Prolonged manganese exposure induces severe deficits in lifespan, development and reproduction possibly by altering oxidative stress response in *Caenorhabditis elegans*

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Abstract

We examined the possible multiple defects induced by acute and prolonged exposure to high levels of manganese (Mn) solution by monitoring the endpoints of lifespan, development, reproduction, and stress response. Our data suggest that acute exposure (6 h) to Mn did not cause severe defects of life span, development, and reproduction, similarly, no significant defect could be found in animals exposed to a low concentration of Mn (2.5 μmol/L) for 48 h. In contrast, prolonged exposure (48 h) to high Mn concentrations (75 and 200 μmol/L) resulted in significant defects of life span, development, and reproduction, as well as the increase of the percentage of population with *hsp-16.2::gfp* expression indicating the obvious induction of stress responses in exposed animals. Moreover, prolonged exposure (48 h) to high concentrations (75 and 200 μmol/L) of Mn decreased the expression levels of antioxidant genes of *sod-1*, *sod-2*, *sod-3*, and *sod-4* compared to control. Therefore, prolonged exposure to high concentrations of Mn will induce the severe defects of life span, development, and reproduction in nematodes possibly by affecting the stress response and expression of antioxidant genes in *Caenorhabditis elegans*.

Key words: manganese toxicity; prolonged exposure; stress response; antioxidant gene; *Caenorhabditis elegans*

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Introduction

Manganese (Mn), an abundant element, makes up about 0.1% of the earth's crust. As an essential element it is widely used in industries. Mn is needed for the regulation of reproduction and normal brain functions, and required for the activation of several enzymes, such as arginase and pyruvate carboxylase (Lin *et al.*, 2006). The ubiquitous Mn is normally found in low levels in water, air, soil, and food. The most Mn intake usually occur through diet, bypass the gut, and can enter different organs, such as brain. In addition, chronic exposure to high atmospheric levels of Mn is also toxic. The clinical features of Mn neurotoxicity, linked primarily to inhalation exposure, are almost totally based on high doses, such as those experienced by miners (Weiss, 2006). The people living close to the Mn mines and processing plants suffer from an incipient motor deficit, as a result of their inhaling Mn-rich dust in Mexico (Rodríguez-Agudelo *et al.*, 2006).

Mn exposure can cause cell death which may eventually due to the necrosis, even if Mn-treated cells have activated apoptosis signaling pathways (Roth and Garrick, 2003). Mn also interferes with mitochondrial function in the cellular energy production since it inactivates complex I of the electron transport chain, 4Fe-4S cluster containing aconitase and F1-ATPase (Zheng *et al.*, 1998). Overload of Mn causes a Parkinson-like syndrome or psychiatric damage. Moreover, although the features of Mn-induced Parkinsonism differ from those of idiopathic Parkinson diseases (PD), they overlap considerably (Weiss, 2006). Mergler *et al.* (1999) observed a persuasive influence of age on how Mn exposure is expressed by neurobehavioral deficits. The age may be an important variable that should be considered in assessing the toxicity of Mn (Dorman *et al.*, 2006). Compared to young animals, senescent (16 months old at the start of the exposure) rats showed significantly decreased striatal glutathione (GSH) levels with subchronic MnSO₄ inhalation and similar decrease in GSH concentrations in the cerebellum and hypothalamus with the exposure to hureaulite (Erikson *et al.*, 2004).

The toxicity from Mn exposure could be successfully assessed in soil invertebrates. Tests performed by Kuperman *et al.* (2004), including the earthworm cocoon production test, the enchytraeid reproduction test, and the

collembolan reproduction test, were conducted in sandy loam soil that supports a relatively high bioavailability of metals. *Caenorhabditis elegans*, a free-living soil nematode, is one of the best-characterized animals at the genetic, molecular, and developmental levels (Riddle *et al.*, 1988), and has been chosen as a bioindicator for toxicity tests because of the properties of short life cycle, ease of generating mass cultures and low cost (Hitchcock *et al.*, 1997; Mutwakil *et al.*, 1997; Wang and Yang, 2007). LC$_{50}$ was used by Donkin and Dusenbery (1993), which provides information regarding *C. elegans* survival in metal-contaminated soil. A more sensitive test using reproduction and growth as endpoints was developed by Trauspurgr *et al.* (1997) to assess sediment toxicity in samples from Elbe River and could detect 88% of polluted sites. Locomotion behavior and chemotaxis plasticity were monitored using a computer tracking system after metal exposure in nematodes (Boyd *et al.*, 2003; Anderson *et al.*, 2004; Wang *et al.*, 2007). The availability of recombinant technology can make it possible to improve the sensitivity of assays by transgenesis, and economic way with nematodes to assay heavy metals has been established by taking advantage of transgenic and fluorescent microscopy technique (Chu and Chow, 2002; Wang and Wang (2008). For the effects of Mn exposure, Lin *et al.* (2006) found that supplementation with MnSO$_4$ in the nematode growth medium (NGM) fed with *Escherichia coli* lawn on the surface did not negatively affect the lifespan, development, fertility, and thermotolerance at the assayed levels used in *C. elegans*, which is largely different from Mn toxicity observed in other organisms as mentioned above.

Therefore, in the present study, we selected the organism of *C. elegans* to analyze the possible multiple defects induced by prolonged exposure to high levels of Mn solutions by monitoring the endpoints of lifespan, development, reproduction (brood size and generation time), and stress response. The objective of this study is to determine the possible relationship between prolonged exposure to high Mn levels and Mn exposure-induced stress responses in *C. elegans*.

1 Materials and methods

1.1 Chemicals

Three different MnCl$_2$ concentrations 2.5, 75, and 200 μmol/L were used in the current work. The selected concentrations were referred in our previous study (Wang *et al.*, 2007). Metal concentrations of exposed solutions were analyzed by atomic absorption spectrophotometry (AAS; Pye-Unicam model SP9, Cambridge, UK). All the chemicals were obtained from Sigma-Aldrich (St. Louis, USA).

1.2 Strains

Wild-type N2 was originally obtained from the *Caenorhabditis* Genetics Center (CGC). Transgenic line KC136 was from the laboratory of Dr. King L. Chow. Strains were maintained on NGM plates seeded with *E.*

coli OP50 at 20°C as described by Brenner (1974). Gravid nematodes were washed off the plates into centrifuge tubes and were lysed with a bleaching mixture (0.45 mol/L NaOH, 2% HOCl). Age synchronous populations of larva (L4-stage) were obtained by the collection as described by Donkin and Williams (1995). The L4-larva stage nematodes were washed with double-distilled water twice, followed by washing with modified K medium once (50 mmol/L NaCl, 30 mmol/L KCl, 10 mmol/L NaOAc, pH 5.5) (Williams and Dusenbery, 1990). Exposures were performed in sterile culture plates. Approximately 100 animals were transferred in 100 μL to each exposure solution in micropipetter. All exposures were 6 or 48 h long, and were carried out at 20°C incubator in the presence of food. Endpoints of lifespan, body size, brood size, generation time, and stress response were used for toxicity testing in *C. elegans*.

1.3 Determination of lifespan

The method for determination of lifespan was performed as described by Wang and Wang (2008). Thirty control or exposed animals were transferred into NGM plates at 20°C, and the time was recorded as $t = 0$. Assayed animals were transferred every 2 d during the brood period. Animals were monitored daily throughout development and post maturity by tapping the nematode on the head with a hair. Animals were considered dead if no movement was observed following repeated probing. For life span, graphs are representative of at least three trials. The data was statistically analyzed using a 2-tailed 2 sample *t*-test (Minitab Ltd., Coventry, UK).

1.4 Body size

The method was performed as described by Wang and Wang (2008). We picked out approximately thirty control and exposed animals to new NGM plates. Body size was determined by measuring the flat surface area of nematodes along the axis of anterior-posterior using the Image-Pro® Express software (North Reading, MA, USA).

1.5 Brood size and generation time

The methods were performed as described previously (Wang and Wang, 2008). Brood size was assayed by placing single tested animals onto individual well of tissue culture plates. The P0 (parental) animals were transferred to a new plate every 1.5 d. Progeny were counted the day following transfer. The generation time refers to the time from P0 egg to the first F1 (final) egg. For both the brood size and the generation time test, at least 20 replicates were performed for statistical purposes.

1.6 Analysis of transgenic strain

The method was performed as described early (Wang and Wang, 2008; Wang *et al.*, 2007). If the metal exposure was toxic, it would affect animals through environmental stress and thus result in a stress response (Chu and Chow, 2002). To analyze the changes of *hsp-16.2* expression patterns, the treated KC136 animals were allowed to settle for 10 min, and then pipetted onto an agar pad on a

glass slide, mounted and observed for the fluorescent signals with a fluorescent microscope. Observations of the green fluorescent protein (GFP) were recorded and color images were taken for the documentation of results with Magnafire® software (Olympus, Irving, USA). More than 50 animals were counted for the statistical analysis.

1.7 Analysis of expression of antioxidant genes to oxidative stress

Poly(A)$^+$ RNA of nematodes was prepared and analyzed as described previously (Yanase and Ishii, 1999). A total of 1 μg of poly(A)$^+$ RNA per lane was separated by electrophoresis in a 1.2% agarose gel containing 2 mol/L formaldehyde and 0.02 mol/L MOPS, and transferred onto a nylon membrane by a mild alkaline transfer method. Northern hybridizations of transferred poly(A)$^+$ RNA were performed using PCR product probes labeled with ^{32}P-dCTP at 65°C overnight. The Northern blot analysis of mRNAs for some antioxidant genes was carried out in nematodes under concentrated oxygen as described by Yanase *et al.* (2002). The investigated genes were *sod-1* (encoding a copper/zinc-superoxide dismutase, Cu/Zn-SOD), *sod-2* (encoding a manganese-superoxide dismutase, Mn-SOD), *sod-3* (encoding a Mn-SOD), and *sod-4* (encoding an extracellular Cu/Zn-SOD).

1.8 Statistical analysis

All data in this article were expressed as means ± SD. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA). One-way analysis of variance (ANOVA) followed by a Dunnett's *t*-test was used to determine the significance of the differences between the groups. The probability levels of 0.05 and 0.01 were considered statistically significant.

2 Results

2.1 Effect of prolonged exposure on life spans

Life span is an important endpoint to assess heavy metal toxicity in *C. elegans*. Here, maximum and median life spans were first explored to evaluate the toxicity of Mn

exposure. As shown in Fig. 1a, compared to control (22.4 ± 0.9 d), acute exposure to Mn at different concentrations (2.5 μmol/L (22.1 ± 1.4 d), 75 μmol/L (21.5 ± 1.5 d), and 200 μmol/L (20.9 ± 1.7 d)) for 6 h did not cause severe defects of maximum life spans. Similarly, no significant median life spans were observed in animals exposed to different assayed concentrations of Mn for 6 h compared to control (Fig. 1b). In contrast, prolonged exposure to Mn at concentrations of 75 μmol/L (18.5 ± 1.6 d) and 200 μmol/L (16.2 ± 2.1 d) for 48 h significantly shortened the maximum life spans of nematodes (Fig. 1a). The median life spans of animals exposed to Mn for 48 h at concentrations of 75 μmol/L ($P < 0.05$) and 200 μmol/L ($P < 0.01$) were also significantly reduced (Fig. 1b), compared to control (0 μmol/L). The prolonged exposure to Mn at a low concentration of 2.5 μmol/L would not affect the maximum and median life spans (Fig. 1). Therefore, prolonged exposure, together with high concentrations, will induce the shortened life spans in Mn exposed nematodes.

2.2 Effect of prolonged exposure on developmental morphology and body sizes

The possible toxic effects of prolonged exposure to Mn on development were examined by observing the morphology and body sizes. Acute exposure to Mn at different assayed concentrations for 6 h did not affect the developmental morphology of exposed animals (data not shown). No abnormal developmental morphology could be also observed in the animals exposed to Mn at concentrations of 2.5 and 75 μmol/L for 48 h (data not shown), whereas prolonged exposure to Mn resulted in abnormal morphology in animals exposed to 200 μmol/L of Mn for 48 h. At least three types of abnormal morphologies could be observed. Usually, compared to vulva with slightly smooth surface in control animals, abnormal vulva with the formation of large or huge protuberance could be observed in Mn exposed animals at the ratio of 4%–5% (Fig. 2a, type I). In addition, we noticed that prolonged exposure to high concentration of Mn could occasionally induce the appearance of dumpy body (Fig. 2a, type II) or abnormality with a cutaway-like tail (Fig. 2a, type III). Therefore,
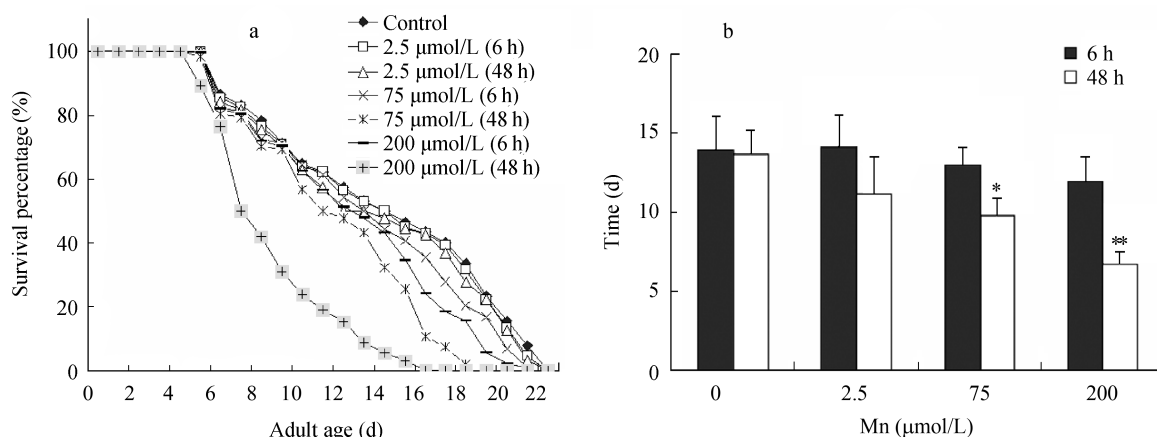

Fig. 1 Maximum (a) and median (b) life spans of control and manganese-treated animals for 6 and 48 h, respectively, in *C. elegans*. In Fig. 1b, bars represent means ± S.D. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control.

Mn might still be able to be considered as teratogenic in nematodes.

Acute exposure to Mn for 6 h would not induce severe body size defects at all assayed concentrations; however, for 48 h, there are significant body size defects at concentrations of 75 μmol/L ($P < 0.05$) and 200 μmol/L ($P < 0.01$) (Fig. 2b). Those phenomena suggested that the prolonged exposure will cause the significantly reduced body sizes in animals exposed to high concentrations of Mn in *C. elegans*.

2.3 Effect of prolonged exposure on reproduction

Endpoints of brood size and generation time were selected to assess the reproduction in nematodes. Brood size reflects the reproductive capacity; generation time reflects the reproductive speed (Wang and Yang, 2007). Acute exposure to Mn at different concentrations for 6 h did not induce severe defects of brood size and generation time (Fig. 3). Similarly, prolonged exposure to 2.5 μmol/L of Mn for 48 h could not significantly affect the endpoints of brood size and generation time (Fig. 3). However, prolonged exposure to high concentrations of Mn for 48 h significantly decreased the brood sizes by approximately 73.3% at 75 μmol/L ($P < 0.05$) and by approximately 41.3% at 200 μmol/L ($P < 0.01$) (Fig. 3a), and increased the generation time by approximately 25% at 75 μmol/L ($P < 0.05$) and by approximately 67% at 200 μmol/L ($P < 0.01$) (Fig. 3b), compared to control. Therefore, prolonged exposure will cause the significantly reduced brood sizes and increased generation times in animals exposed to high concentrations of Mn in *C. elegans*.

2.4 Effect of prolonged exposure on stress responses

We further investigated the stress responses by observing the induction of percentage of population with *hsp-16.2::gfp* expression in transgenic reporter to obtain a rapid perception of the environmental stress. The *gfp* production in KC136 strain could be induced rapidly and conveniently monitored by fluorescent microscopy. For KC136 strain, only metal concentrations that could induce 50% of the transgenic animals to display strong *gfp* signal were considered as having positive effects (Chu and Chow, 2002) (Fig. 4, above the line). Acute exposure to Mn at all assayed concentrations for 6 h could not activate a significant induction of percentage of population with *hsp-16.2::gfp* expression in transgenic KC136 strain compared to control, whereas prolonged exposure to Mn at high concentrations of 75 μmol/L ($P < 0.05$) and 200 μmol/L ($P < 0.01$) resulted in a significant induction (Fig. 4). Therefore, prolonged exposure can activate a significant induction of stress responses in animals exposed to high concentrations of Mn in *C. elegans*.
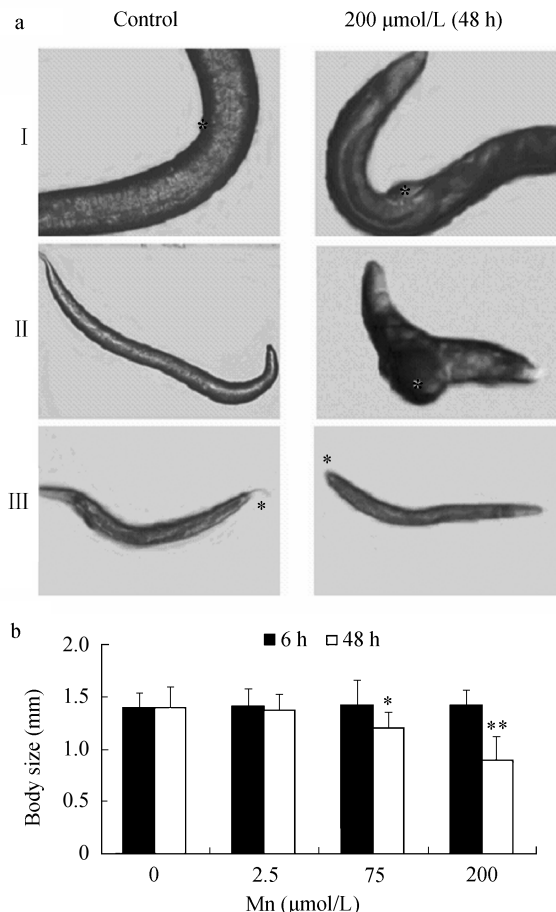

Fig. 2 Effects of acute (6 h) and prolonged (48 h) Mn exposure on animals' morphology (a) and body size (b) in *C. elegans*. Prolonged Mn exposure could cause three lines of abnormal developmental phenotypes. Phenotype I, compared to control, Mn exposure induced an obvious apophysis at the vulva position; phenotype II, compared to control, Mn-exposed animal had a dumpy body and a huge apophysis at the vulva position; phenotype III, compared to control with a acidotus tail, Mn exposure resulted in the appearance of a cutaway-like tail in exposed animals. Asterisks in (I) and (II) indicate the position of vulva, and asterisks in (III) indicate the position of tail. All images were representative of three-day post hatch worms. Bars represent means ± S.D. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control.
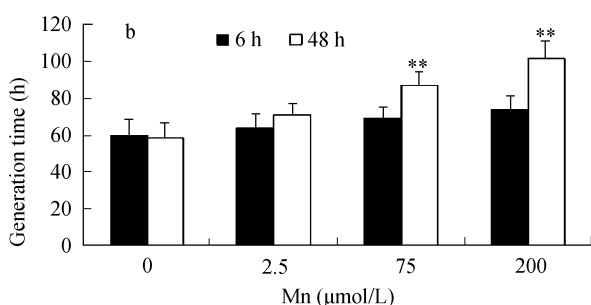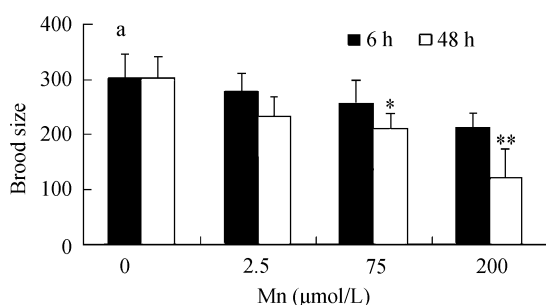

Fig. 3 Brood sizes (a) and generation time (b) of control and manganese-treated animals for 6 and 48 h, respectively, in *C. elegans*. Bars represent means ± S.D. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control.
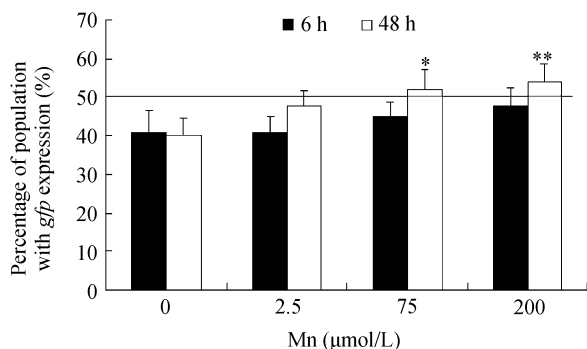
Fig. 4 Significant induction of *hsp-16.2::gfp* expression (50% of a population, above the line) was observed in wild-type N2 animals exposed to high concentrations (75 and 200 μmol/L) of Mn for 48 h. The control gave a signal beyond the detection limit of photography. Bars represent means ± S.D. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control.

2.5 Expression of antioxidant genes under the stress of acute and prolonged Mn exposure

We finally investigated the expression pattern of antioxidant genes in nematodes exposed to Mn by Northern blotting assay. The mRNA levels of four antioxidant genes (*sod-1* through *sod-4*) known to be important in combating oxidative stress were measured. As shown in Fig. 5, no obvious alteration of *sod-1*, *sod-2*, *sod-3*, and *sod-4* expression could be observed in 200 μmol/L Mn exposed nematodes for 6 h, as well as in 2.5 and 75 μmol/L Mn exposed nematodes for 6 h (data not shown), compared to control. Similarly, exposure to 2.5 μmol/L of Mn for 48 h also would not noticeably affect the expression levels of antioxidant genes of *sod-1*, *sod-2*, *sod-3*, and *sod-4* compared to control. However, exposure to 75 and 200 μmol/L of Mn for 48 h could significantly ($P < 0.01$) decreased the transcript levels of antioxidant genes of *sod-1*, *sod-2*, *sod-3*, and *sod-4* compared to control, indicating that prolonged exposure to Mn would obviously alter the expression patterns of antioxidant genes in *C. elegans*. Therefore, our results suggest that the altered life span, development, and reproduction in prolonged Mn exposed nematodes are possibly the result from changed responses to oxidative stress.
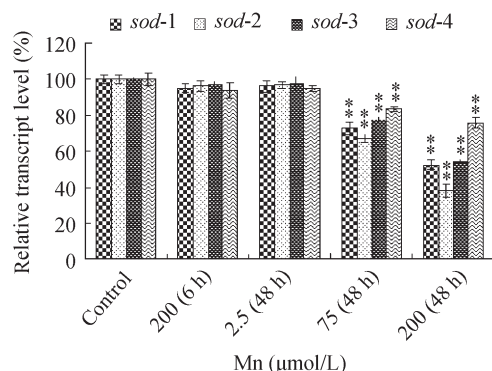

Fig. 5 Response of antioxidant genes to acute and prolonged manganese exposure. The mRNA levels of four antioxidant genes (*sod-1* through *sod-4*) were examined. Bars represent means ± S.D. ** $P < 0.01$ vs. control.

3 Discussion

Mn is both an essential nutrient and a potent toxicant, which make it present a special conumdrum for risk assessment. In the current work, we examined the effects of acute (6 h) and prolonged (48 h) exposure to Mn on the life span, development, and reproduction with the aid of endpoints of maximum life span, median life span, developmental morphology, body size, brood, and generation time. It was shown that both acute exposure (6 h) and prolonged exposure at a low Mn concentration (2.5 μmol/L, 48 h) did not result in defects of life span, development, and reproduction. In contrast, prolonged exposure to high concentrations of Mn (75 and 200 μmol/L) induced significant defects of life span, development, and reproduction. Thereby, it can be concluded that prolonged exposure, together with the high concentrations, will induce the shortened life span, abnormal development, reduced body size, decreased brood size, and increased generation time, compared to control. In addition, the Mn toxicity can cause multi-biological defects with a concentration-dependent manner in *C. elegans*.

Moreover, the observations on the induction of significant percentage of population with *hsp-16.2::gfp* expression further support our conclusions. The *hsp-16.2* gene expression represents a rapid read-out sensitive to sub-lethal doses of stress caused by environmental insults (Chu and Chow, 2002). Only prolonged exposure to 75 μmol/L ($P < 0.05$) and 200 μmol/L ($P < 0.01$) of Mn could activate significant increase of the percentage of population with *hsp-16.2::gfp* expression. The expression patterns of *hsp-16.2::gfp* in Mn exposed animals suggest that acute exposure for 6 h at concentrations lower than 200 μmol/L would not induce obvious stress responses, whereas prolonged exposure for 48 h at concentrations more than 75 μmol/L would activate remarkable stress responses in *C. elegans*.

Some studies have claimed that Mn exposure could cause Parkinson's disease–like syndrome, DNA fragmentation, and interferes with cellular energy production (Zheng *et al.*, 1998; Roth and Garrick, 2003; Weiss, 2006), whereas there have been other reports that Mn acts as a radical scavenger in unicellular organisms. For example, Lin *et al.* (2006) indicated that Mn supplemented to the growth medium did not appear to be toxic for the development, fertility, life span, and thermotolerance. Our data suggest that Mn may have dual roles in affecting exposed animals' development. When a low level of accumulation and/or bioavailability is formed in Mn exposed animals, we suppose that Mn can serve as a protection factor for exposed animals. The protection effect for lifespan could be observed in *mev-1* mutants exposed to Mn (MnCl$_2$) at concentrations of 75 and 200 μmol/L for 6 h (data not shown), suggesting that the protection effect from Mn exposure can be enlarged in animals subjecting to severe stresses. However, when a high level of accumulation and/or bioavailability is formed in Mn exposed animals, Mn may have toxic effects on exposed animals' development. Prolonged exposure (48 h) to 200 μmol/L of

Mn would sharply increase the Mn accumulation and/or bioavailability compared to that in animals exposed to 200 µmol/L of Mn for 6 h (data not shown). At the same time, the severe stress response as indicated by the induction of significant percentage of population with *hsp-16.2::gfp* expression was observed in animals exposed to 200 µmol/L of Mn for 48 h compared to control and that for 6 h (Fig. 4). Moreover, we could not observe again the protection effects on lifespan in animals subjecting to prolonged Mn exposure (data not shown).

The protection effects of Mn supplement on the development, fertility, life span, and thermotolerance as indicated by Lin *et al*. (2006) were investigated on the NGM medium fed with OP50 *E. coli* strain. In the current work, exposures were performed in sterile culture plates, and approximately 100 animals were transferred to each exposure solution in the micropipetter. The reason is that both the presence of salts and a food source have been found to influence the results of toxicity tests using other invertebrates (Sprague, 1985). Donkin and Williams (1995) reported that the most important environmental variable in determining metal toxicity was the medium in which the tests were conducted, since the supplement of potassium and sodium salts in medium could significantly ($P < 0.05$) decrease the metal toxicity. Donkin and Williams (1995) suggest that a low ionic concentration in the test medium may be desired to increase sensitivity and more closely mimic conditions that nematodes is likely to encounter in nature. Moreover, although addition of 5-fluoro-2-deoxyuridine (FUDR) alone does not alter wild type life span (Mitchell *et al*., 1979), it is interesting to note that, in the presence of FUDR, Mn is beneficial to the wild-type reproduction at concentrations greater than 0.1 mmol/L and also to *mev-1* at concentrations greater than 0.1 mmol/L, suggesting that FUDR may possibly interfere with Mn uptake, accumulation, bioavailability, and/or localization (Lin *et al*., 2006).

Furthermore, we provide evidence to indicate that the altered stress response by prolonged exposure to Mn may be mainly oxidative stress response. The expression levels of *sod-1*, *sod-2*, *sod-3*, and *sod-4* were significantly ($P < 0.01$) decreased by Mn exposure at concentrations of 75 and 200 µmol/L for 48 h, compared to those in control and Mn exposed nematodes at different concentrations (2.5, 75, and 200 µmol/L) for 6 h, as well as in Mn exposed nematodes at a concentration of 2.5 µmol/L for 48 h (Fig. 5). Because the response of antioxidant genes can largely reflect the oxidative stress in *C. elegans* (Yanase *et al*., 2002), the observed severe defects of life span, development, and reproduction in prolonged Mn exposed nematodes are possibly the main result from altered responses to oxidative stress.

The toxic effects from the overload of Mn on development and reproduction can also be observed in other organisms or investigations. Reinecke and Reinecke (1996, 1997) reported a reduction in the growth and development of *Eisenia fetida* fed cattle manure spiked with Mn at 152 mg/kg and the damage to spermatozoan structure from treatment containing food spiked with 62 mg/kg Mn. Definitive toxicity tests conducted with Mn-amended

weathered/aged natural soil showed that the order of chemical toxicity based on EC_{20} (the effect concentration at which 20% reduction occurs) values for reproductive endpoints was *Enchytraeus crypticus* > *Eisenia fetida* > *Folsomia candida*, and reproductive endpoints in the earthworm and enchytraeid tests were most sensitive than adult survival (Kuperman *et al*., 2004). Our data on Mn toxicity were largely agreed to these observations. In addition, the endpoints of lifespan, body size, generation time, brood size, and expression of *hsp-16.2::gfp* will be useful for the assessment of Mn chronic toxicity at high concentrations in *C. elegans*.

In addition, for the Mn toxicity induced by prolonged exposure, the developmental phenotypes were very specific. Prolonged Mn exposure specially caused the appearance of abnormal vulva, dumpy body and animals with a cutaway-like tail. Among these phenotypes, the appearance of dumpy body and cutaway-like tail has never been observed in nematodes exposed to other metals (Wang *et al*., 2007; Wang and Wang, 2008; Wang and Yang, 2007). Therefore, these abnormal developmental morphologies will be valuable to be explored as parameters to identify the specific toxicity from Mn exposure in *C. elegans*.

4 Conclusions

In summary, our data suggest that prolonged exposure to 75 µmol/L ($P < 0.05$) and 200 µmol/L ($P < 0.01$) of Mn for 48 h will induce the severe defects in life span, development, and reproduction in nematodes possibly by affecting the oxidative stress response and metal accumulation and/or bioavailability. In contrast, acute exposure to Mn for 6 h may not significantly influence the accumulation and/or bioavailability, and can not cause severe defects in life span, development, and reproduction.

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