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Combinational effect of titanium dioxide nanoparticles and nanopolystyrene particles at environmentally relevant concentrations on nematode *Caenorhabditis elegans*

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ABSTRACT

The possible adverse effects of nanoplastics have received the great attention recently; however, their effects at environmentally relevant concentration on organisms are still largely unclear. We here employed *Caenorhabditis elegans* to investigate the combinational effects of titanium dioxide nanoparticles (TiO_2-NPs) and nanopolystyrene particles at environmentally relevant concentrations on organisms. In wild-type nematodes, prolonged exposure to nanopolystyrene particles (1 μg/L) could enhance the toxicity of TiO_2-NPs (1 μg/L) in decreasing locomotion behavior and in inducing intestinal reactive oxygen species (ROS) production. Meanwhile, combinational exposure to TiO_2-NPs (1 μg/L) and nanopolystyrene particles (1 μg/L) altered the molecular basis for oxidative stress in wild-type nematodes. Moreover, prolonged exposure to nanopolystyrene particles (0.1 μg/L) could further enhance the toxicity of TiO_2-NPs (1 μg/L) in decreasing locomotion behavior and in inducing intestinal ROS production in *sod-3* mutant nematodes. Our data suggest the potential role of nanopolystyrene particles at environmentally relevant concentrations in enhancing the toxicity of ENMs in the environment.

1. Introduction

Nematode *Caenorhabditis elegans* has typical properties for classic model animals (Brenner, 1974; Zhao et al., 2013). More importantly, *C. elegans* is very sensitive to environmental exposure, which allows us to determine the in vivo toxicological performances of different environmental toxicants or stresses (Leung et al., 2008; Wang et al., 2014; Yu et al., 2015; Du et al., 2015; Tan et al., 2015; Zuo et al., 2016; Shakoor et al., 2016; Qu et al., 2017; Ren et al., 2017; Zhao et al., 2017a; Li et al., 2018). The rich in the availability of genetic mutants is further helpful for detecting the potential toxicity of environmental toxicants at environmentally relevant concentrations (Li et al., 2012; Wu et al., 2014; Zhi et al., 2016b; Xiao et al., 2017).

Microplastics are solid synthetic organic polymers (less than 5 mm) (Wright and Kelly, 2017; Horton et al., 2017). Recently, the possible adverse effects of nano-sized plastics (nanoplastics) derived from the degradation of microplastic particles in the environment on organisms have received the great attention (Cole et al., 2011; Browne et al., 2013; Lee et al., 2013; Mattsson et al., 2015; Hu et al., 2016; Sussarellu et al., 2016). The predicted environmentally relevant concentrations for microplastics are in the range ≤ 1 μg/L (Lenz et al., 2016). *C. elegans* has been further employed to assess the toxicity of nanoplastic particles (Zhao et al., 2017b; Lei et al., 2018). In nematodes, prolonged exposure (from L1-larvae to adult day-1) to nanopolystyrene particles (≥ 10 μg/L) induced intestinal reactive oxygen species (ROS) production, decreased locomotion behavior, and reduced brood size (Zhao et al., 2017b).

The widespread application of engineered nanomaterials (ENMs) will confer their enormous potential for human exposure and environmental release. Titanium dioxide nanoparticles (TiO_2-NPs) have been widely used as pigments and additives (Lomer et al., 2004; Oberdorster et al., 2005). The predicted environmental concentrations of TiO_2-NPs were reported as 16 or 24.5 μg/L in the water (Mueller and Nowack, 2008; Tiede et al., 2009). Prolonged exposure (from L1-larvae to adult day-1) to TiO_2-NPs (10 nm) at concentrations ≥ 0.01 μg/L could also induce intestinal ROS production, decrease in locomotion behavior, and reduction in brood size (Li et al., 2012; Wu et al., 2013, 2014). However, the possible combinational effects of TiO_2-NPs and nanopolystyrene particles at environmentally relevant concentrations on environmental organisms are still unclear.

In this study, we aimed to investigate the combinational effects of TiO_2-NPs and nanopolystyrene particles at environmentally relevant

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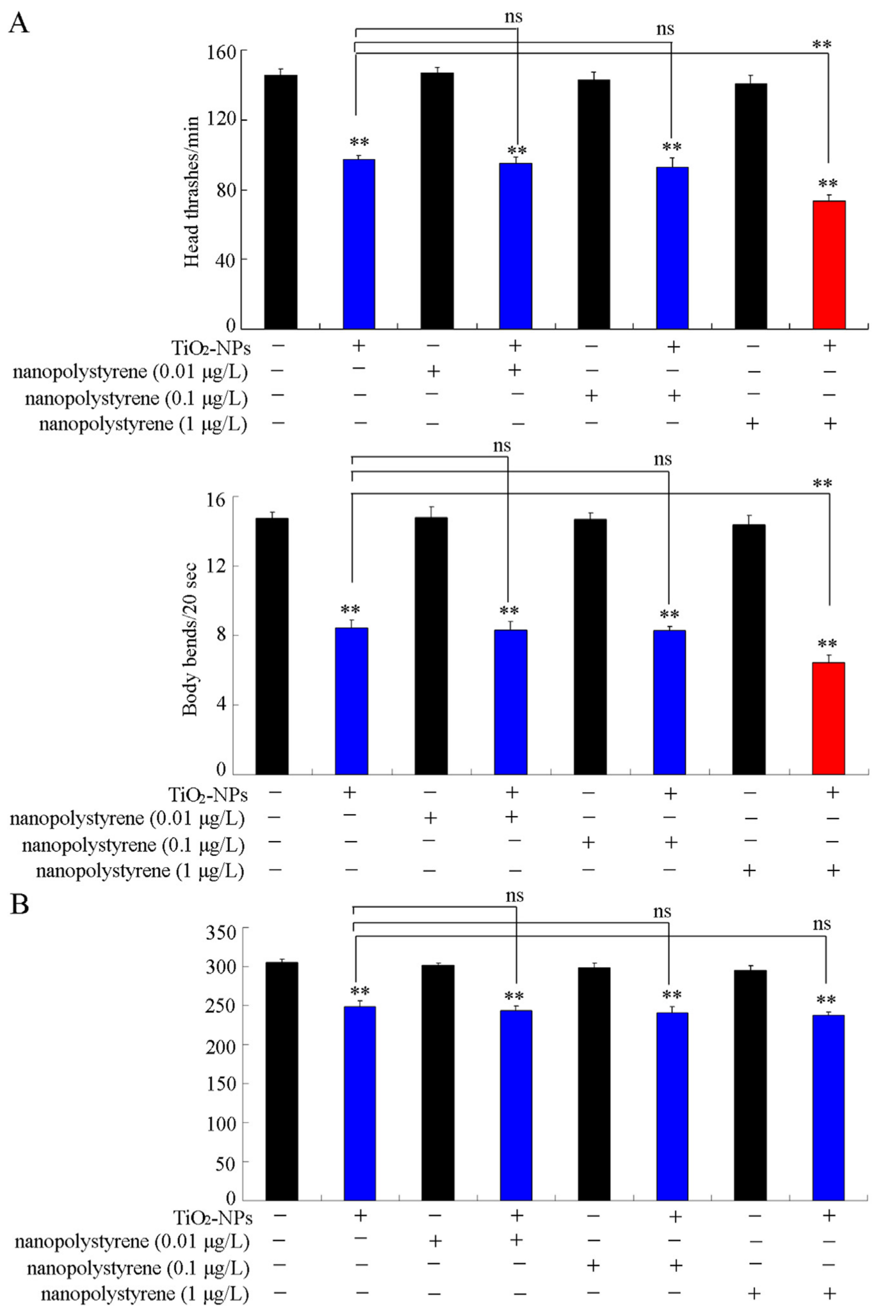

Fig. 1. Combinational effects of TiO_2-NPs and nanopolystyrene particles on locomotion behavior (A) and brood size (B) in wild-type nematodes. Prolonged exposure was performed from L1-larvae to adult day-1. Exposure concentration of TiO_2-NPs was 1 μg/L. Control, without TiO_2-NPs and nanopolystyrene particles exposure. Bars represent means ± SD. $^{**}P < 0.01$ *vs* control (if not specially indicated); ns, no significant difference.

concentrations on nematodes. Our results suggest that nanopolystyrene particles at predicted environmentally relevant concentration may potentially enhance the toxicity of TiO_2-NPs at environmentally relevant concentrations on nematodes. Our study implies the potential adverse effect of nanopolystyrene particles at predicted environmentally relevant concentrations in enhancing the toxicity of environmental ENMs on organisms.

2. Materials and methods

2.1. Properties of TiO_2-NPs and nanopolystyrene particles

Purity of TiO_2-NPs powder (10 nm, Zhejiang Wanjin Material Technology Co., Ltd) was 99.5%. Particle size of TiO_2-NPs was 10 ± 2 nm based on dynamic light scattering (DLS) measurement (Li et al., 2012; Wu et al., 2014). Surface properties of TiO_2-NPs were hydrophobic (Li et al., 2012; Wu et al., 2014). Surface area of TiO_2-NPs was 160 m^2/g based on the measurement by N2 sorption at 77 K (Li et al., 2012; Wu et al., 2014).

The used nanopolystyrene was from Janus New-Materials Co. (Nanjing, China), which is 1% solid suspension in water. The size of nanopolystyrene particles was 108.2 ± 4.5 nm based on the analysis by Nano Zetasizer (Zhao et al., 2017a), and the zeta potential of nanopolystyrene particles was − 9.698 ± 0.966 mV (Zhao et al., 2017b).

2.2. Strains and exposure

Wild-type N2, mutant of *sod-3(gk235)*, and transgenic strains of