–HCl buffer (pH 7.5). Each homogenate was briefly sonicated (2–3 s) using an ultrasonic processor and centrifuged at 9000 g at 4 °C for 15 min. After centrifugation, supernatants were collected and immediately used for the biochemical analyses. Total protein content was determined by a colorimetric method (Biorad Protein Assay), using bovine serum albumin (BSA) as a standard to normalize all biochemical results.

2.4. Enzymatic activities

Catalase activity (mK/mg protein; K = (s^{-1})) was measured by means of Aebi (1984), based on the decomposition of H_2O_2. Glutathione reductase (GRd) activity was measured by a modification of the Goldberg and Spooner's spectrophotometric method (Goldberg and Spooner, 1984). Glutathione peroxidase (GPx) was determined following the method described by Flohe and Gunzler (1984). Measurements were recorded at a wavelength of 550 nm. Superoxide dismutase (SOD) (pmol/min/mg protein) activity was determined by the degree of inhibition of the reduction of cytochrome C by the superoxide anion generated by the xanthine oxidase/hypoxanthine system (Flohe and Otting, 1984). Glutathione-S-transferase (GST) activity was determined at 314 nm using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Habig et al., 1974). AChE activity (μmol/min/mg) was determined in gills using the method described by Ellman and collaborators (Ellman et al., 1961) with slight modifications. Briefly, thiocholine derivatives are hydrolyzed by acetylcholineterase to yield thiocholine. Subsequent combination with 5,5 dithiobis-2-dinitrobenzoic acid (DTNB) forms the yellow anion 5-thio-2-nitrobenzoic acid, which strongly absorbs at 412 nm. All antioxidant enzyme activities and GST activity were determined with a Shimadzu UV-2100 spectrophotometer at 25 °C.

2.5. Malondialdehyde (MDA) assay

Malondialdehyde (MDA) levels in gills, as lipid peroxidation marker, were analyzed by a colorimetric assay for MDA determination based on the reaction of MDA with a chromogenic reagent to yield a stable chromophore with maximal absorbance at 586 nm. Briefly,