Aquatic Toxicology 158 (2015) 218–229

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Genotoxicity, potential cytotoxicity and cell uptake of titanium dioxide nanoparticles in the marine fish *Trachinotus carolinus* (Linnaeus, 1766)

Caroline P. Vignardi[a,*], Fabio M. Hasue[a], Priscila V. Sartório[a], Caroline M. Cardoso[a], Alex S.D. Machado[b], Maria J.A.C.R. Passos[a], Thais C.A. Santos[a], Juliana M. Nucci[a], Thiago L.R. Hewer[d], Ii-Sei Watanabe[c], Vicente Gomes[a], Ngan V. Phan[a]

[a] *Department of Biological Oceanography, Oceanographic Institute, University of São Paulo, Praça do Oceanográfico 191, Cidade Universitária, Butantã, São Paulo, SP 05508900, Brazil*
[b] *Faculty of Veterinary Medicine, Integrated College North of Minas Osmane Barbosa Avenue, 11111, JK, Montes Claros, MG 39404006, Brazil*
[c] *Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, Prof. Lineu Prestes Avenue, 2415, Cidade Universitária, Butantã, São Paulo, SP 05508900, Brazil*
[d] *Institute of Chemistry, University of São Paulo, Prof. Lineu Prestes Avenue, 748, Cidade Universitária, Butantã, São Paulo, SP 05508000, Brazil*

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form 5 November 2014
Accepted 14 November 2014
Available online 23 November 2014

Keywords:
Cytogenotoxicity
Marine fish
Nanotoxicology
TiO_2

ABSTRACT

Nanoparticles have physicochemical characteristics that make them useful in areas such as science, technology, medicine and in products of everyday use. Recently the manufacture and variety of these products has grown rapidly, raising concerns about their impact on human health and the environment. Adverse effects of exposure to nanoparticles have been reported for both terrestrial and aquatic organisms, but the toxic effects of the substances on marine organisms remain poorly understood. The main aim of this study was to evaluate the genotoxicity of TiO_2–NP in the marine fish *Trachinotus carolinus*, through cytogenotoxic methods. The fish received two different doses of 1.5 μg and 3.0 μg–TiO_2–NP g^{-1} by intraperitoneal injection. Blood samples were collected to analyze erythrocyte viability using the Trypan Blue exclusion test, comet assay (pH > 13), micronucleus (MN) and other erythrocyte nuclear abnormalities (ENA) 24, 48 and 72 h after injection. The possible cell uptake of TiO_2–NP in fish injected with the higher dose was investigated after 72 h using transmission electron microscopy (TEM). The results showed that TiO_2–NP is genotoxic and potentially cytotoxic for this species, causing DNA damage, inducing the formation of MN and other ENA, and decreasing erythrocyte viability. TEM examination revealed that cell uptake of TiO_2–NP was mainly in the kidney, liver, gills and to a lesser degree in muscle. To the extent of the authors' knowledge, this is the first in vivo study of genotoxicity and other effects of TiO_2–NP in a marine fish.

1. Introduction

Nanoparticles are substances with at least one dimension that measures between 1 and 100 nm (Arora et al., 2012). Natural substances of nanometric size have existed on earth for millions of years, and have been used by man for thousands of years. Engineered nanoparticles are a recent product of nanotechnology. Due to their tiny size, these particles have physicochemical characteristics that are beneficial for human use, and are used in products of everyday use as well as in science, technology and medicine. On the other hand, these characteristics also make nanoparticles more chemically reactive, resulting in unexpected and harmful effects on biological systems, when compared with sub-micron sized particles of the same material (Gosens et al., 2010).

Titanium dioxide nanoparticles (TiO_2–NP) are widely used in the manufacture of paints, paper, plastic, laminate flooring materials, food coloring and cosmetics (Jugan et al., 2012). Due to their photocatalytic properties these nanoparticles are also used in wastewater and as environmental disinfectants (Cho et al., 2004). They are also used extensively in a wide range of pharmaceuticals, medical devices and treatments (Zhang and Sun, 2004).

* Corresponding author. Tel.: +1 805 895 4977.
E-mail addresses: carolpatvig@usp.br (C.P. Vignardi), humbigutis@gmail.com (F.M. Hasue), pri.sartorio@gmail.com (P.V. Sartório), camargonato@gmail.com (C.M. Cardoso), mamiferomarinho@gmail.com (A.S.D. Machado), zezemar@usp.br (M.J.A.C.R. Passos), tcsantos@yahoo.com (T.C.A. Santos), junucci@gmail.com (J.M. Nucci), hewer@iq.usp.br (T.L.R. Hewer), watanabe@icb.usp.br (I.-S. Watanabe), vicgomes@usp.br (V. Gomes), phanvn@usp.br (N.V. Phan).

http://dx.doi.org/10.1016/j.aquatox.2014.11.008
0166-445X/

The rapid development of nanotechnology and the corresponding increase in the use of manufactured nanomaterials in commercial products has led to concerns about possible environmental contamination caused by these substances. It is therefore important to understand the impact that these products may have on human health and the environment (Thomas et al., 2011; Nel et al., 2013). Available data on the release of TiO_2–NP from human usage (Mueller and Nowack, 2008; Kaegi et al., 2008; Kiser et al., 2009; Johnson et al., 2011) suggests that TiO_2–NP may represent a risk when exposed to aquatic environments, particularly freshwater and coastal environments, because of the proximity of such environments to human populations, industry and wastewater discharge (Ward and Kach, 2009).

With respect to freshwater organisms, the cytotoxicity and/or genotoxicity of TiO_2–NP has been studied in vitro and in vivo in algae (Miao et al., 2009), crustaceans (Lovern and Klaper, 2006), and a number of fish species (Reeves et al., 2008; Vevers and Jha, 2008; Paterson et al., 2011). The acute and/or chronic effects of nanoparticles on marine organisms remain poorly understood (Klaine et al., 2008; Ward and Kach, 2009). The effects of TiO_2 have been studied in marine algae (Rodríguez-González et al., 2010), marine phytoplankton (Miller et al., 2012), polychaete (Galloway et al., 2010), and bivalves (Canesi et al., 2010). Studies of the effects of nanoparticles on marine fish are extremely rare. The body burdens of citrate capped AgNPs were measured in the juvenile estuarine sheepshead minnow *Cyprinodon variegates* (Griffitt et al., 2012). The larvae of the brackish medaka fish *Oryzias melastigma* were used to compare the effects of nano and bulk zinc oxide (Wong et al., 2010). Joo et al. (2013) studied the effects of AgNPs on the diadromous fish *Oncorhynchus mykiss* at different salinities. Estuarine fish were used to study the effects of exposure to dietary CDSe/ZnS quantum dot on reproduction, development, and physiological functions (Blickley et al., 2014). Only one in vitro study of the effects of TiO_2–NP on cetaceans has been carried out (Bernardeschi et al., 2010).

No in vivo studies of cytotoxicity and genotoxicity of TiO_2–NP in marine fish were found, despite the fact that these animals play an important role in the energy flow of coastal ecosystems (Preez et al., 1990) and are a source of proteins for human consumption. Moreover, there are a number of fundamental differences between the physicochemical characteristics of freshwater and seawater, which affect the fate, behavior, bioavailability and toxicity of nanoparticles (Farré et al., 2009). Therefore, the effects of exposure to nanoparticles may vary in different habitats where aquatic organisms are found. The lack of data on the effects of TiO_2–NP on marine organisms prevents a full assessment of the ecological risk of TiO_2–NP released into the aquatic environment.

Studies have shown that TiO_2–NP is genotoxic to aquatic and terrestrial organisms. Gurr et al. (2005) demonstrated that in the absence of photo-activation, acute exposure to TiO_2–NP resulted in oxidative DNA damage to human bronchial epithelial cells (BEAS-2B). Vevers and Jha (2008) reported that TiO_2–NP caused an increase in DNA damage in the gonadal cells of rainbow trout in the presence of UV light. TiO_2–NP also caused DNA damage in the skin cells of goldfish (GFSK-S1) in the absence of UV light (Reeves et al., 2008). Long-term exposure to TiO_2–NP has been shown to induce chromosomal instability and cell transformation in in vitro (Huang et al., 2009) and in vivo experiments (Trouiller et al., 2009). The exact mechanism of the genotoxicity of TiO_2–NP has not been established. TiO_2–NP may cause DNA damage through direct interaction or through repair enzymes that cause breakage in the chain (Hartwig, 1998; Reeves et al., 2008). Much more likely, radicals induced by TiO_2–NP are responsible for DNA damage in exposed cells (Reeves et al., 2008). There is therefore a consensus that studies on the effects of TiO_2, as well as standardized protocols in nanoecotoxicology are necessary (Clemente et al., 2013). The most common method for the in vivo exposure of small aquatic organisms to pollutants is immersion. One drawback to this method for nanoparticles, however, is that TiO_2–NP aggregates/agglomerates and sinks rapidly in seawater, thus changing its concentration. Another common method is through injection. For small animals, however, intravenous injections cannot be applied, and so intraperitoneal injection is the preferred method for the exposure of small mammals and fish when testing the toxicity and genotoxicity of different substances under laboratory conditions (Ayllon and Garcia-Vazquez, 2000).

The present study evaluates the genotoxicity, cell viability and cell uptake of intraperitoneal injected TiO_2–NP in the tissues of juvenile pompano *T. carolinus*, a marine fish. This specie is found in abundance during the summer months (Bellinger and Avault, 1971) in surf zones, and in the neritic zone as an adult. This life cycle makes the fish vulnerable to coastal pollution and also makes it a possible carrier of pollutants between coastal and oceanic zones. The species is widely geographically distributed in the western Atlantic, from Massachusetts in the United States to the coast of Central and South America (Hoese and Moore, 1977) and has great commercial and sport fishing value, fulfilling an important ecological role (Watanabe, 1995; Sanches et al., 2007). The genotoxicity of TiO_2–NP was studied by comet assay to assess DNA strand breaks and by micronucleus test and evaluation of other nuclear erythrocyte abnormalities to study the chromosome loss and breakage. Cell viability and cell uptake were studied using the Trypan Blue exclusion test and transmission electron microscopy, respectively.

2. Material and methods

2.1. Reagents

TiO_2 nanopowder (ref T8141) was obtained from Sigma–Aldrich. Other chemical reagents used in the assays were obtained from Sigma–Aldrich and Merck.

2.2. Characterization

Crystalline phase and crystallite size were identified by the X-ray diffraction technique - XRD (Rigaku X-ray Diffractometer, Ultima IV, type $\theta - \theta$) using the Scherrer equation (Klug and Alexander, 1962). The X-ray beam was produced by pivoting a copper X-ray tube ($\lambda_{\alpha K} = 0.15428$ nm), varying the sample sweep angle of incidence θ, coupled to a diffraction angle 2θ, with a step of $\Delta 2\theta = 0.05^{\circ}$, and a counting time of 10 s per step from $2\theta_{min} = 20^{\circ}$. The morphology and size of TiO_2–NP were determined by transmission electron microscopy (TEM), using Jeol 1010 and Morgagni 268D microscope models. A suspension of 150 mg L^{-1} in saline solution for marine fish (Hoar and Hickman, 1967) was sonicated for 20 min at sonication power 7 Watt (XL-2000 Microson TM, Misonix). After this period, with the sonicator still functioning, 5 μl of the suspension was placed on MET 200 mesh screens with a carbon-coated blade. The screens were then stored for 24 h in a dry chamber at room temperature. Photomicrographs were taken (50,000-fold increase) and the diameters of 150–200 nanoparticles were randomly measured using Comet Score^{TM} Version 1.5 image analysis software. The behavior of TiO_2–NP in exposure suspension was evaluated by dynamic light scattering (DLS) using a ZetasizerNano ZSP (Malvern Instruments Ltda) particle size analyzer. The TiO_2–NP suspension (300 mg L^{-1}) was prepared as described in Section 2.4

2.3. Capture and maintenance of fish

Juvenile pompano, *T. carolinus*, with an average length of 7.31 ± 1.10 cm and mass of 5.04 ± 2.57 g were collected from Lazaro and Enseada beaches (23°30′ S; 45°07′ W, Ubatuba, Sao Paulo, Brazil), and transported to the Clarimundo de Jesus research station