J. ENVIRON. TOXICOL.
Vol. 23, No. 2, 87～91 (2008)

Ecotoxicological Effects of CeO_2 Nanoparticles on Soil Nematode *Caenorhabditis elegans*

Ji-Yeon Roh, Young-Kwon Park and Jinhee Choi※

Faculty of Environmental Engineering, College of Urban Science, University of Seoul, Seoul 130-743, Korea

토양선충 *Caenorhabditis elegans*를 이용한 세리아(CeO_2) 독성연구

노 지 연, 박 영 권, 최 진 희※

서울시립대학교 환경공학부 독성학연구실

ABSTRACT

In this study, three different sizes of cerium oxide (CeO_2) nanoparticles were synthesized and exposed to *Caenorhabditis elegans* to investigate the potential harmful effect of CeO_2 nanoparticles on the environment. The effects of the CeO_2 nanoparticles on *C. elegans* were assessed at multiple levels, such as with respect to stress response gene expression, growth, reproduction and mortality. Moreover, to test the ecotoxicological relevance of CeO_2-induced gene expression. The overall results suggest that CeO_2 nanoparticles may provoke ecotoxicity in *C. elegans* especially with respect to gene expression, reproduction and survival, which can comprise an important contribution to knowledge on the ecotoxicity of CeO_2 nanoparticles, about which little data are available. This is particularly valuable in the biomarker research on ecotoxicology, as ecological relevance is a crucial criterion for the applicability of the biomarker in field biomonitoring and ecological risk assessment.

Key words : ceria, *Caenorhabditis elegans*, stress related gene expression

INTRODUCTION

Nanoparticle toxicity studies are among the fastest growing areas of environmental toxicology research, because of the fast applications of the nanotechnology in a wide variety of fields (Nohynek *et al*., 2007; Rogueda and Traini, 2007; Warheit *et al*., 2007). The potential health and environmental risks related to the widespread production and use of the nanomaterials created by these technologies, need to be investigated in terms of their toxic mechanism, as well as, long term consequences which will cause a physiological response in the organism that can alter the fitness of certain organisms, and ultimately leads to population/community level changes. Ecotoxicological studies with NPs (nanoparticles) are much more limited with few reports on the toxic effects of NPs to aquatic organisms, such as, Fish and Daphnia us-

※ To whom correspondence should be addressed.
Tel: +82-2-2210-5622, Fax: +82-2-2244-2245
E-mail: jinhchoi@uos.ac.kr

ing organism level endpoints (Hund-Rinke and Simon, 2006; Lovern and Klaper, 2006; Lovern *et al*., 2007; Smith *et al*., 2007). However, few studies have been performed on the ecotoxicity of soil organism. In the current study, ecotoxicological assessments of nanoparticles were conducted on soil nematode, *Caenorhabditis elegans*, including sub-organism level endpoint.

C. elegans, a free-living nematode that lives mainly in the liquid phase of soils, is the first multicellular organism to have its genome completely sequenced. The genome showed an unexpectedly high level of conservation with the vertebrate genome and functional genomic tools (gene knock out and RNA interference), which makes *C. elegans* an ideal system for biological studies, such as those in genetics, molecular biology, and development biology (Bettinger *et al*., 2004; Leacock *et al*., 2006; Schafer, 2006; Schroeder, 2006). Due to its abundance in soil ecosystems, its convenient handling in the laboratory, and its sensitivity to different kinds of stresses, it is frequently used in ecotoxicological studies utilizing various exposure media, including soil and water (Williams and Dusenbery 1990; Peredney and Williams 2000; Boyd and Williams 2003)

As model nanoparticle, cerium oxide (CeO_2) was selected as the cerium oxide is one of the most important rare-earth oxides that have been widely investigated due to the unique properties and multiple applications, such as, acting as the three-way catalysts in vehicle emission-control systems, electrolyte materials of solid oxide fuel cells, and ultraviolet blocking materials (Yu *et al*., 2001; Corma *et al*., 2004). In this study, CeO_2 NPs were synthesized and exposed to *C. elegans*. Effect of CeO_2 NPs was assessed on stress-related gene expression growth, reproduction, and mortality in *C. elegans*.

MATERIALS AND METHODS

1. Organisms

The wild-type *C. elegans* Bristol strain N2 was used. *C. elegans* (Caenorhabditis Genetics Center, USA) were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50, at 20˚C, using the standard method previously described by Brenner (1974). Young adults (3 days old) from an age-synchronized culture were used in all the experiments. Worms were incubated at 20˚C for 24 h without a food source, and were then subjected to the analysis.

2. Sample preparation

The cerium oxide was prepared as described previously (Park *et al*., 2008). Nematodes were exposed to CeO_2 NPs prepared in a K-medium (0.032 M KCl, 0.051 M NaCl, Williams and Dusenbery, 1990) for assessment of 4 types of endpoints (stress-related gene expression, growth, reproduction, and mortality). For homogeneous dispersion of NPs, CeO_2 containing K-media was pretreated with 20 min of sonification prior to introduction of *C. elegans*. Three replicates for each treatment and a control were conducted for all the test types.

3. RNA extraction and semi-quantitative reverse transcription-polymerase chain reaction

Total RNA was prepared by phenol-chloroform extraction, according to the manufacturer's standard protocol. RNA concentrations were determined by the absorbance at 260 nm. The two-step reverse transcription-polymerase chain reaction (RT-PCR) method was used with RT Premix (Bioneer Co., Seoul, Korea) and PCR Premix kits (Bioneer Co.), using a PTC-100 thermal cycler (MJ Research, Lincoln, MA, USA). The primers were designed on the basis of the sequences retrieved from the *C. elegans* database (www.wormbase.org). Actin mRNA was used for expression-level normalization of the studied genes. The PCR products were separated through electrophoresis on 1.5% agarose gel (Promega, Madison, WI, USA) and were visualized with ethidium bromide (Bioneer Co.). All the tests were replicated at least three times, and the relative densities of each

band were determined with the use of a Kodak EDAS 290 image analyzer (Kodak, Rochester, NY, USA), with a TFX-20.M UV transilluminator (Vilber Lourmat, Marne la Vallee, France).

4. Growth reproduction and mortality

Following the 24 h incubation with exposure to the test solution of each nanoparticle size, growth reproduction and mortality were assessed. Growth was assessed by measuring the length of the worms that had been killed by the heat through microscopy, with a scaled lens in each sample. The average length of the unexposed control worm was in the range of 1.0 ~1.2 mm. Reproduction was assessed by counting the eggs of each worm through the microscopic inspection of the transparent *C. elegans* body in each sample. Although this procedure differs from more commonly used reproduction tests of offspring counting from an age-synchronized single worm, this simple detection method seems appropriate for the rapid screening of the reproduction effect (Roh *et al*., 2006). The average number of eggs per worm in the unexposed controls was in the range of 10 ~ 25. One hundred worms were examined per treatment for growth and reproduction experiments. Mortality was assessed by counting the numbers of live and dead worms, which were determined through visual inspection by probing the worms with a platinum wire under a dissecting microscope.

5. Data analysis

The statistical differences between the control and treated worms were determined with the aid of the parametric *t* test.

RESULTS AND DISCUSSION

1. Stress-related gene expression analysis

Recently, Gene expression as environmental stress response has been increasingly used in modern ecotoxicolgy, as it offers high sensitivity and mechanistic values for diagnose environmental contamination (Snell *et al*., 2003; Lee *et al*., 2006; Roh *et al*., 2006, 2007; Poynton *et al*., 2007). However, relating such a molecular level response to ecological effects represents a substantial challenge that can only be met by a large scale investigation with an appropriate group of organisms at all scales (molecular, individual organism and population level). *C. elegans* is an attractive animal model for the study of the ecotoxicologi-

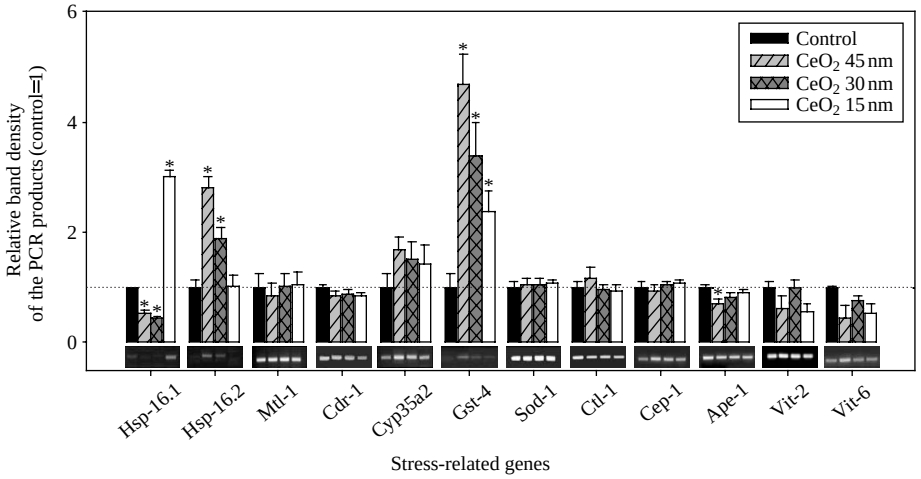

Fig. 1. Stress response gene expression in CeO_2 NPs exposed *C. elegans* for 24 h (control=1, number=3; mean±stansdard error of the mean; *$p < 0.05$).

cal relevance of chemical-induced molecular-level responses (Menzel *et al*., 2005; Reichert and Menzel, 2005). In this study, stress-related gene expression was investigated as toxicity of CeO_2 NPs in *C. elegans*. Stress-related gene expression profiling analysis was conducted on the selected genes, based on our previous studies (Roh *et al*., 2006, 2007). We investigated alteration on the gene expression of heat shock proteins (*hsp-16.1*, *hsp-16.2*), metal response proteins (*mtl-1*, *cdr-1*), xenobiotic metabolism enzymes (*cyp35a2*, *gst-4*), antioxidant enzymes (*sod-1*, *ctl-2*), tumor suppressor and apoptosis proteins (*cep-1*, *ape-1*) and vitellogenins (*vit-2*, *vit-6*) in CeO_2 exposed *C. elegans* (Fig. 1). An increase in the expression of *hsp16-1*, *hsp16-2*, and *gst-4* was observed. Increase of *cyp35a2* gene was also observed, but with much lesser degree. Although some minor fluctuation was observed, expression of other genes was not significantly changed by CeO_2 NPs exposure. It seems that the expression of *hsp* was increased also by CeO_2 NPs, which was expected since this protein is known to be induced by various environmental stimuli. The phase II enzyme, *gst* seems to play important role in CeO_2 toxicity in *C. elegans*, as the expression of this gene was the most pronounced. However, our tested gene sets are rather limited to fully understand transcriptional level toxicity by CeO_2 exposure in *C. elegans*, response was assessed only from 12 genes with 24 h exposure. If the more genes with longer exposure period had been tested, this could probably be better evaluated and explained. In this regard, in order to investigate unbiased and genome-wide response on CeO_2 induced gene expression, microarray experiment may be needed, which is ongoing project of our laboratory.

2. Growth and reproduction

As growth indicator, the changes in the worms' body lengths after CeO_2 NPs exposure are presented in Fig. 2A. No significant effect of CeO_2 NPs exposure was observed on the body length. As far as reproduction indicator, we counted the number of eggs per worm (Fig. 2B). Significant decrease in egg number was observed in CeO_2 treated worms compared to control. Reduced reproduction capacity may be explained as a part of defense and/or compensatory mechanism to metabolite and/or detoxify the toxicity induced by CeO_2 NPs.

In this study potential harmful effect of CeO_2 NPs was investigated in *C. elegnas*. Overall result suggest NPs, CeO_2 may provoke toxicity in *C. elegans* espe-

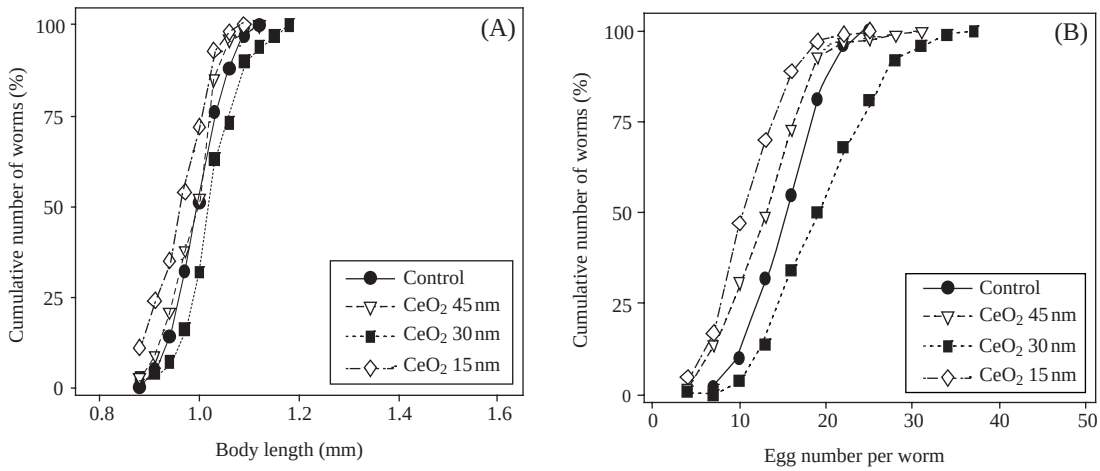

Fig. 2. Growth (A) and reproduction (B) indicators examined in the young adult of *C. elegans* exposed to CeO_2 nanoparticles for 24 h.

cially on gene expression, growth and reproduction. This is particularly valuable in the biomarker research on ecotoxicology, as ecological relevance is a crucial criterion of the applicability of the biomarker in field biomonitoring and ecological risk assessment.

REFERENCES

Bettinger JC, Carnell L, Davies AG and McIntire SL. The use of *Caenorhabditis elegans* in molecular neuropharmacology, Int Rev Neurobiol 2004; 62: 195-212.

Boyd WA and Williams PL. Availability of metals to the nematode *Caenorhabditis elegans*: toxicity based on total concentrations in soil and extracted fractions, Environ Toxicol Chem 2003; 22: 1100-1106.

Brenner S. The genetics of *Caenorhabditis elegans*, Genetics 1974; 77(1): 71-94.

Corma A. Atienzar P, Garcia H and Chane-Ching JY. Hierarchically mesostructured doped CeO_2 with potential for solar-cell use, Nat Mater 2004; 3: 394-397.

Hund-Rinke K and Simon M. Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids, En-viron Sci Pollut Res Int 2006; 13: 225-232.

Leacock SW and Reinke V. Expression profiling of MAP kinase-mediated meiotic progression in *Caenorhabditis elegans*, PLoS Genet 2006; 2: e174.

Lee SB and Choi J. Multilevel evaluation of nonylphenol toxicity in fourth-instar larvae of *Chironomus riparius* (*Diptera, Chironomidae*), Environ Toxicol Chem 2006; 25: 3006-3014.

Lovern SB and Klaper R. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles, Environ Toxicol Chem 2006; 25: 1132-1137.

Lovern SB, Strickler JR and Klaper R. Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC70Hx), Environ Sci Technol 2007; 41: 4465-4470.

Menzel R, Stürzenbaum S, Bärenwaldt A, Kulas J and Steinberg CE. Humic material induces behavioral and global transcriptional responses in the nematode *Caenorhabditis elegans*, Environ Sci Technol 2005; 39: 8324-8332.

Nohynek GJ, Lademann J, Ribaud C and Roberts MS. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety, Crit Rev Toxicol 2007; 37: 257-277.

Park EJ, Choi J, Park YK and Park K. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells, Toxicology 2008; 12: 90-100.

Poynton HC, Varshavsky JR, Chang B, Cavigiolio G, Chan S, Holman PS, Loguinov AV, Bauer DJ, Komachi K, Theil EC, Perkins EJ, Hughes O and Vulpe CD. *Daphnia magna* ecotoxicogenomics provides mechanistic insights into metal toxicity, Environ Sci Technol 2007; 41: 1044-1050.

Peredney CL and Williams PL. Utility of Caenorhabditis elegans for assessing heavy metal contamination in artificial soil, Arch Environ Contam Toxicol 2000; 39: 113-118

Reichert K and Menzel R. Expression profiling of five different xenobiotics using a *Caenorhabditis elegans* whole genome microarray, Chemosphere 2005; 61: 229-237.

Rogueda PG and Traini D. The nanoscale in pulmonary delivery. Part 1: deposition, fate, toxicology and effects, Expert Opin Drug Deliv 2007; 4: 595-606.

Roh JY, Jung IH, Lee JY and Choi J. Toxic effects of di (2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode *Caenorhabditis elegans*, Toxicology 2007; 237: 126-133.

Roh JY, Lee J and Choi J. Assessment of stress-related gene expression in the heavy metal-exposed nematode Caenorhabditis elegans: a potential biomarker for metal-induced toxicity monitoring and environmental risk assessment. Environ, Toxicol Chem 2006; 25: 2946-2956.

Schafer WF. Genetics of egg-laying in worms, Annu Rev Genet 2006; 40: 487-509.

Schroeder FC. Small molecule signaling in *Caenorhabditis elegans*, ACS Chem Biol 2006; 1: 198-200.

Smith CJ, Shaw BJ and Handy RD. Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects, Aquat Toxicol 2007; 82: 94-109.

Snell TW, Brogdon SE and Morgan MB. Gene expression profiling in ecotoxicology, Ecotoxicology 2003; 12: 475-483.

Warheit DB, Borm PJ, Hennes C and Lademann J. Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop, Inhal Toxicol 2007; 19: 631-643.

Williams PL and Dusenbery DB. Aquatic toxicity testing using the nematode *Caenorhabditis elegans*, Environ Toxicol Chem 1990; 9: 1285-1290

Yu JC, Yu J, Ho W and Zhang L. Preparation of highly photocatalytic active nano-sized TiO_2 particles via ultrasonic irradiation, Chem Commun (Camb) 2001; 19: 1942-1943.