Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Linking cortisol response with gene expression in fish exposed to gold nanoparticles

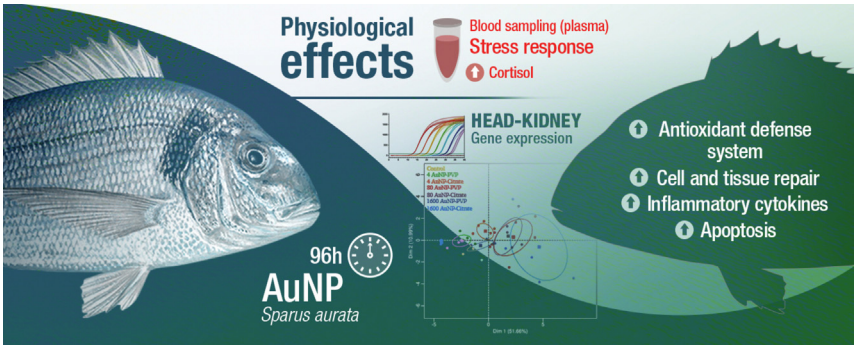
M. Teles [a,b,*], A.M.V.M. Soares [c], L. Tort [b], L. Guimarães [a,**], M. Oliveira [c,**]

[a] CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[b] Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
[c] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

HIGHLIGHTS

- Gold nanoparticles (AuNP) induce increases in plasma cortisol.
- Different coatings induce distinct cortisol responses.
- AuNP exposure cause altered gene expression in the head kidney.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 17 January 2017
Accepted 22 January 2017
Available online xxxx

Editor: Henner Hollert

Keywords:
Sparus aurata
Head kidney
Stress response
Glucose
Cytokines
Apoptotic markers

ABSTRACT

Fish exposure to environmental stressors (e.g. chemicals, hypoxia, temperature) induce responses enabling them to cope with alterations in their environment. A stress response involves a wide array of changes, from molecular to physiological and behavioural, set to counteract the effect of the stressor and recover homeostatic equilibrium. Among other processes, there is activation of the hypothalamus–pituitary–interrenal (HPI) axis, resulting in stimulation of the steroidogenic pathway and release of cortisol, important mediator of the adaptive response to stress. The purpose of this study was to evaluate if exposure of a marine teleost (gilthead sea bream) to gold nanoparticles (AuNP) could interfere with the HPI axis eliciting an acute stress response and how this response would be linked with alterations in the mRNA levels of target genes in the head kidney, important centre of endocrine response in fish. Fish were exposed via water, for 96 h, to four concentrations (0, as control, 4, 80 and 1600 $\mu g \cdot L^{-1}$) of 40 nm spherical AuNP, covered with two different types of coatings (citrate and PVP). At the end of the exposure, fish were anesthetized and blood and the head kidney sampled. Results showed that exposure to 1600 $\mu g \cdot L^{-1}$ AuNP-citrate and 80 $\mu g \cdot L^{-1}$ AuNP-PVP increased plasma cortisol levels, compared to controls, but caused no change in glucose levels. AuNP modulated the expression of target genes related to oxidative stress, cell-tissue repair, immune function and apoptosis in the head kidney of fish. The patterns of response were distinct for the two coatings tested. Unlike AuNP-citrate, AuNP-PVP elicited an inverted U-shaped response. Present findings demonstrated that AuNP were able to activate the fish HPI axis and alter a battery of related molecular markers in the head kidney.

* Corresponding author at: Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain.
** Corresponding authors.
E-mail addresses: mteles0@gmail.com (M. Teles), lguimaraes@ciimar.up.pt (L. Guimarães), migueloliveira@ua.pt (M. Oliveira).

http://dx.doi.org/10.1016/j.scitotenv.2017.01.153
0048-9697/

1. Introduction

Changes in the hypothalamus–pituitary–interrenal (HPI) axis function, measured as cortisol levels, are important indicators of fish condition. Generally, fish respond to stress with increases in plasma cortisol, their main corticosteroid, which is considered a primary response to stress (Tort, 2010). High cortisol levels mobilize and elevate glucose production in fish through glycogenesis and glycogenolysis to handle the energy request generated by the stress agent for the "fight or flight" reaction (Mommsen et al., 1999). Regardless of the use of glucose as an indicator of stress in fish, some studies reported an increased plasma cortisol level accompanied by unaltered or even decreased plasma glucose (Teles et al., 2016a; Teles et al., 2003). These discrepancies occur because fish plasma glucose also depends on other factors, such as the nutritional status, life stage, and the glucose clearance rates (Mommsen et al., 1999). In addition to the stress response, cortisol is involved in many aspects of the endocrine-mediated immune response of fish, causing alteration of various pro-inflammatory cytokines and inflammatory markers also observable at the genomic level (Yada and Tort, 2016). Plasma cortisol and glucose levels are therefore broadly used as main biomarkers of physiological stress in fish, including in animals exposed to environmental contaminants. Indeed, endocrine disrupting compounds interfering with fish HPI axis were also found to impact the adaptive cortisol response, highlighting its interest to evaluate also exposure to toxicant stress (Levesque et al., 2003; Teles et al., 2006).

In recent years, gold nanoparticles (AuNP) have been recognised as emerging contaminants of concern for their numerous ever-growing applications and potential environmental and health impacts. They have been successfully employed in biomedical research, water remediation and aquaculture practices (Alex and Tiwari, 2015; Saleh et al., 2016), for example. Nonetheless, environmental monitoring data is still lacking for AuNP due to analytical limitations. Estimates were derived for risk assessment using environmental exposure models and global information on consumer products in the UK, assuming 10% market penetration (Boxall et al., 2007). According to these, 0.14 $\mu g.L^{-1}$ and 5.99 $\mu g.kg^{-1}$ could be expected for water and soil, respectively, but other applications of AuNP have emerged over the latest years. More recent studies refer levels of 1–20 $\mu g.L^{-1}$ as environmentally relevant (Baalousha et al., 2016). As to effects on aquatic organisms, several studies suggest that exposure to AuNP can have harmful effects on aquatic organisms, including fish (Dedeh et al., 2015; Teles et al., 2016b; Volland et al., 2015). Production of reactive oxygen species (ROS) elicited by AuNP has been indicated as primary cause of nanotoxicity in mammals and aquatic organisms (Siddiqi et al., 2012; Volland et al., 2015). Increases in HSP70 protein levels have also been found in the brain of rats exposed to AuNP (Siddiqi et al., 2012). Inflammatory potential of AuNP was observed in the rat brain (Siddiqi et al., 2012), as well as mouse (Cho et al., 2009) and fish (Teles et al., 2016b) liver. It is, however, not clear if AuNP could affect cortisol levels in the plasma and mRNA expression of cytokine signalling molecules and immune-related genes in the head-kidney (organ of cortisol synthesis and secretion) of fish, and marine species in particular.

Sparus aurata, gilthead sea bream, is an edible marine teleost and a common aquaculture species. It is a top predator and a valuable species in the Mediterranean area. Because of its availability and ecological importance in marine ecosystems it is also widely employed in toxicity evaluations to the marine environment.

The purpose of this research work was to determine if AuNP could be recognised as a stressor by the HPI axis of *S. aurata*, by measuring hormonal (cortisol) and intermediary metabolism (glucose) biomarkers in the plasma after exposure to AuNP. Expression of target genes involved in several stress-related key functions (i.e., immune response, haematopoiesis, and stress-related hormone secretion) was subsequently evaluated in the head kidney. Two different AuNP coatings were evaluated in this study due to their application in several areas of activity and previous knowledge of the behaviour of these nanoparticles in high ionic strength media (Barreto et al., 2015). AuNP-citrate coated are prone to adsorption of serum proteins onto their surface (Chen et al., 2013) with a low stability in high ionic strength media. PVP is recognised as biocompatible coating, considerably increasing AuNP stability (Behera and Ram, 2013).

2. Materials and methods

2.1. Preparation and characterization of gold nanoparticles

AuNP of approximately 40 nm were prepared by reduction of $HAuCl_4$ by citrate using known procedures (Barreto et al., 2015). After synthesis AuNP-citrate were centrifuged (Sorvall Lynx 4000, Thermo) to remove any remaining reagents and resuspended in ultrapure water. The final concentration of AuNP-citrate was determined based on their absorption spectra and sizes (Liu et al., 2007; Paramelle et al., 2014). AuNP-citrate were coated with a PVP layer and quantified as described previously by Barreto et al. (2015). Characterization of AuNP was performed by UV–vis spectrophotometry (Cintra 303, GBC Scientific), dynamic light scattering, assessing hydrodynamic size and zeta potential (Zetasizer Nano ZS, Malvern), and by transmission electron microscopy (Hitachi, H9000 NAR). Based on previous results with similar AuNP (Barreto et al., 2015), and the recognition that AuNP intensity and position of the surface plasmon resonance peaks are related to the size, shape as well as colloidal stability (Pereira et al., 2014), a stability test was performed at a concentration of 15 mg AuNP$\cdot L^{-1}$. AuNP-citrate displayed a round shape with a size of approximately 37 nm size and polydispersity index of 0.3. The average value of zeta potential in ultrapure water was −44.5 mv. The PVP coating process increased the total average size to approximately 50 nm and decreased the polydispersity index to 0.2. The zeta potential was also altered (−17 mv). In saltwater, AuNP-citrate turned into light blue, whereas AuNP covered with PVP presented no colour alteration. The characteristic plasmon absorption of AuNP-citrate disappeared within a few minutes after placement in saltwater unlike AuNP-PVP that maintained the peak position and intensity during 96 h. Dynamic light scattering data revealed the formation of AuNP-citrate agglomerates/aggregates with sizes up to 600 nm in the first 24 h in saltwater and no variation in the size of AuNP-PVP during 96 h (Supplementary Fig.1).

2.2. Fish maintenance, exposure protocol and sampling

Gilthead sea bream (*Sparus aurata*) were purchased from a Spanish fish farm and acclimatized in the laboratory for 3 weeks. During this period, fish (9 ± 0.5 g, mean weight ± SD) were kept under standard conditions of 20 ± 1 °C on a 12 h light/12 h dark photoperiod in artificial saltwater (Ocean Fish, Prodac) with continuous aeration. Artificial saltwater was prepared by dissolving the salt in reverse osmosis water until reaching a salinity of 35. Fish were fed with a commercial diet (Sorgal, Portugal) once a day at a ratio of 1 g per 100 g of fish. After acclimatization and an additional two days of starvation, fish were exposed to four different concentrations of AuNP (0, as control, 4, 80, and 1600 $\mu g \cdot L^{-1}$) during 96 h, generally following OECD guideline 203 (OECD, 1992). Exposure solutions were prepared by dilution of the stock solutions in artificial saltwater. One of the test treatments was near the predicted environmental levels for water and soil (Boxall et al., 2007).

Ten fish were randomly distributed into duplicate 80 L experimental tanks (5 fish per tank, at a density of 1 $g.L^{-1}$) containing 50 L of either artificial seawater (control group) or AuNP test solutions and exposed for

Please cite this article as: Teles, M., et al., Linking cortisol response with gene expression in fish exposed to gold nanoparticles, Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.01.153