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Long-term exposure to thiolated graphene oxide in the range of μg/L induces toxicity in nematode *Caenorhabditis elegans*

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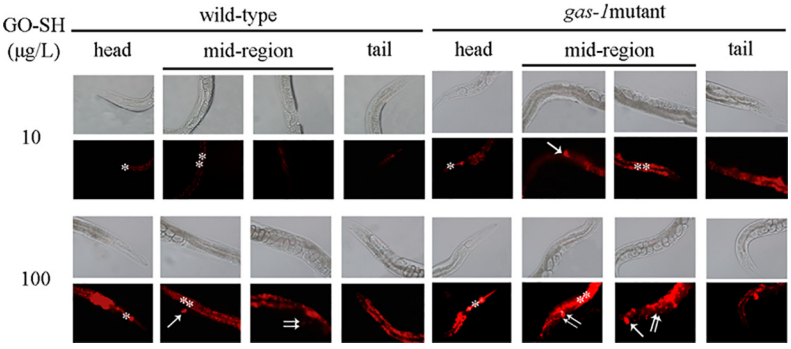
HIGHLIGHTS

- Prolonged exposure to GO-SH (≥100 μg/L) resulted in toxicity in wild-type nematodes.
- Exposure to GO-SH (100 μg/L) decreased *gas-1* expression in wild-type nematodes.
- Mutation of *gas-1* caused the GO-SH toxicity at concentration > 10 μg/L.
- Our data highlight GO-SH toxicity in the range of μg/L after long-term exposure.

GRAPHICAL ABSTRACT

Our results highlight the potential adverse effects of GO-SH on environmental organisms after long-term exposure.



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ABSTRACT

The *in vivo* toxicity and translocation of thiolated graphene oxide (GO-SH) are still largely unclear. We hypothesized that long-term exposure to GO-SH may cause the adverse effects on environmental organisms. We here employed *in vivo* assay system of *Caenorhabditis elegans* to investigate the possible toxicity and translocation of GO-SH after long-term exposure. In wild-type nematodes, we observed that prolonged exposure to GO-SH at concentrations > 100 μg/L resulted in the toxicity on functions of both primary targeted organs such as the intestine and secondary targeted organs such as the neurons and the reproductive organs. The severe accumulation of GO-SH was further detected in the body of wild-type nematodes. The translocation of GO-SH into secondary targeted organs such as reproductive organs through intestinal barrier might be associated with the enhancement in intestinal permeability in GO-SH exposed wild-type nematodes. Prolonged exposure to GO-SH (100 μg/L) decreased the expression of *gas-1* encoding a subunit of mitochondrial complex I, and mutation of *gas-1* caused the formation of GO-SH toxicity at concentration > 10 μg/L and more severe accumulation of GO-SH in the body of animals. Therefore, our results confirm the possibility for prolonged exposure to GO-SH in inducing adverse effects on nematodes. Our data highlight the potential adverse effects of GO-SH in the range of μg/L on environmental organisms after long-term exposure.

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1. Introduction

Graphene and its derivatives have a two-dimensional (2-D) carbon nanostructure. Graphene family has the unique physical and chemical properties, such as large surface area, attractive thermal and mechanical properties, high electrical conductivity, and ease of chemical modification on the surface (Geim, 2009). It has been supposed that graphene engineered nanomaterials (ENMs) may overtake carbon nanotubes in the future commercial applications (Geim and Novoselov, 2007). Considering the fact that graphene oxide (GO) possess some functional groups to allow the thiolation, thiolated graphene oxide (GO-SH) can be generated as one of the important derivatives of graphene (de Sousa et al., 2016). GO-SH has been shown to be potentially applied in drug delivery, biosensor, perovskite solar cells, and 3-D heterostructural assembly (Debgupta and Pillai, 2013; Wang et al., 2015; Cao et al., 2015; de Sousa et al., 2016). Moreover, GO-SH or GO-SH-based nanocomposites exhibited highly efficient photocatalytic property for detection and removal of environmental toxicants (You et al., 2012; Fang et al., 2013; Li et al., 2014), which implies the potential release of GO-SH in the environment. However, so far, it is still largely unclear for the possible environmental toxicity of GO-SH on organisms.

Caenorhabditis elegans is an important invertebrate model animal with the typical properties of model animal, such as the small size, short life-cycle, short lifespan, transparent body, self-fertilization, and ease of culture (Brenner, 1974). It has been shown that *C. elegans* is very sensitive to environmental toxicants, including the ENMs (Leung et al., 2008; Yu et al., 2015; Wang, 2016; Chatterjee et al., 2017), suggesting the potential value of *C. elegans* in the toxicity assessment of environmental toxicants. *C. elegans* has been successfully used in toxicity assessment and toxicological study of different ENMs (Zhang et al., 2012; Chen et al., 2013; Shu et al., 2015; Shakoor et al., 2016; Zhao et al., 2016a; Zhuang et al., 2016; Zhi et al., 2016a). Moreover, *C. elegans* has well-described genetic and molecular background, which will be helpful for deeply elucidating the underlying mechanisms for the observed toxicity induced by certain environmental toxicant (Leung et al., 2008; Chatterjee et al., 2017). In nematodes, exposure to carbon-based ENMs could result in toxicity on the functions of both primary (such as the intestine) and secondary (such as the neuron and the reproductive organs) targeted organs (Zhang et al., 2012; Wu et al., 2013; Zhao et al., 2015a, 2015b, 2015c; Zhao et al., 2015b; Yang et al., 2016). Additionally, activation of oxidative stress, bioavailability, intestinal permeability, and defecation behavior may play important roles in the toxicity induction of carbon-based ENMs in nematodes (Zhang et al., 2012; Wu et al., 2013; Chatterjee et al., 2017).

We hypothesized that GO-SH may result in the adverse effects on environmental organisms after long-term exposure. The aim of this study is to investigate the possible toxicity and translocation of GO-SH after long-term exposure using *in vivo* assay system of *C. elegans*. Our results suggest that long-term exposure to GO-SH in the range of μg/L could cause the toxicity and the severe GO-SH accumulation in the body in nematodes. Prolonged exposure to GO-SH in the range of μg/L altered the expression of GAS-1, a subunit of mitochondrial complex I, and mutation of *gas-1* induced a susceptibility to GO-SH toxicity. Our data implies the potential of environmental toxicity of GO-SH in the range of μg/L on organisms.

2. Materials and methods

2.1. Characterization of GO-SH

GO-SH was purchased from the JCNANO Co. (Nanjing, China). GO-SH was characterized by atomic force microscopy (AFM, SPM-9600, Shimadzu, Japan), Fourier transform infrared spectroscopy (FTIR, Avatar 370, Thermo Nicolet, USA), size distribution, and zeta potential. Zeta potential was analyzed by dynamic light scattering (DLS) using a Nano Zetasizer (Malvern Instrument Ltd., Malvern, UK).

2.2. C. elegans strains and maintenance

Nematode strains used were wild-type N2, and mutant of *gas-1(fc21)*, which were from *Caenorhabditis* Genetics Center. Nematodes were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20 °C (Brenner, 1974), and lysed with a bleaching mixture (0.45 M NaOH, and 2% HOCl). The L1-larvae populations were prepared as described previously (Donkin and Williams, 1995).

2.3. Exposure and toxicity assessment

The stock solution of GO-SH (1 mg/mL) in K medium was sonicated for 30 min (40 kHz, 100 W). GO-SH at the working concentrations (0.01, 0.1, 1, and 10 mg/L) were prepared by diluting the stock solution with K medium. Prolonged exposures to GO-SH were performed from L1-larvae to adult day-1 (approximately 4.5 days) in the liquid in wells of 12-well sterile tissue culture plates at 20 °C in the presence of food (OP50).

The endpoint of intestinal reactive oxygen species (ROS) production was used to reflect the functional state of the primary targeted organ of intestine (Zhao et al., 2016b), and analyzed as described previously (Zhao et al., 2015c). Fifty nematodes were examined per treatment. The endpoint of locomotion behavior was used to reflect the functional state of motor neurons (Li et al., 2013). Head thrash and body bend were selected to evaluate the locomotion behavior, and analyzed under the dissecting microscope as described previously (Sun et al., 2015; Liu et al., 2015). Fifty nematodes were examined per treatment. The endpoint of brood size was used to reflect the functional state of reproductive organs (Ruan et al., 2012). The brood size was analyzed as described previously (Zhao et al., 2017; Wu et al., 2016). Thirty nematodes were used for each reproduction assay.

2.4. Nile red staining

Nile Red staining results were used to reflect the intestinal permeability (Zhao et al., 2015a). Nile Red staining was performed as described previously (Zhao et al., 2015a). Fifty nematodes were examined per treatment.

2.5. Analysis of triglyceride content

Lipid was extracted in nematodes as described previously (Bligh and Dyer, 1959). The triglyceride content was measured using an enzymatic kit (Wako Pure Chemical Ltd., Osaka, Japan). Ten replicates were performed.

2.6. Distribution and translocation of GO-SH

To investigate the *in vivo* distribution and translocation of GO-SH, Rhodamine B (Rho B) was loaded on GO-SH by mixing Rho B solution (1 mg/mL, 0.3 mL) with aqueous GO-SH suspension (0.1 mg/mL, 5 mL) as described (Zhi et al., 2016b). The unbound Rho B was removed by dialysis against the distilled water over 72 h. Nematodes were exposed to GO-SH/Rho B from L1-larvae to adult day-1, and then washed with three times of M9 buffer. After treatment, the nematodes were observed and analyzed under a laser scanning confocal microscope.

2.7. Reverse-transcription and quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA was isolated from nematodes using Trizol (Invitrogen, UK) according to the manufacturer's protocol. Purity and concentration of RNA were evaluated by a ratio of OD260/280 using a spectrophotometer. After cDNA synthesis, the relative expression levels of targeted