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Effect of titanium dioxide nanoparticles on the bioavailability and neurotoxicity of cypermethrin in zebrafish larvae

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ABSTRACT

In aquatic environment, the presence of nanoparticles (NPs) has been reported to modify the bioavailability and toxicity of the organic toxicants. Nevertheless, the combined toxicity of NPs and the pesticides that were used world-widely still remains unclear. Cypermethrin (CYP), a synthetic pyrethroid insecticide, is commonly used for controlling agricultural and indoor pests. Therefore, the effects of titanium dioxide NPs ($nTiO_2$) on CYP bioconcentration and its effects on the neuronal development in zebrafish were investigated in our study. Zebrafish embryos (2- hour-post-fertilization, hpf) were exposed to CYP (0, 0.4, 2 and 10 μg/L) alone or co-exposed with $nTiO_2$ (1 mg/L) until 120-hpf. $nTiO_2$ is taken up by zebrafish larvae and also it can adsorb CYP. The zebrafish body burdens of CYP was observed and CYP uptake was increased by $nTiO_2$, indicating that the $nTiO_2$ could accelerate the bioaccumulation of CYP in larvae. Co-exposure of $nTiO_2$ and CYP induced the generation of reactive oxygen species. Exposure to CYP alone significantly decreased the mRNA expression of genes, including *glial fibrillary acidic protein (gfap)*, *α1-tubulin*, *myelin basic protein (mbp)* and *growth associated protein (gap-43)*. Besides, reductions of serotonin, dopamine and GABA concentrations were observed in zebrafish and the larval locomotion was significantly decreased in response to the lower level of the neurotransmitters. Moreover, co-exposure of $nTiO_2$ and CYP caused further significantly decreased in the locomotion activity, and enhanced the down-regulation of the mRNA expression of specific genes and the neurotransmitters levels. The results demonstrated that $nTiO_2$ increased CYP accumulation and enhanced CYP-induced developmental neurotoxicity in zebrafish.

1. Introduction

Pesticides are used mainly in agriculture, household and hygiene to protect plants, animals and human from insects and vector diseases (Fernandez-Cornejo et al., 2014; Narayan et al., 2013; Thomas, 2001). As a type II pyrethroid insecticide, cypermethrin (CYP) has been widely used to control pests, also including indoor pests, since its superior insecticidal activity and broad insecticidal range (Jin et al., 2011a). Owing to the restrictions on the application of organophosphorus (OPs) pesticides, CYP is expected to emerge as a major agricultural pesticide in many countries (Shi et al., 2011). Unfortunately, CYP being released directly into the environment mainly via run-off and entering a watershed and water bodies, and has emerged as one common aquatic contaminants (Jin et al., 2011b). CYP are now ubiquitous organic contaminants in water, sediment, animal tissue, even in the urine of children, pregnant women and infants (Alonso et al., 2015; Yang et al., 2014; Marino and Ronco, 2005; Vryzas et al., 2011., Lu et al., 2006; Qi et al., 2012; Berkowitz et al., 2003). In surface water, the concentration of CYP was usually detected at μg/L levels in most cases, e.g, average concentration of CYP was found at 0.325 μg/L in five main surface systems in Beijing (Ge et al., 2010). However, as high as 194 μg/L CYP was also detected in the runoff of farmed areas in Argentina (Marino and Ronco, 2005). Therefore, it has raised widespread concerns of the adverse environmental impacts and potential ecological health risks induced by CYP exposure. Pyrethroids has been regarded as a category of pesticides that associated causing adverse effects on nervous system of nontarget organisms (Singh et al., 2012). For examples, CYP induced neuroproteins levels alteration and neurobehavioral abnormalities in mice (Lee et al., 2015). Previous studies also reported that exposure of CYP can cause change in the activity of acetylcholinesterase in a freshwater fish (Kumar et al., 2009). However, the effect of CYP on the fish nervous system still poorly understood, especially when interact with other substances, which may modify the toxicity of CYP and reflect the risk of CYP exposure in the aquatic environment more accurately.

Because of their widely usage in a variety of consumer products such as sunscreens, cosmetics, paints and industrial products, titanium dioxide nanoparticles ($nTiO_2$) have attracted a great deal of research interests over the past four decades (Faria et al., 2014). Besides, $nTiO_2$

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also has been documented as an excellent catalyst in the photodegradation of organic pollutants in environmental applications (Chen and Mao, 2007). During their manufacture, transport and disposal, $nTiO_2$ is undoubtedly enter the environment, including air, soil and water (Lopez-Serrano Oliver et al., 2015). the environmental concentrations of $nTiO_2$ would range in the particles per pertrillion (ppt) to low part-per-billion (ppb) in surface water, (e.g. 0.078–2.8 μg/L in UK river; Mueller and Nowack 2008; Gondikas et al., 2014; Jaensson et al., 2007). Due to their characteristic physicochemical properties, including extremely small size, large surface area and photocatalysis, unpredictable health outcomes of $nTiO_2$ would appear (Qu et al., 2013). Except for the toxicity of $nTiO_2$ themselves, they are tending to interact with other environmental toxicants (Chen et al., 2011; Fang et al., 2015). The interaction of $nTiO_2$ may influence the bio-availability, environmental behavior, and toxic effects of other toxicants, which raising concerns for environmental risk assessments. Several study results demonstrated that adsorptive interactions really exist between $nTiO_2$ and other contaminants. Enhanced retention and toxicity of copper were reported when the presence of $nTiO_2$ in *Daphnia magna* (Fan et al., 2011). Several studies have showed that the uptake and toxicity of cadmium in *Lumbriculus variegatus*, *Daphnia magna* and fish is increased when cadmium is adsorbed on $nTiO_2$ (Nigro et al., 2015; Hu et al., 2011; Hartmann et al., 2012). Moreover, some evidence indicates that $nTiO_2$ could interact with organic pollutants. For example, previous studies have indicated that enhanced toxicity of trybutiltin in marine abalone embryos was associated with the presence of $nTiO_2$ (Zhu et al., 2011; Miller et al., 2012). $nTiO_2$ can enhance the bioavailability and bioconcentration of bisphenol A, polybrominated diphenyl ether, leading to endocrine disruption and developmental toxicity in zebrafish (Fang et al., 2016; Yan et al., 2014; Wang et al., 2014).

All in all, $nTiO_2$ is getting more and more attention because of their potential risk to wildlife and human health. Pyrethroids as mention above are ubiquitous in the environment, while the potential influences of co-exist nanoparticles on these pesticides in aquatic organisms remain unknown. Therefore, in order to better understand the environmental fate of pesticides in present of nanoparticles, it is essential to get data for the accumulation, and toxicity of typical pesticide in biota. In this regard, we evaluated the uptake and bioconcentration of CYP in zebrafish larvae when co-exposure with $nTiO_2$. In addition, we also investigated the potential effects of co-exposure of $nTiO_2$ and CYP on the neural development of zebrafish embryos. Several genes (*glial fibrillary acidic protein [gfap], synapsin IIa [syn2a], myelin basic protein [mbp], α1-tubulin, growth associated protein [gap-43], neural differentiation factor [neuro-D]*) related to the central nervous system development, differentiation and growth were assessed in the present study. In addition, neurotransmitter levels, and locomotion activity were measured in the present study. Taken together, our results show that $nTiO_2$ enhanced the bioavailability and neurotoxicity of CYP in zebrafish.

2. Materials and methods

2.1. Chemicals

Cypermethrin (CAS: 52315-07-8), tricaine mesylate (MS-222; CAS: 886-86-2) and $nTiO_2$ (CAS: 13463-67-7) were supplied by Aladdin (USA), Sigma Aldrich (St.Louis, MO, USA) and Hangzhou Wan Jing New Material Company (China) respectively. According to the manufacturer, the diameter of the $nTiO_2$ was 7.04 nm. More information of $nTiO_2$ have been provided in 3.1. Dimethyl sulfoxide (DMSO; CAS: 67-68-5; Sigma Aldrich) was used to prepare the stock solutions.

2.2. Preparation of $nTiO_2$ suspensions

A stock solution of 1 mg/mL $nTiO_2$ was obtained by dispersing the $nTiO_2$ in ultrapure water (Millipore, Billerica, MA, USA) with sonication (20 min; 50 W/L at 40 kHz, Tan et al., 2012). Exposure solution of $nTiO_2$ was prepared immediately prior to use and fresh charcoal-filtered water was used to dilute the stock suspension. In order to maintain the concentration of $nTiO_2$, the solution was renewed every day with new exposure solution. The information of $nTiO_2$ have been characterized in our previous study and a detailed method is provided in the supplementary Materials (Text S1).

2.3. Zebrafish maintenance and embryo exposure

The maintenance of adult zebrafish (*Danio rerio*, AB strain) and embryos exposure were performed in accordance with a published protocol (Wang et al., 2015b). Briefly, at about 2 hpf (blastula stage), the developed normally embryos (400 for each treatment) were chosen for this experiment. A stock solution of 100 mg/L CYP was prepare in DMSO. In the CYP exposure groups, the embryos were randomly transferred to glass beakers containing 200 mL CYP solution at four nominal concentrations (0, 0.4, 2 and 10 μg/L). Combination of CYP with $nTiO_2$ exposure was also performed, CYP test solutions were prepared with 1 mg/L (0.013 mmol/L) $nTiO_2$ prior to exposure. The concentration of $nTiO_2$ was chosen according to some previous studies, which reported that after 2 days exposure to 10 mg/L $nTiO_2$, no apparent abnormality was observed in zebrafish embryos (Wang et al., 2011). Four replicates were employed for each treatment and all the groups contained 0.01% (v/v) DMSO. During exposure, the temperature was maintained at 28 ± 0.5 °C with a 14:10-h light/dark cycles throughout the entire experiment. And the exposure solutions were changed daily. The embryos were exposed until 120 hpf when the yolk was absorbed completely. At 120 hpf, the larvae were sampled for subsequent gene expression, ROS generation, neurotransmitter contents, locomotor activity and chemical analysis. Besides, the developmental endpoints (hatching, malformation, survival and body length) were also recorded.

2.4. Adsorption experiments of CYP on $nTiO_2$

A mixed solution of 1 mg/L $nTiO_2$ and 10 μg/L CYP was used to measure the adsorption of CYP on $nTiO_2$. Briefly, 100 mL of the mixed solution was collected and centrifuged (5 min, 14 000 rpm) to separate the $nTiO_2$ particles at 0, 2, 4, 8, 16 and 24 h. The CYP concentrations in the supernatant were further analyzed by Agilent GC7890A (Palo Alto, CA).

2.5. $nTiO_2$ analysis in larvae

In larvae, the concentration of $nTiO_2$ was measured as previously described with slight modifications (Wang et al., 2014). Briefly, freeze-dried 100 larvae were weighed after washing 5 times. To decompose TiO_2 into Ti^{4+}, the samples were digested in 5 mL HNO_3 with a microwave digestion system (Multiwave 3000, Anton Paar, Austria) and then evaporated to dryness at 140 °C. The residue was re-dissolved with 1 mL 2% nitric acid. The concentrations of Ti^{4+} in water and larvae were determined by Atomic Absorption Spectrometer (PinAAcle 900T, PekinElmer, USA). The molecular weight conversion was used to change Ti concentrations to TiO_2 concentrations.

2.6. Transmission electron microscopy

Larvae were euthanized in MS-222 after exposure with $nTiO_2$ and washed with PBS (0.1 M, pH 7.4). Then the samples were fixed in PBS (2.5% glutaraldehyde, 2% paraformaldehyde) overnight at 4 °C. Later, they were post-fixed in 1% osmium tetroxide, dehydrated and embedded in Spurr's epoxy resin. Sections (60–80 nm in thickness) were prepared using a Leica EM UC6 ultramicrotome, then they were observed by a Hichachi (HT-7700) TEM at 80 kV (Wang et al., 2014).