Ecotoxicology (2018) 27:430–439
https://doi.org/10.1007/s10646-018-1907-7

Protein profiling as early detection biomarkers for TiO_2 nanoparticle toxicity in *Daphnia magna*

Paula Sá-Pereira[1] · Mário S. Diniz[2] · Liliana Moita[3] · Teresa Pinheiro[4] · Elsa Mendonça[3,5] · Susana M. Paixão[3] · Ana Picado[3]

Accepted: 22 January 2018 / Published online: 23 March 2018

Abstract
The mode of action for nanoparticle (NP) toxicity in aquatic organisms is not yet fully understood. In this work, a strategy other than toxicity testing was applied to *Daphnia magna* exposed to TiO_2-NPs: the use of nuclear microscopy and the assessment of protein profile. *D. magna is* a keystone species broadly used as a model system in ecotoxicology. Titanium (Ti) was found in the *D. magna* digestive tract, mainly in the gut. The penetration of Ti into the epithelial region was greater at higher exposure levels and also observed in eggs in the brood pouch. The protein profile of individuals exposed to different concentrations showed that 2.8 and 5.6 mg/L TiO_2-NP concentrations induced an over-expression of the majority of proteins, in particular proteins with molecular weight of ~120, 85 and 15 kDa, while 11.2 mg/L TiO_2-NP had an inhibitory effect on protein expression. The Matrix-assisted laser desorption ionization with tandem time of flight mass spectrometry (MALDI-TOF/TOF MS) analysis of these proteins consistently identified them as vitellogenin (Vtg)-like proteins, associated with enzymes involved in redox balance. These results indicate that Vtg-like proteins are up-regulated in *D. magna* exposed to TiO_2-NPs. Vitellogenesis is associated with the reproduction system, suggesting that TiO_2-NP exposure can impair reproduction by affecting this process. The precise mode of action of TiO_2-NPs is still unclear and the results from this study are a first attempt to identify specific proteins as potential markers of TiO_2-NP toxicity in *D. magna*, providing useful information for future research.

Keywords TiO_2 nanoparticles · *Daphnia magna* · 1D-electrophoresis · Protein profiling · Early biomarkers

✉ Paula Sá-Pereira
paula.sapereira@iniav.pt

✉ Ana Picado
ana.picado@lneg.pt

[1] INIAV—Instituto Nacional de Investigação Agrária e Veterinária, IP, Av. da República, Quinta do Marquês, 2784-505 Oeiras, Portugal

[2] REQUIMTE, Departamento de Química, Centro de Química Fina e Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal

[3] LNEG–Laboratório Nacional de Energia e Geologia, IP, Estrada da Portela, Bairro do Zambujal Ap 7586, 2720-999 Amadora, Portugal

[4] Instituto de Bioengenharia e Biociências, Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal

[5] APA-Agência Portuguesa do Ambiente, I.P. Rua da Murgueira 9/9ª, 2610-124 Amadora, Portugal

Introduction

The growing application of commercial nanotechnology has led to concerns about health risks and the impact of nanoscience-based products on the environment, though there are limited data on the interaction of nanoparticles with biological systems and their toxicological effects. The prospect of engineered nanomaterials such as nanoparticles (NPs) affecting biological systems has been recognized in the last decade, as indicated in several scientific reports showing their toxicity (Kang 2010; Menard et al. 2011; Zhang et al. 2012; Ivask et al. 2014). The potential harmful effects of NPs to living organisms seems to be related to their physical and chemical properties, such as surface area, size, surface modification and the formation of free radicals. The induction of oxidative stress associated with NP properties can damage lipids, carbohydrates, proteins and DNA, in which lipid peroxidation is considered most dangerous, leading to alterations in the enzymic machinery and cell membrane properties (Farhadian et al. 2012; Li et al. 2013).