Science of the Total Environment 643 (2018) 119–126

Contents lists available at ScienceDirect

Science of the Total Environment

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

Using *acs-22* mutant *Caenorhabditis elegans* to detect the toxicity of nanopolystyrene particles

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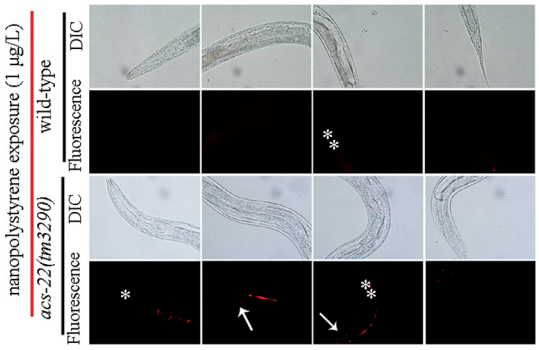
HIGHLIGHTS

- We investigated effect of functional deficit in intestinal barrier on nanoplastic toxicity.
- Nanopolystyrene (≥1 μg/L) caused toxicity in *acs-22* mutant nematodes.
- Nanopolystyrene (1 μg/L) was translocation into targeted organs in *acs-22* mutant.

GRAPHICAL ABSTRACT

Our results highlight potential toxicity of nanoplastic particles at predicted environmental concentration (1 μg/L) on environmental organisms with deficit in intestinal barrier after long-term exposure.



ARTICLE INFO

Article history:
Received 27 March 2018
Received in revised form 12 June 2018
Accepted 13 June 2018
Available online xxxx

Editor: Henner Hollert

Keywords:
Intestinal barrier
Translocation
Nanopolystyrene particles
Caenorhabditis elegans

ABSTRACT

In this study, we employed *Caenorhabditis elegans* with *acs-22* mutation to examine the *in vivo* effect of functional deficit in intestinal barrier on toxicity and translocation of nanopolystyrene particles. Mutation of *acs-22* leads to deficit in intestinal barrier. After prolonged exposure, nanopolystyrene particles at concentrations ≥1 μg/L could cause toxicity on *acs-22* mutant nematodes. *acs-22* mutation resulted in translocation of nanopolystyrene particles into targeted organs through intestinal barrier in nanopolystyrene particles (1 μg/L) exposed nematodes. After prolonged exposure, nanopolystyrene particles (1 μg/L) dysregulated expressions of some genes required for the control of oxidative stress and activated expression of Nrf signaling pathway. Therefore, under certain pathological conditions, our results suggest the potential toxicity of nanoplastic particles at predicted environmental concentration on organisms after long-term exposure.

1. Introduction

Microplastics, solid synthetic organic polymer with a size between 100 nm and 5 mm, are usually derived from human materials, such as cosmetics (Browne et al., 2011). Besides this, microplastics can also be derived from breakdown of larger plastic debris in the environment (Lambert and Wagner, 2016; Zhang et al., 2017). It has been widely recognized that a global plastic resin production is sharply increasing, and a huge amount of plastic waste has been released into the environment, such as the ocean (Cole et al., 2011; Jambeck et al., 2015; Bouwmeester et al., 2015). After the release into the environment,

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https://doi.org/10.1016/j.scitotenv.2018.06.173
0048-9697/

microplastic particles may be potentially degraded into nano-sized plastic particles, which implies the bioavailability of nanoplastic particles to the organisms in the environment (Mattsson et al., 2015). In the environment, the environmentally relevant concentrations of microplastics or nanoplastics have been predicted in the range ≤ 1 μg/L (Lenz et al., 2016).

Invertebrate *Caenorhabditis elegans* not only has properties of classic model animals (Brenner, 1974; Li et al., 2018), but also exhibits the sensitivity to environmental toxicants or stresses (Leung et al., 2008; Vanduyn et al., 2010; Mcelwee et al., 2013; Du et al., 2015; Luz and Meyer, 2016; Shakoor et al., 2016; Zhao et al., 2017a; Xiao et al., 2017). In nematodes, some useful sublethal endpoints, including development, reproduction, intestinal reactive oxygen species (ROS) production, and locomotion behavior, can be used to assess toxic effects of certain toxicants or stresses (Hall et al., 2012; Saldanha et al., 2013; Boyd et al., 2016; Yu and Liao, 2016; Zhi et al., 2017; Xiao et al., 2018a). *C. elegans* shows the potential for evaluating ecological risk of certain toxicants at environmentally relevant concentrations (Zhou et al., 2016; Huang et al., 2017). *C. elegans* has been widely and successfully used in the ecological risk assessment in water or river (Bragigand et al., 2006; Wolfram et al., 2012; Xiao et al., 2018b). We can also directly visualize and analyze accumulation and translocation of some chemicals in the body of nematodes (Zhi et al., 2016a; Wu et al., 2016; Ding et al., 2018). Additionally, some genetic mutants are available, and can be employed to assess the toxicity of environmental toxicants under certain pathological conditions (Leung et al., 2008; Zhao et al., 2013).

In the recent several years, nanoplastic particles in the environment have received an increasing attention. Nevertheless, most of the related studies have been performed in wild-type organisms (Wegner et al., 2012; Besseling et al., 2014; Della Torre et al., 2014; Ma et al., 2016). So far, the possible effects of nanoplastic particles on organisms with deficits in certain biological barrier, such as intestinal barrier or epidermal barrier, are still largely unclear. We hypothesize that deficits in biological barrier will strengthen the toxicity of nanoplastic particles on organisms.

In *C. elegans*, *acs-22* encodes a protein homologous to mammalian fatty acid transport protein. *acs-22* is required for the control of functional state of biological barrier (Kage-Nakadai et al., 2010; Zhi et al., 2016b). For example, mutation of *acs-22* could enhance the intestinal permeability and the accumulation of certain nanomaterials, such as multi-walled carbon nanotubes, in the body of nematodes (Zhi et al., 2016b). *acs-22* is mainly expressed in the intestine (Kage-Nakadai et al., 2010). Our previous study has demonstrated that prolonged exposure (from L1-larvae to adult day-1) to nanopolystyrene particles at concentrations ≥10 μg/L could cause the toxicity on wild-type nematodes (Zhao et al., 2017b). In this study, we aimed to investigate the effect of functional deficit in intestinal barrier caused by *acs-22* mutation on toxicity and translocation of nanopolystyrene particles and the underlying mechanisms. Our study suggested that functional deficit in intestinal barrier caused by *acs-22* mutation will strengthen the toxicity of nanopolystyrene particles on nematodes. In the *acs-22* mutant nematodes, we could detect the toxicity of nanopolystyrene particles at a predicted environmental concentration (1 μg/L) after prolonged exposure. Our results highlight the potential toxicity of nanoplastic particles at low concentrations under certain pathological conditions after long-term exposure.

2. Materials and methods

2.1. Physicochemical characterizations of nanopolystyrene particles

Size of used nanopolystyrene particles (Janus New-Materials Co., Nanjing, China) was 108.2 ± 4.5 nm based on analysis by transmission electron microscopy (TEM) (Fig. S1) and Nano Zetasizer (Nano ZS90, Malvern Instrument, UK). In these nanopolystyrene particles, Rhodamine B (Rho B) was enveloped by the polystyrene. The commercial nanopolystyrene particles were 1% solid suspension. An absorption band at *ca.* 530 nm was observed in UV–Vis absorption spectrum for nanopolystyrene particles (Zhao et al., 2017b). An absorption band at *ca.* 590 nm (the maximum wavelength) was observed in fluorescence spectrum for nanopolystyrene particles (Zhao et al., 2017b). Zeta potential of nanopolystyrene particles was −9.698 ± 0.966 mV (Zhao et al., 2017b). In K medium, nanopolystyrene particles were stably suspended for at least one week, and no obvious aggregation of nanopolystyrene particles and the leakage of dye could be detected.

2.2. C. elegans strains and culture

Wild-type N2, mutant of *acs-22(tm3290)*, and transgenic strains of LD1/*idIs7*[skn-1::GFP, and CL2166/*dvIs19*[gst-4::GFP] were used in this study. The strains were from *Caenorhabditis* Genetics Center. We maintained the nematodes on normal nematode growth medium (NGM) plates seeded with the food of *Escherichia coli* OP50 (Brenner, 1974). Age synchronous L1-larvae or L2-larvae population was prepared after lysis of adult hermaphrodite nematodes with bleaching mixture solution (0.45 M NaOH, 2% HOCl) (Donkin and Williams, 1995).

2.3. Exposure and toxicity assessment

The used working concentrations for nanopolystyrene particles were 0.1, 1, and 10 μg/L, and prepared after the dilution of stock solution (1 mg/mL) with the K medium. Prolonged exposure to nanopolystyrene particles was performed from L1-larvae to adult day-1 in the liquid solutions (500 animals/500 μL) at 20 °C with the addition of food (OP50).

Lethality was evaluated by the percentage of survival animals. After the exposure to nanopolystyrene particles, the lethality was assessed. For the lethality assay, the nematodes are judged to be dead if they cannot respond to the stimulus using a small metal wire. One hundred animals were examined per treatment.

Environmental toxicants can induce toxicity on the functions of both primary and secondary targeted organs in nematodes (Yang et al., 2016a; Yang et al., 2016b). Intestinal ROS production reflecting the functional state of intestinal cells was analyzed as described (Ren et al., 2017). The 5′,6′-chloromethyl-2′,7′dichlorodihydro-fluorescein diacetate (CM-H_2DCFDA) can detect the presence of intracellular produced ROS species. The examined nematodes were incubated with 1 μM CM-H_2DCFDA solution for 3 h in the dark. After that, the examined nematodes were mounted on a 2% agar pad, and analyzed at 488 nm of excitation wavelength and at 510 nm of emission filter under a laser scanning confocal microscope (Leica, TCS SP2, Bensheim, Germany). Relative fluorescence intensity of intestinal ROS signals was semi-quantified in comparison to the intestinal autofluorescence. Forty nematodes were examined per treatment.

Head thrash and body bend reflecting the functional state of motor neurons were analyzed as described (Chen et al., 2017; Wu et al., 2017). Head thrash and body bend were analyzed under the dissecting microscope by eyes as described (Qu et al., 2017). A head thrash is defined as a change in the direction of bending at the mid body, and a body bend is defined as a change in the direction of the part of the nematodes corresponding to the posterior bulb of the pharynx along the y axis, assuming that nematode was traveling along the x axis. Fifty nematodes were examined per treatment.

2.4. Intestinal permeability assay

Enhancement in intestinal permeability is an important cellular contributor to toxicity formation of environmental toxicants (Zhao et al., 2013; Zhi et al., 2016a). The method was performed basically as described (Gelino et al., 2016). The examined nematodes were suspended in blue food dye of erioglaucine disodium (5.0% wt/vol in water) in the presence of OP50 for 3 h. After that, the whole nematodes were