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InP/ZnS QDs exposure induces developmental toxicity in rare minnow (*Gobiocypris rarus*) embryos

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ABSTRACT

We investigated the in vivo toxicity of InP/ZnS quantum dots (QDs) in Chinese rare minnow (*Gobiocypris rarus*) embryos. The 72 h post-fertilization (hpf) LC_{50} (median lethal concentration) was 1678.007 nmol/L. Rare minnows exposed to InP/ZnS QDs exhibited decreased spontaneous movement, decreased survival and hatchability rates, and an increased malformation rate. Pericardial edema, spinal curvature, bent tails and vitelline cysts were observed. Embryonic *Wnt8a* and *Mstn* mRNA levels were significantly up-regulated after InP/ZnS QDs treatment at 48 hpf (200 nmol/L) ($p < 0.05$). The superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels at 96 hpf (800 nmol/L) had an increasing trend. *Hsp70* mRNA expression was significantly changed at 48 hpf (200 nmol/L), but compared with the blank control, the different InP/ZnS QDs treatments did not significantly change the Olive tail moments ($p > 0.05$). Thus, InP/ZnS QDs caused teratogenic effects and death during the development of Chinese rare minnow embryos, but InP/ZnS QDs did not cause significant genetic toxicity during Chinese rare minnow development.

1. Introduction

Among the many nanomaterials, quantum dots (QDs) have unique optical properties, such as continuous excitation spectra, narrow emission spectra, and strong emission (Chan et al., 2002; Gao and Nie, 2004). QDs are widely used in cell imaging, biomedicine and other fields due to their unique optical properties (Erogbogbo et al., 2008; Michalet et al., 2005; Zarco-Fernández et al., 2016). QDs are usually composed of two parts: a core and a shell. At present, studies have predominantly focused on group II–VI QDs (Yang et al., 2011) and IV–VI QDs (Hardman, 2006); however, since these QDs cores contain toxic heavy metal elements (e.g., Cd, Pb, Hg, Te and As), their applications are limited. Therefore, designing and developing low-toxicity and high-performance QDs materials is one of the current frontiers in research (Fan et al., 2014; Mushonga et al., 2012; Tamang et al., 2016; Tang and Sargent, 2011). Group III–V QDs are believed to be potential replacements for heavy-metal-based QDs due to the optical properties of II–VI QDs and their lack of heavy metal elements. InP/ZnS QDs are the most widely used materials in group III–V due to their strong covalent bonds, indicating they are ideal for the substitution of II–VI QDs (Heath and Shiang, 1998). High-performance InP QDs with high sensitivity and low toxicity have already been used as fluorescent probes in cell imaging and cell diagnosis applications (Michalet et al., 2005; Mushonga et al., 2012).

During production and applications, QDs enter the human body through respiration, skin contact, consumption and other routes. In addition, drug treatment and biological monitoring are also potential sources of exposure to QDs. There are substantial variations in the absorption, transfer, accumulation and removal of QDs in different organisms (Alberola and Rädler, 2009). These differences depend the specific particle characteristics, such as particle size, traits (Amrite and Kompella, 2005), and surface chemical properties (Ballou et al., 2004). Several studies have shown that QDs could induce cytotoxicity by altering cell morphology (Su et al., 2009), reducing cell viability (Derfus et al., 2004), inducing cells to produce reactive oxygen species (ROS) (Kirchner et al., 2005) or autophagy (Li et al., 2011) and altering gene expression (Chen et al., 2012) at various levels.

Research into the biological toxicity of InP/ZnS QDs has been reported. Brunnetti et al. indicated that water-soluble InP/ZnS QDs were less toxic than CdSe/ZnS QDs to human A594 cells, SH-SY5Y cells, and *Drosophila* (Brunetti et al., 2013). Liu et al. found that InP/ZnS QDs at concentrations of less than 1.0 μm did not notably reduce the viability of human A549 cells (Liu et al., 2015). Huang's et al. studied the toxicity of InP/ZnS QDs on human serum albumin (HSA), and their results indicated that InP/ZnS QDs strongly bound to HSA and caused damage by interfering with the normal physiological function and

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conformational changes of HSA (Huang et al., 2015). InP/ZnS QDs were injected into BALB/c mice, and the results showed that InP/ZnS QDs did not cause observable toxicity within the evaluation period, but the QDs primarily accumulated in the spleen (Lin et al., 2015). InP/ZnS QDs were shown to be less cytotoxic than CdTe/ZnS QDs and CdSe/ZnS QDs (Chibli et al., 2011). Liu et al. treated human cancer cells (SH-SY5Y) with InP-Phos QDs for 48 h and did not observe any obvious cytotoxicity (Liu et al., 2015).

Rare minnow (*Gobiocypris rarus*) is the only native biological test subject in China recommended by *Chemical Testing Methods* and *Water and Wastewater Detection Methods*. A protocol for the acute toxicity test of rare minnow has been established in national standards (GB/T 29763-2013). Rare minnow is a freshwater cyprinid with several useful features, such as ease of maintenance in a laboratory, short life cycle, high fecundity, large egg size, transparent eggs, rapid development, and sensitivity to environmental pollutants; thus, rare minnow has been regarded as a suitable aquatic organism for biological toxicity assessments. The embryonic period is one of the most sensitive periods to various drugs in the life cycle of aquatic organisms (Sehonova et al., 2017a,b; Chromcova et al., 2015; Fiorino et al., 2018). When the body is stimulated by exogenous substances, the levels of active oxygen free radicals (ROS) will increase. Excessive a lot of ROS can induce oxidative stress in the body, often accompanied by lipid oxidation and, structural damage to the structure of the protein and DNA damage (Bartoskova et al., 2013; Savorelli et al., 2017; Burgos-Aceves et al., 2018). Therefore, we chose several key indicators of embryonic health: spontaneous movements, heart rate, survival rate, body length, malformation rate, relative enzyme activity (superoxide dismutase [SOD] and malondialdehyde [MDA] levels), gene expression (*Hsp70*, *Wnt8a*, *Mstn*) and DNA damage. This study assessed the toxicity of InP/ZnS QDs to the development of rare minnow embryos to improve awareness of the toxicity of InP/ZnS QDs exposure to aquatic biological development.

2. Materials and methods

2.1. Characterization of InP/ZnS QDs

Water-soluble InP/ZnS QDs (with ZnS shell layers) were purchased from Najing Technology Co., Ltd. (Hangzhou, China). InP/ZnS nanocrystals are a group of InP/ZnS core/shell QDs with a carboxylate ligand (3-mercaptopropionic acid). The concentration of the water-soluble InP/ZnS QDs was 7140 nmol/L. Transmission electron microscopy (TEM) showed that these nanocrystals were approximately 4.5 nm in diameter (Fig. 1).

2.2. Fish culture and embryo collection

Rare minnows were obtained from the Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education) at Southwest University in Chongqing, and the water temperature was maintained at 25 ± 0.5 °C with an oxygen content exceeding 6.0 mg/L. The body length (cm) and weight (g) were 3.60 ± 0.71 and 0.69 ± 0.20, respectively. The fish were fed twice daily with hatched brine shrimps at 9:00 and 15:00 with a 12 h light: 12 h dark artificial photoperiod. The rare minnow embryos were collected during natural spawning periods (fertilization rate > 95%) and washed twice with standard dilution buffer (ISO1996; 5.5 mg/L KCl, 294.0 mg/L $CaCl_2{\cdot}2H_2O$, 123.3 mg/L $MgSO_4{\cdot}7H_2O$, and 63.0 mg/L $NaHCO_3$).

2.3. Fish embryo toxicity test

The toxicity of InP/ZnS QDs on the embryonic development of rare minnow was evaluated by exposing fertilized eggs to the QDs. Rare minnow embryos were collected immediately after fertilization at the blastocyst stage. The rare minnow embryos were observed and photographed under a stereomicroscope (SMZ25 Nikon, Japan) after exposure to 0, 50, 100, 200, 400 or 800 nmol/L QDs. In our study, the control group was exposed to standard dilution buffer. The InP/ZnS QDs were diluted in standard dilution buffer (pH 7.5–8.0) for the test group. Embryos were selected and transferred to 24-well plates at the blastocyst stage. Twenty embryos were transferred to each well with 1 mL of renewed test medium. The plates were incubated at 25 ± 1 °C under a 16:8 h (light/dark) photoperiod until 96 h post-fertilization (hpf). The test solution was changed every 24 h to maintain the appropriate water quality and concentration of InP/ZnS QDs. Dead eggs were removed daily. Embryos or larvae were randomly sampled at 12, 24, 48, 72 and 96 hpf and immediately prepared for use in gene expression, enzyme activity and single-cell gel electrophoresis (SCGE) assays. The samples were stored at −80 °C for mRNA and enzymatic activity analyses.

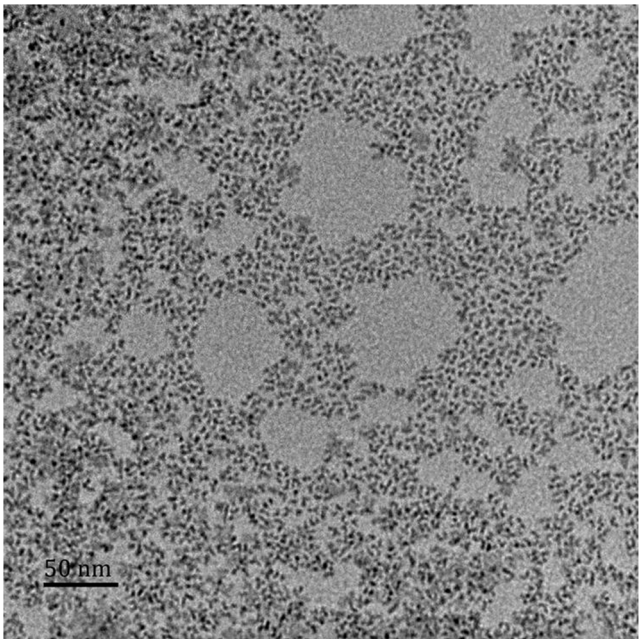

Fig. 1. TEM image of InP/ZnS QDs. Bar = 50 nm.

2.4. Developmental toxicity assays

Developmental parameters (survival rate, spontaneous movement, average heart rate, and body length), enzymatic activity, DNA damage and mRNA levels were selected to further explore the toxicity of InP/ZnS QDs in embryos. The embryos were exposed to 0, 50, 100, 200, 400 or 800 nmol/L QDs for 96 hpf. After the incubation period, embryos were selected at random for morphological observation using microscopy (SMZ25 Nikon, Japan). The hatching, mortality, and the malformation rates were calculated. The body length (mm) was measured, the heart rate (beats/min) was recorded (Zhu et al., 2013), spontaneous movement and heartbeat recordings were made for 30 s and 10 s, respectively, and the results were extrapolated to 1 min. Thirty intact embryos per tested concentration in the treatment and control groups were selected for enzymatic activity assays after exposure until 12, 24, 48, 72 or 96 hpf. The embryos were homogenized. The MDA content and the SOD activity were determined using commercial kits according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Gene expression analysis

Primers were designed according to previous studies (Liu et al., 2016; Zhu et al., 2013) and are listed in (Table 1). Total RNA was