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Aluminum nanoparticle exposure in L1 larvae results in more severe lethality toxicity than in L4 larvae or young adults by strengthening the formation of stress response and intestinal lipofuscin accumulation in nematodes

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ABSTRACT

Toxicity of Al_2O_3-NPs, as compared to that of Al_2O_3, to L1-larval, L4-larval or young adult nematodes was evaluated. When exposure was performed at L1-larval stage, the significant increases of lethality, stress response, and intestinal lipofuscin autofluorescence were observed in 6.3–203.9 mg/L of Al_2O_3-NPs exposed nematodes. In contrast, when exposure was performed at L4-larval or young adult stage, the significant increases of lethality and intestinal lipofuscin autofluorescence were observed in 12.7–203.9 mg/L of Al_2O_3-NPs exposed nematodes, and the significant inductions of stress response were detected in 25.5–203.9 mg/L of Al_2O_3-NPs exposed nematodes. Moreover, the lethality was significantly correlated with the stress response and the intestinal lipofuscin autofluorescence in Al_2O_3-NPs exposed nematodes. These data imply that Al_2O_3-NPs exposure in L1 larvae causes more severe lethality toxicity than in L4 larvae or young adults by strengthening the formation of stress response and intestinal lipofuscin accumulation in nematodes.

1. Introduction

The research on bioavailability, uptake, tissue distribution, food-chain transportation, and effects of nanoparticles (NPs) has been identified as a near-term priority by the U.S. Environmental Protection Agency (2005). Manufactured NPs, defined as particles with one or more dimensions of less than 100 nm, are currently produced in metric tons per year and widely used in biomedical applications as carries for gene delivery, hyperthermia treatment for tumor destruction, and bio-detection of pathogens, and may have adverse effects on organisms because of their composition, shape, or unique size (Ma et al., 2009). Studies have suggested that NPs can affect biota at the cellular, subcellular, and protein levels (Service, 2004), and influence the respiratory and cardiovascular systems in mammals (Holsapple et al., 2005). Widely used NPs, such as Al_2O_3-NPs, may produce a physiological response in certain organism by entering the environments. Thus, the possible toxic effects and ecological consequences from exposure to NPs should be carefully considered and investigated before the commercial benefits of these materials can be fully

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realized (Thomas and Sayre, 2005; Ma et al., 2009). Nevertheless, still few studies have been conducted regarding the possible adverse effects of NPs to ecological receptors, such as invertebrates and vertebrates from terrestrial and aquatic environments (Ma et al., 2009).

Recently, the nematode *Caenorhabditis elegans* has been used for the evaluation of toxicity from NPs exposure. *C. elegans*, a free-living soil nonparasitic nematode surviving in terrestrial, benthic, and aquatic environments, is one of the most thoroughly studied animals, and the detailed knowledge of its biology, including movement, feeding, development, and reproduction is available (Riddle et al., 1997), which make it an excellent candidate for ecotoxicological studies for a wide range of environmental toxicants (Li et al., 2009a,b; Ruan et al., 2009; Xiao et al., 2009). Using *C. elegans* as a bioindicator, the manufactured zinc oxide NPs have toxicity to the nematodes similar to that of aqueous $ZnCl_2$ with the aid of lethality, movement, reproduction, and transgene expression as endpoints (Ma et al., 2009). The functional genomic studies using mutant analysis suggested that the *sod-3* and *daf-12* gene expressions may have been related to the silver NPs-induced reproductive failure (Roh et al., 2009). The silica-NPs could further induce an age-related degeneration of reproductive organs (Pluskota et al., 2009). Moreover, both NPs (ZnO-NPs, Al_2O_3-NPs, and TiO_2-NPs) and their bulk counterparts were toxic, inhibiting growth and especially the reproductive capability of the nematodes (Wang et al., 2009).

The choice of an optimal endpoint for testing environmental toxicants depends on several factors, including sensitivity, and the sensitive endpoints can permit detection of lower levels of toxicants (Anderson et al., 2001). With the aid of lethality and feeding as endpoints, L1 *C. elegans* larvae may represent one of the most sensitive invertebrates so far adopted for metal toxicity assay (Jones and Candido, 1999; Chu and Chow, 2002). Younger larvae (L1, L2, and L3) nematodes could exhibit more severe lethality and neurobehavioral toxicities than L4 larvae and young adult nematodes after 4 h of metal exposure (Xing et al., 2009a,b).

Moreover, stress response is a universal phenomenon characterized by the induced synthesis of heat shock proteins. In *C. elegans*, reporter transgene consisting of a fragment of the promoter from the *C. elegans* heat-shock protein of *hsp-16.2* that control the transcription of a green fluorescent protein (*gfp*) has been generated and successfully used for assessment of toxicological response to metals or other toxicants (Chu and Chow, 2002; Wang et al., 2007a,b; Wang and Wang, 2008; Shen et al., 2009; Wang and Xing, 2009; Li et al., 2009a). For example, chronic exposure to Ag, Cr, Pb, Cu, Hg, and Cd resulted in a significant induction of *hsp-16.2::gfp* expression at concentrations from 1 μM to 10 μM (Shen et al., 2009). In addition, chronic exposure to Ag, Cr, Pb, Cu, Hg, and Cd would induce a more severe stress response than exposure to Zn and Mn in intestine, and chronic exposure to Pb, Hg, Cr, Zn, and Mn would induce a more severe stress response than exposure to Ag, Cu, and Cd in head neurons (Shen et al., 2009).

In the present study, toxicity of Al_2O_3-NPs, as compared to that of Al_2O_3, to L1-larval, L4-larval or young adult *C. elegans* was evaluated using lethality as an endpoint. The stress responses in nematodes exposed to Al_2O_3-NPs or Al_2O_3 at different developmental stages were monitored by the P*hsp-16.2::gfp* expression. Moreover, the intestinal autofluorescence caused by lipofuscin accumulation was further measured in Al_2O_3-NPs or Al_2O_3 exposed nematodes. The linear regression analysis was performed to investigate the possible associations of percentage of survival animals with stress response or intestinal autofluorescence in Al_2O_3-NPs and Al_2O_3 exposed nematodes. The data presented in this study will be helpful for our further understanding the mechanism explaining the possible sensitivity of larvae nematodes to Al_2O_3-NPs exposure.

2. Materials and methods

2.1. Regents and preparation of Al_2O_3-NPs suspensions

The Al_2O_3-NPs (purity, >99.99%; diameter, 60 nm) were purchased from Hongchen Material Sci. & Tech. Co., China. Bulk Al_2O_3 (purity, >98.5%; diameter, 429 nm) was purchased from ACROS, Fisher Scientific and Baker Chemical Co. The surface areas of the Al_2O_3-NPs and Al_2O_3 provided by the producer were 180 m^2/g and 11 m^2/g, respectively (Wang et al., 2009). The wide distributions of particle size for Al_2O_3-NPs and Al_2O_3 were from 470 to 1126 and 565 to 974 nm, respectively (Wang et al., 2009). The zeta potentials for Al_2O_3-NPs and Al_2O_3 were 33.1 and 5.6 mV, respectively (Wang et al., 2009). All the other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

The stock suspensions of Al_2O_3-NPs and bulk Al_2O_3 (6.3, 12.7, 25.5, 51, 102 and 203.9 mg/L, respectively) were prepared as described (Wang et al., 2009). The stock suspensions of Al_2O_3-NPs and bulk Al_2O_3 were dispersed in ultrapure water by probe sonication (Sonic Dismembrator, Model 100, USA) at 100 W and 40 kHz for 30 min to form homogeneous suspensions. The NPs were then serially diluted in water and additionally sonicated for 30 min. Small magnetic bars were placed into the suspensions for stirring during dilution to avoid particle aggregation and deposition. To avoid precipitation of $Al(OH)_3$, the pH value of Al_2O_3-NPs and bulk Al_2O_3 was adjusted to 5.6.

2.2. Strain preparation

Nematodes used in the present study were wild-type N2, originally obtained from the *Caenorhabditis* Genetics Center (funded by the NIH National Center for Research Resource, USA), and KC136 (*Ex*(P*hsp-16.2::gfp*)), a gift from Dr. King L. Chow. They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20 °C as described (Brenner, 1974). Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (0.45 M NaOH, 2% HOCl). Age synchronous populations of L1-larval, L4-larval or young adult nematodes were obtained by the collection as described (Donkin and Dusenbery, 1993). The collected nematodes were washed with double-distilled water twice, followed by washing with modified K medium once (50 mM NaCl, 30 mM KCl, 10 mM NaOAc, pH 5.5) (Williams and Dusenbery, 1988). Exposures were performed on L1-larval, L4-larval or young adult nematodes in 12-well sterile tissue culture plates at 20 °C incubator in the presence of food. When