Ecotoxicology and Environmental Safety 80 (2012) 103–110

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles

Linhua Hao [a,b,*], Lei Chen [c,d]

[a] First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China
[b] Ocean University of China, Qingdao 266100, China
[c] Qingdao Municipal Hospital, Qingdao 266001, China
[d] Shandong University School of Medicine, Jinan 250012, China

ARTICLE INFO

Article history:
Received 19 September 2011
Received in revised form
15 February 2012
Accepted 18 February 2012
Available online 15 March 2012

Keywords:
Nano-ZnO
Carp
Antioxidant defense system
Lipid peroxidation
Oxidative stress

ABSTRACT

Changes in activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic antioxidant reduced glutathione (GSH) content and levels of Lipid peroxidation (LPO) in gill, liver, brain and intestine of juvenile carp (*Cyprinus carpio*) were evaluated after exposure to different concentrations (0.5, 5.0 and 50.0 mg/L) of waterborne nano-ZnO for 1, 3, 7, 10 and 14 day. The results showed that the variation trendency of antioxidant defense systems and LPO levels would be more significant with increasing concentration and exposure time. 50.0 mg/L nano-ZnO caused significant decrease of several enzymes activities and GSH content and increase of LPO level. As a result, these biomarkers were all appropriate for monitoring oxidative stress status of fish after exposure to nano-ZnO. Gill, liver and brain might be more sensitive response organs, being intestine the least altered organ. Further ecotoxicological evaluation should be made concerning the risk of nano-ZnO on aquatic environment.

1. Introduction

The rapid growth of nanotechnology is stimulating research concerning the potential environmental impacts of nanoparticles (NPs). Manufactured NPs are inevitably released and present in the environment during manufacturing, transport, use, and disposal operations, suggesting that a fundamental understanding of their mode and range of toxicity is needed (Handy et al., 2008; Lin et al., 2010). Following environmental release, these NPs are likely to represent a possible danger to aquatic life (Baun et al., 2008; Blaise et al., 2008; Wiqqinton et al., 2009; Farré et al., 2009). Metal oxide NPs are manufactured in large scale for both industrial and household use. They are finding increasing application in various commercial products, leading to concerns for their environmental fate and potential toxicity (Dreher, 2004; Kahru et al., 2008).

Nanoparticulate Zinc Oxide (nano-ZnO) particles are typical metal oxide NPs and they are noncombustible and odorless white powders. They are produced abundantly and widely applied in a range of products including sunscreens, cosmetics, paint, paper, plastics and building materials because of its high stability, anticorrosion and photocatalytic property. Nevertheless, this very property may cause nano-ZnO hazard in the environment. Recent studies have shown that nano-ZnO might pose significant risks to aquatic organisms (Reddy et al., 2007; Handy et al., 2008; Wang et al., 2009; Farré et al., 2009; Xiong et al., 2011). Although there have been a number of studies on the potential hazards of nano-ZnO to aquatic life, their environmental impacts and toxicity mechanisms still have not been fully elucidated. Moreover, those emerging literatures on the toxicity of nano-ZnO have mainly focused on acute exposure or early developmental stages to aquatic organisms (Adams et al., 2006; Heinlaan et al., 2008; Zhu et al., 2008; Aruoja et al., 2009; Kasemets et al., 2009; Zhu et al., 2009; Wiench et al., 2009; Ji et al., 2011). It is likely that a sub-acute or chronic exposure may more clearly reveal the toxicity of nano-ZnO and be of more ecological importance in expanding our understanding. And also, there are no detailed studies on the toxic effects of nano-ZnO on antioxidant defense system of the different body systems of organisms. Therefore, in the current study, we aimed to assess the changes of antioxidant defense systems including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), etc. antioxidant enzymatic activities and non-enzymatic antioxidant viz., reduced glutathione (GSH) content. Level of Lipid peroxidation (LPO) was also evaluated in response to exposure to different concentrations of nano-ZnO. Accordingly, the possible biomarkers as warning of nano-ZnO pollution in aquatic ecosystem would be discussed. Additionally, the other main goal was to determine the differences in the response between different organs to evaluate which

* Corresponding author at: First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China. Fax: +86 532 88965276.
E-mail address: haolh619@yahoo.com.cn (L. Hao).

0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2012.02.017

one was more severely affected to nano-ZnO. Very few studies to date have investigated the effects of exposure to nano-ZnO in intact free-swimming fish. These studies take together provide support to the hypothesis that the toxicity of metal oxide NPs is related to reactive oxygen species (ROS)-mediated pathway and provide insight into possible mechanism of toxicity as well as providing information for evaluating the risk to aquatic organisms exposed to nano-ZnO.

2. Materials and methods

2.1. Materials and particle suspension preparation

Nano-ZnO (30-nm sized particle, a surface area of 50 m^2/g, with a purity of 99.90%) was purchased from Beijing DK nano technology Co. LTD, China. The particle size was confirmed by transmission electron microscopy (TEM, JEOL 1200EXII). As indicated in Fig. 1, the determined ZnO nanoparticle was nearly spherical and very fit with the nano-scale, and the measured particle size was close to the manufacturer information.

A stock solution of 10 g/L nano-ZnO was prepared by dispersing the nano-particles dry powders in ultra-pure water (Millipore, ion free and unbuffered) with sonication for 6 h in a bath-type sonicator (100 W, 40 kHz), and different concentrations of the exposure solutions were obtained by diluting the stock solution with aerated tap water and a further 30-min sonication immediately prior to dosing each day.

2.2. Experimental fish

Carp (*Cyprinus carpio*), which is a kind of freshwater fish abundant in China, was chosen as the test organism. The fish was obtained from a local aquatic breeding base, Qingdao, China. The same batch of juvenile carp with mean values of 5.0–6.0 cm in length and 3.0–5.0 g in weight were acclimatized to laboratory conditions for 2 weeks prior to the experiments. In the toxicity study, they were maintained in 10 L aerated glass aquarium containing dechlorinated tap water. The water quality used to culture fish was controlled as follows: pH 7.0–7.8, dissolved oxygen concentration 100% saturation (aerated), temperature 20–22 °C, hardness of $CaCO_3$ 200 mg/L, conductivity 650 μS/cm. All fish were cultured under a natural 12-h light and 12-h dark cycle.

2.3. Exposure protocol

Juvenile carps (50 fish per group) in triplicate were randomly administered to three concentrations (0.5, 5.0, and 50.0 mg/L) of nano-ZnO for consecutive 14 day. The above experimental concentrations were designed to allow for sub-lethal physiological effects over the exposure period rather than fish mortality. The exposure time of 14 day was chosen to enable some physiological or biochemical responses to the exposure, but also considering the ethical constraint of using the minimum exposure period likely to achieve the scientific objectives. The entire experiment was subject to ethical approval, and independently monitored by a fish health expert. To maintain a relative stable aqueous phase concentration of nano-ZnO, the exposure solution was completely refreshed daily. The control group was provided with fresh aerated tap water without any NPs under the same conditions. All the fish were not fed during the experiment period in order to minimize the risk of nano-ZnO absorbing to food or fecal material and help maintain water quality.

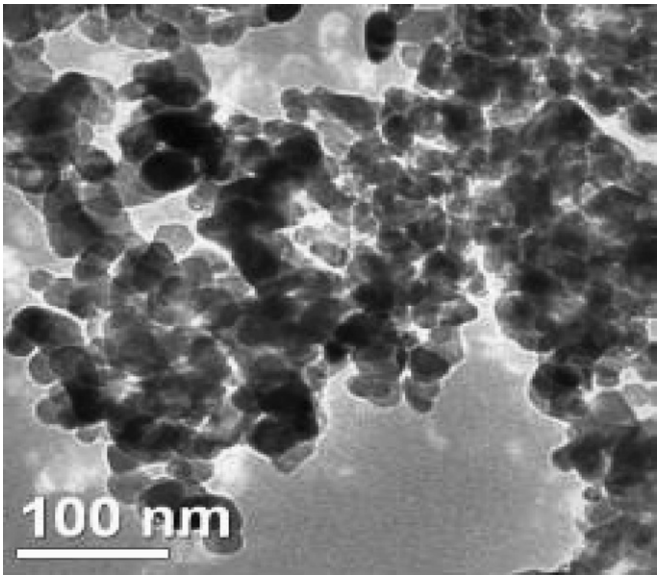

Fig. 1. TEM image of nano-ZnO sample (scale: 100 nm).

Fish from each group were sampled on days 1, 3, 7, 10, and 14, respectively. All the fish samples were sacrificed in ice-water, dried with filter paper, weighed and finally anatomized for the collection of target organs including liver, gill, brain and intestine. These organs were carefully removed and washed with physiological saline (0.9% NaCl) and immediately frozen into liquid nitrogen and stored at −80 °C until analysis.

2.4. Analytical procedure

The concentration of nano-ZnO in the exposure solution was quantified by the combination of microwave-assisted digestion (MAD, Mars5 HP500, CEM Corporation, USA) with inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500). Nano-ZnO samples (1 mL) with the combination of 3 mL of concentrated nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) were added into each of PTFE digestion tubes. After 15 min, the vessels were thereafter sealed to avoid any acid leakage and put into the microwave. Then the samples were digested using a three-stage digestion protocol (5 min at 150 °C, 5 min at 180 °C and 5 min at 200 °C). Afterwards the vessels were cooled down. Subsequently, Zn concentration in digested samples was determined by ICP-MS. The instrumental parameters were: RF power 1.0 kW, Nebulizer pressure 1.0 kPa, carrier gas (Ar) flow rate 15.0 L/min, peristaltic pump rate 15 rpm/min, Integration time High WL Range 5 s, Low WL Range 30 s, and wavelength 336.12 nm. The pretreatment method could recover (95 ± 5)% Zn concentration in the tested samples. The detection limit of ICP-MS for Zn concentration was 10 μg/L.

All tests of biomarkers were conducted within 2 day after the preparation of samples. The frozen organs (about 0.5 g) were homogenized in 5-fold chilled 100 mmol/L, pH 7.4 sodium phosphate buffer solution containing 20% (v/v) glycerol, 1 mmol/L EDTA, and 1.4 mmol/L dithioerythritol (DTE) by hand on ice using a glass tissue homogenizer. The homogenates were centrifuged at 10,000g for 10 min at 4 °C and the resultant supernatant was stored in 0.5 mL aliquots to assess possible effects on oxidative stress and antioxidant defense. All biochemical assays were preformed in triplicates.

SOD activity was estimated based on its ability to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated using xanthine/xanthine oxidase. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50% (Sun et al., 1988). CAT activity was determined using a modified method based on the method of Claiborne (1985) by measuring the initial rate of the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption over 1 min. GSH content was measured using the 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB)-oxidized GSH (GSSG) recycling assay (Anderson, 1985). GPx activity was assessed using H_2O_2 as a substrate according to Drotar et al. (1985). This method is based on the oxidation of GSH by H_2O_2 via GPx. Protein contents were determined by the Bradford dye-binding assay with bovine serum albumin as standard (Bradford, 1976). The absorbances of SOD, CAT, GSH, GPx and protein were respectively measured at 550, 240, 412, 412 and 590 nm using a microplate reader (Thermo, Varioskan Flash, USA). The level of LPO was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the TBA reaction with minor modification of the method Fatima et al. (2000). Commercial kits of all biochemical parameters were purchased from Nanjing Jiancheng Bioengineering Institute (NJBI, China).

2.5. Statistical analysis

All data were expressed as mean ± SD ($n=3$). Statistical analyses were carried out using Excel 2003 and SPSS version12.0 software package. The differences between the experimental and the control groups were tested for significance using one way analysis of variance (ANOVA) and Student's T-test. P values below 0.05 were regarded as significant.

3. Results

3.1. Aqueous exposure to ZnO nanoparticles

During the sub-acute toxic experiment period, the actual nano-ZnO concentrations in the water column were determined at (0.48 ± 0.05), (4.86 ± 0.2) and (47.9 ± 2.1) mg/L, respectively. Some loss of nano-ZnO was likely due to nanoparticle aggregates