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Behavioral deficits and neural damage of *Caenorhabditis elegans* induced by three rare earth elements

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HIGHLIGHTS

- Neurotoxicity of three rare earth elements (REEs) was tested on *C. elegans*.
- 10–30 mg/L $NdCl_3$, $PrCl_3$ and $ScCl_3$ caused significant declines in motor behaviors.
- REEs resulted in loss of dentrite and soma in dopaminergic and GABAergic neurons.
- Three REEs induced down-expression of *dat-1::GFP* and *unc-47::GFP* in *C. elegans*.

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ABSTRACT

Rare earth elements (REEs) are widely used in industry, agriculture, medicine and daily life in recent years. However, environmental and health risks of REEs are still poorly understood. In this study, neurotoxicity of trichloride neodymium, praseodymium and scandium were evaluated using nematode *Caenorhabditis elegans* as the assay system. Median lethal concentrations (48 h) were 99.9, 157.2 and 106.4 mg/L for $NdCl_3$, $PrCl_3$ and $ScCl_3$, respectively. Sublethal dose (10–30 mg/L) of these trichloride salts significantly inhibited body length of nematodes. Three REEs resulted in significant declines in locomotor frequency of body bending, head thrashing and pharyngeal pumping. In addition, mean speed and wavelength of crawling movement were significantly reduced after chronic exposure. Using transgenic nematodes, we found $NdCl_3$, $PrCl_3$ and $ScCl_3$ resulted in loss of dendrite and soma of neurons, and induced down-expression of *dat-1::GFP* and *unc-47::GFP*. It indicates that REEs can lead to damage of dopaminergic and GABAergic neurons. Our data suggest that exposure to REEs may cause neurotoxicity of inducing behavioral deficits and neural damage. These findings provide useful information for understanding health risk of REE materials.

1. Introduction

Rare earth elements (REEs) are a set of seventeen chemical elements which tend to occur in the same ore deposits and exhibit similar chemical properties. Scandium (Sc), yttrium (Yb), lanthanum (La), neodymium (Nd) and praseodymium (Pr) are common REEs. In recent years, rare earth elements are widely used in various fields including agriculture, animal husbandry, electronics, energy, fuel additives and modern biomedicine (Du and Graedel, 2011; USEPA, 2012; Celik et al., 2015). China has the largest deposits of rare earth elements and supplies more than 90% REEs for the world. Large-scale exploitations of REE resources have resulted in substantial increases in the contamination levels in soil and water around mining areas (Miao et al., 2011). The REEs are regarded as emerging trace pollutants (Pagano et al., 2015a; USEPA, 2012). In addition, wide usage of REEs has gradually increased the pollution of the environment which in turn has caused an accumulation in organisms, therefore making it possible to enter the food chain and induce health risks (Kumar et al., 2011; Rim, 2016). Low levels of REEs were reported to accumulate in blood, brain and

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bone after entering into human body (Chen and Zhu, 2008; Aquino et al., 2009). Hence, the potential risks of REEs to the environment and human health have attracted much attention due to the extensive applications of REEs.

Despite the growing interest and the dominant role of REEs in technological progress, the information about environmental and health issues is relatively scant (Rim, 2016). There is also the current controversy regarding the health benefits *vs* toxic effects of REEs (Pagano et al., 2015b). The application of REEs as feed additives caused beneficial effects, including an increase in body weight, and milk or egg production in some poultry (He et al., 2001). However, increasing studies showed bioaccumulation of REEs and adverse effects on organisms (Rim, 2016). Populations in a heavy rare earth area showed a significant decline of liver function in terms of population-based study (Zhu et al., 2005). In addition, exposure to lanthanoids elicited oxidative injury of the lungs and kidneys in animals and humans (Zhao et al., 2013; Hong et al., 2015). REEs also influenced the oxidative stress, could cross the blood testosterone barrier, and induced big biomolecules tangling (Chen et al., 2015). La^{3+} had some biotoxicity effects on mitochondria (Wu et al., 2015); La^{3+} and Yb^{3+} delayed zebrafish embryo and larval development, decreased survival and hatching rates, and caused tail malformation (Cui et al., 2012). Comparatively, the potential effects of REEs on the nervous system were scarcely studied (Rim, 2016). Actually, REEs can cross the blood brain barrier and reach the brain; so neurotoxicity risks of REEs need to be elucidated *in vivo*.

In recent years, *Caenorhabditis elegans* has emerged as an important animal model for environmental and toxicological studies, basing on both the whole-animal level and the single-cell level. It has the advantage of the properties of a short life cycle, transparent body, ease of handling, and well-described genetic and molecular backgrounds (Leung et al., 2008; Wu et al., 2014; Li et al., 2016). Moreover, *C. elegans* has been shown to be very sensitive to environmental toxicants. In addition, *C. elegans* contains 302 neurons and its neuronal lineage is fully described; so it is advantageous as a model organism for neurotoxicology. *C. elegans* lacks a functional blood brain barrier. Once chemical molecules are taken up, they may quickly diffuse into the nervous system. So far, *C. elegans* has been considered as an important non-mammalian alternative model for toxicity assessment and elucidation of the underlying mechanisms (Xu et al., 2017).

The objective of this study is to investigate and compare the potential neurotoxicity of three rare earth elements, neodymium (Nd), praseodymium (Pr) and scandium (Sc). *C. elegans* were respectively exposed to trichloride salts, $NdCl_3$, $PrCl_3$ and $ScCl_3$, for 48 h in sub-lethal dosage. Behavior changes were assayed by testing the endpoints of body bends, head thrashes, pharyngeal pumping and crawling behavior. Using fluorescently labeled transgenic nematodes, the effects on dopaminergic neurons and GABAergic neurons were further investigated. Finally, the potential neurotoxicity of three rare earth elements were fully evaluated and compared.

2. Materials and methods

2.1. Chemicals

$NdCl_3$, $PrCl_3$ and $ScCl_3$ were purchased from Sigma Aldrich Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemicals used in this study were of analytical grade.

2.2. C. elegans strains and exposure

All strains of *C. elegans* were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA) and maintained in term of standard protocols as previously described (Brenner, 1974). Wild-type (Bristol, N2) and transgenic strains were used, including *BZ555* (*dat-1::GFP*; green fluorescent protein [GFP] visible in dopaminergic neurons) (Maduro and Pilgrim, 1996) and *EG1285* (*unc-47*:GFP + lin-15(+); GFP expressed in GABAergic neurons) (White et al., 1986). Nematodes were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli OP50* at 20 °C. According to the previous methods (Chen et al., 2014; Xu et al., 2017), adult animals were collected into tubes by washing plates with M9 buffer, and then dissolved with bleaching solution (0.45 M NaOH, 2% HClO) to collect eggs. The eggs were hatched on the plates with food, and then synchronized for experiments.

$NdCl_3$, $PrCl_3$ or $ScCl_3$ solutions were prepared in K-medium (32 mmol/L KCl, 51 mmol/L NaCl), and the control group was K-medium. *C. elegans* were exposed to a series of concentrations of $NdCl_3$, $PrCl_3$ or $ScCl_3$ in the 24-well plates. Each well contained 50–100 age-synchronized nematodes. Four replicates were used for each exposure group. Nematodes were exposed for 48–96 h from L1 stage.

2.3. Lethality assays

Synchronized nematodes were exposed to a series of concentrations (0, 10, 50, 75, 100, 200 mg/L) of $NdCl_3$, $PrCl_3$ or $ScCl_3$ from L1 stage in 24-well plates for 96 h. Each well contained about 30 nematodes with food. To prevent eggs from hatching, 0.33 μL of fluoro-29-deoxyuridine (150 mM) was added to each well. Dead nematodes were identified as unresponsive to a stimulation to their head with a metal needle under the observation by the dissecting microscope. Numbers of dead nematodes were recorded on the second and fourth day, respectively. Each experiment was run in quadruplicate. The median lethal concentrations (LC_{50}) of $NdCl_3$, $PrCl_3$ and $ScCl_3$ were determined by linear regression analysis with the Hill model of Graphpad Prism (Li et al., 2016; Xu et al., 2017).

2.4. Body length assay

According to the median lethal concentrations (LC_{50}), a series of sublethal concentrations of $NdCl_3$, $PrCl_3$ and $ScCl_3$ (0, 0.5, 1, 10, 30, 50 mg/L) were exposed to nematodes from L1 stage for 48 h. Then nematodes were washed three times with M9 buffer and transferred to a fresh NGM medium surface. Then body lengths of nematodes were measured by Motic microscope, and analyzed by Image Advanced. 8–10 worms were measured in each group. Experiments were run in quadruplicate.

2.5. Local movement

Head thrashing, body bending and pharyngeal pumping were used to evaluate locomotion behaviors of nematodes in term of previous methods (Li et al., 2016; Xu et al., 2017). After exposure, *C. elegans* was transferred to fresh (NGM) plates without food. After 1 min of recovery, head thrashes and body bending were counted for 1 min and 30 s, respectively. For the pharyngeal pumping assay, nematodes were transferred to another NGM plates with food and the number of pumps was counted in 30 s. In each group, 8–10 worms were assayed for local movements. Head thrashing was defined as a change in the direction of bending in the mid body. Body bending was defined as a change in the direction of the worm corresponding to the posterior bulb of the pharyngeal along the y axis, assuming the worm was travelling along the x axis. Experiments were run in quadruplicate.