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Inhibition of ROS elevation and damage to mitochondrial function prevents lead-induced neurotoxic effects on structures and functions of AFD neurons in *Caenorhabditis elegans*

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Abstract

Here we investigated the possible roles of oxidative stress in the formation of decreased thermotaxis to cultivation temperature in lead (Pb)-exposed nematodes *Caenorhabditis elagans*. Exposure to Pb at the examined concentrations decreased thermotaxis behaviors, and induced severe deficits in the structural properties of AFD sensory neurons. Meanwhile, Pb exposure caused the induction of severe oxidative damage, reactive oxygen species (ROS) production, and mitochondrial dysfunction in young adults. Moreover, pre-treatment with the antioxidants dimethyl sulfoxide (DMSO), ascorbate and *N*-acetyl-L-cysteine (NAC), used to inhibit both the ROS elevation and the mitochondrial dysfunction caused by Pb exposure, at the L2-larval stage prevented the induction of oxidative damage and the formation of severe deficits in thermotaxis and structural properties of AFD sensory neurons in Pb-exposed young adults. Therefore, the formation of oxidative stress caused by Pb exposure may be due to both the induction of ROS elevation and damage to mitochondrial function, and oxidative stress may play a key role in inducing the neurotoxic effects on the structures and function of AFD sensory neurons in Pb-exposed nematodes.

Key words: thermotaxis; lead exposure; oxidative stress; AFD sensory neuron; *Caenorhabditis elegans*
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Introduction

Lead (Pb) is a ubiquitous environmental contaminant associated with developmental, cognitive, and motor deficits in humans and animals (Lim et al., 2005). Previous studies have revealed that Pb exposure results in severe deficits in perception behaviors (Wu et al., 2000; Chuang et al., 2000; Lim et al., 2005). For example, significant effects caused by Pb exposure were observed in 2-odor olfactory discrimination acquisition and olfactory-based reversal learning tasks (Lim et al., 2005).

The free-living nematode *Caenorhabditis elegans*, a model animal well-characterized at the genetic, physiological, molecular, and developmental levels (Riddle et al., 1988), has been explored as an excellent candidate for studying neurotoxicology, since it has only 302 neurons and the complete wiring diagram for chemical and electrical connections is available (White et al., 1986; Leung et al., 2008; Du and Wang, 2009). Deficits in movement behavior can be observed using a computer tracking system after exposure to metals or organophosphorus insecticides (Dhawan et al., 2000; Anderson et al., 2001, 2004; Boyd et al., 2004). The neurotoxic effects of metal exposure on locomotion behavior have also been examined by evaluating the endpoints of head thrash, body bend, and basic movements (Wang et al., 2007a; Wang and Wang, 2008a, 2008b; Wang and Xing, 2008; Hu et al., 2008). Nematodes were further used for a test of organophosphate-induced neurotoxicity by an anticholinesterase activity assay (Cole et al., 2004). In nematodes, Pb exposure causes severe deficits in locomotion, learning, and memory behaviors (Wang and Yang, 2007; Ye et al., 2008a, 2008b; Zhang et al., 2010; Wang et al., 2010a).

C. elegans is a model organism for the study of perception (Mori and Ohshima, 1997; Hu et al., 2007). Temperature is a critical modulator of metabolism and behavior, which makes thermotaxis serve as an important perception behavior for the life of the animals. Thermotaxis is based on thermal sensing, and *C. elegans* has an accurate thermosensory system (Hu et al., 2007). Nematodes grown in the presence of food at a particular temperature will actively seek this temperature when presented with a thermal gradient but will avoid this temperature if it is associated with an absence of food (Hedgecock and Russell, 1975; Mori, 1999). To score thermotactic responses, two kinds of temperature gradients, a

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linear temperature gradient and a radial thermal gradient, are usually used (Hedgecock and Russell, 1975). Temperature is sensed by *C. elegans* using the AFD thermosensory neurons (Mori and Ohshima, 1995). TTX-1, a homolog of otd/Otx, specifies the thermotaxis neuron fate (Satterlee et al., 2001). Moreover, our previous study has indicated that exposure to Hg, Cu, Ag, and Cr at certain concentrations decreased thermotaxis and caused damage to the structure of AFD sensory neurons (Xing et al., 2009).

Exposure to heavy metals can induce the formation of severe oxidative stress in nematodes (Wang et al., 2010a, 2010b; Wang and Xing, 2010). Nevertheless, it is still largely unclear how the oxidative stress influences the formation of thermotaxis defects in metal-exposed nematodes. In the present study, we further selected the heavy metal Pb to investigate the possible roles of oxidative stress in forming decreased thermotaxis in metal-exposed nematodes.

1 Materials and methods

1.1 Reagents and strains

Four concentrations (2.5, 50, 100, and 150 μmol/L) of $Pb(NO_3)_2$ solutions were used. Metal concentrations of exposed solutions were analyzed by Atomic Absorption Spectrophotometry (AAS; Pye-Unicam model SP9, Cambridge, UK). The 5, 6-chloromethyl-2',7'dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was from Molecular Probes (Eugene, USA), and other chemicals were obtained from Sigma-Aldrich (USA).

Nematodes used were wild-type N2, and *lin-15(n765); dEx1267*[*lin-15(+)gcy-8::GFP*] labeling AFD neurons, and *Ex*(P*hsp-16.2::gfp*), a gift from Dr. King Chow's lab. They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20°C (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 were obtained by collection as described (Donkin and Williams, 1995). The L2-larvae or young adults were washed with modified K medium (50 mmol/L NaCl, 30 mmol/L KCl, 10 mmol/L NaOAc, pH 5.5) (Williams and Dusenbery, 1990). Exposures were performed in 12-well sterile tissue culture plates as described (Mutwakil et al., 1997). Exposures on young adults were 6-hr long and carried out in a 20°C incubator in the absence of food.

1.2 Body bend

To assay body bend, examined nematodes were picked onto a second plate, and scored for the number of body bends in a 20-sec interval. A body bend was counted as a change in the direction of the part of nematode corresponding to the posterior bulb of pharynx along the *Y* axis, assuming that the nematode was traveling along the *X* axis. Thirty nematodes were examined for each assay.

1.3 Thermotaxis to cultivation temperature

Thermotaxis to cultivation temperature was assayed as described previously (Ye et al., 2008a; Gomez et al., 2001). Approximately 50 nematodes were grown at 20°C overnight in the presence of a fresh lawn of the bacteria strain OP50 on a 6-cm Petri dish. Young adults were transferred onto a fresh plate devoid of bacteria for 2 min, and individual nematodes were then deposited on a 9-cm Petri dish. A radial thermal gradient was created by placing a vial containing frozen acetic acid on the bottom of the plate and incubating at 25°C for 90 min. Upon removal of nematodes from the plate, tracks left were analyzed. A trace is considered as isothermal if more than half of the trace length left on the agar surface by a single nematode is circular or presents an arc of circle near the isotherm of the cultivation temperature. Each data point represents 10 independent assays.

1.4 Fluorescence quantification

To quantify fluorescence intensities, fluorescence images of neurons were captured with a Zeiss Axiocam MRm camera (Carl Zeiss, Gottingen, Germany) on a Zeiss Axioplan 2 Imaging System with a 40× objective using SlideBook software (Intelligent Imaging Innovations, Santa Monica, USA). Images were acquired with a Quantix cooled CCD camera (CoolSNAP CF2, Photometrics, USA), and illumination was provided by a 175W xenon arc lamp and GFP filter sets (Chroma Technology Corp., USA). Exposure times were chosen to fill the 12-bit dynamic range without saturation, and out-of-focus light was removed with a constrained iterative deconvolution algorithm. Background fluorescence from the coverslip and from nonspecific tissue autofluorescence was removed by subtracting an image filtered with a low-pass Gaussian filter. Puncta sizes for cell bodies were measured as the maximum radius for assayed fluorescent puncta. The relative fluorescent intensity of particular fusion proteins at cell bodies or sensory endings was obtained by integrating the pixel intensity in at least 20 nematodes. Relative sizes of fluorescent puncta for cell bodies or relative lengths of sensory endings were examined in at least 20 nematodes.

1.5 Analysis of transgenic strain

It was reasoned that if the exposure was toxic, it would affect nematodes through environmental stress and thus result in a stress response (Chu and Chow, 2002). *Ex*(P*hsp-16.2::gfp*) strain can reveal the presence of toxic stress when the exposed nematodes were examined under a fluorescent microscope. To analyze the changes of *hsp-16.2* expression patterns, the treated *Ex*(P*hsp-16.2::gfp*) animals were allowed to settle for 10 min, and then pipetted onto an agar pad on a glass slide, mounted and observed for fluorescent signals with a fluorescence microscope. Observations of the GFP were recorded and color images were taken for the documentation of results with Magnafire software (Olympus, USA). To distinguish the positive results from the background in the stress test, only stresses that could induce 50% of the transgenic

nematodes to display a strong *hsp-16.2::gfp* expression signal were taken as having a positive effect (Chu and Chow, 2002). Stress response was evaluated by the percentage of population with *gfp* expression. More than 50 nematodes were counted.

1.6 Oxidative damage

Examined nematodes were washed free of bacteria, pelleted and frozen for oxidative damage assay. Oxidative damage was analyzed using an Oxyblot assay kit (Millipore, Boston, MA, USA) to detect carbonylated proteins. The carbonyl groups are derivatized with 2,4-dinitrophenylhydrazone (DNP-hydrazone). The assay was performed according to the manufacturer's protocol. Quantification of carbonylated proteins was obtained by taking the ratio of DNP staining to tubulin staining according to the manufacturer's protocol.

To further quantify whether Pb treatment increases ROS levels in neurons, ROS production was assayed (Liu et al., 2001; Wu et al., 2011). L2-larvae were transferred to 1 mL of M9 buffer containing 1 μmol/L CM-H_2DCFDA and pre-incubated for 3 hr at 20°C. Then, after Pb exposure at the young adult stage, nematodes were mounted on 2% agar pads in 60 μg/mL levamisole and examined with a laser scanning confocal microscope (Leica, TCS SP2, Bensheim, Germany) at excitation wavelength 488 nm and emission filter at 510 nm. The semiquantified ROS was expressed as relative fluorescent units (RFU).

1.7 Oxygen consumption

Oxygen consumption was measured using a Clark electrode (COLORLAB1, UK) for a 10 min period. Nematodes were collected, pelleted and frozen for protein quantification. Proteins were quantified using a bicinchronic acid protein assay kit (Thermo Scientific, UK) according to the manufacturer's protocol.

1.8 Pharmacological assay

Synchronized examined L2-larvae were treated with 0.1% DMSO for 4 hr (Wang et al., 2007b), 10 mmol/L ascorbate, 5 mmol/L *N*-acetyl-L-cysteine (NAC) for 24 hr (Huang and Lemire, 2009), and then exposed to 50 μmol/L Pb for 6 hr when they developed to young adults. Graphs are representative of at least ten trials.

1.9 Statistical analysis

All data were expressed as means ± standard error of the mean (SEM). Graphs were generated using Microsoft Excel (Microsoft Corp., USA). Statistical analysis was performed using SPSS 12.0 (SPSS Inc., USA) by one-way analysis of variance (ANOVA), which was followed by Post Hoc multiple comparisons for the significance of the differences between the groups. LSD Tests were used when there were equal variances in the groups, which can be tested with the Homogeneity-of-variance test; otherwise, Dunnett's T3 Tests were used. Probability levels of 0.05 and 0.01 were considered statistically significant.

2 Results

2.1 Effects of Pb exposure on thermotaxis behaviors

As shown in Fig. 1a, exposure to 50–150 μmol/L Pb for 6 hr caused a significant ($P < 0.01$) decrease of the percentage of nematodes performing isothermal tracking (IT) behavior after overnight feeding at 20°C. Exposure to 2.5 μmol/L Pb also induced a moderate but significant ($P < 0.05$) reduction of the percentage of nematodes performing IT.

To exclude the possible effects of locomotion behavior on IT recording, we examined locomotion behaviors in Pb-exposed nematodes. As shown in Fig. 1b, exposure to 100–150 μmol/L Pb resulted in significant ($P < 0.01$) decreases of body bends; however, exposure to 2.5 and 50 μmol/L of Pb did not noticeably affect body bends of the nematodes, implying that Pb exposure can cause the decrease of thermotaxis, and the severe decrease of thermotaxis induced by high concentrations of Pb may be largely due to the formation of deficits in the locomotion behaviors in nematodes.

Moreover, we did not observe a noticeable intestinal autofluorescence in 2.5 and 50 μmol/L Pb-exposed nematodes; however, a moderate but significant induction of intestinal autofluorescence was detected in 100 and 150 μmol/L Pb-exposed nematodes (data not shown). Intestinal autofluorescence caused by lysosomal deposits of lipofuscin serves as a valuable maker for cellular damage in aging cells, and can accumulate over time in aging nematodes (Garigan et al., 2002; Shen et al., 2010). Thus, some tissues or cells in the 100 and 150 μmol/L Pb-exposed nematodes may be in the process of aging or death, whereas the nematodes exposed to 2.5 and 50 μmol/L Pb did not undergo a severe aging process.

2.2 Effects of Pb exposure on structural properties of AFD sensory neurons

In *C. elegans*, P*gcy-8*::GFP is a specific fluorescent marker to label the AFD sensory neurons (Satterlee et al., 2001). Pb exposure at concentrations from 2.5 to 150 μmol/L caused significant decreases of the relative intensities of cell bodies in AFD sensory neurons (Fig. 1c, d). Similarly, Pb exposure at concentrations from 2.5 to 150 μmol/L caused significant ($P < 0.01$) decreases of the relative sizes of fluorescent puncta of cell bodies in AFD sensory neurons (Fig. 1c, e). Thus, the observed deficit in thermotaxis in nematodes exposed to Pb may be largely due to damage to the development of AFD sensory neurons.

AFD is a thermosensory neuron with a cilium exposed to the environment, and finger-like microvilli can be observed at the end of AFD dendrites (Swoboda et al., 2000). The sensory endings of AFD neurons are typically elaborate, consisting of a single cilium and numerous microvillar "fingers" (Fig. 1f). Exposure to 2.5–150 μmol/L Pb further caused significant ($P < 0.01$) reductions of the relative fluorescent intensities of sensory endings in AFDL neurons (Fig. 1f, g). Exposure to 2.5–150 μmol/L Pb also resulted in significant ($P < 0.01$) decreases of the relative lengths

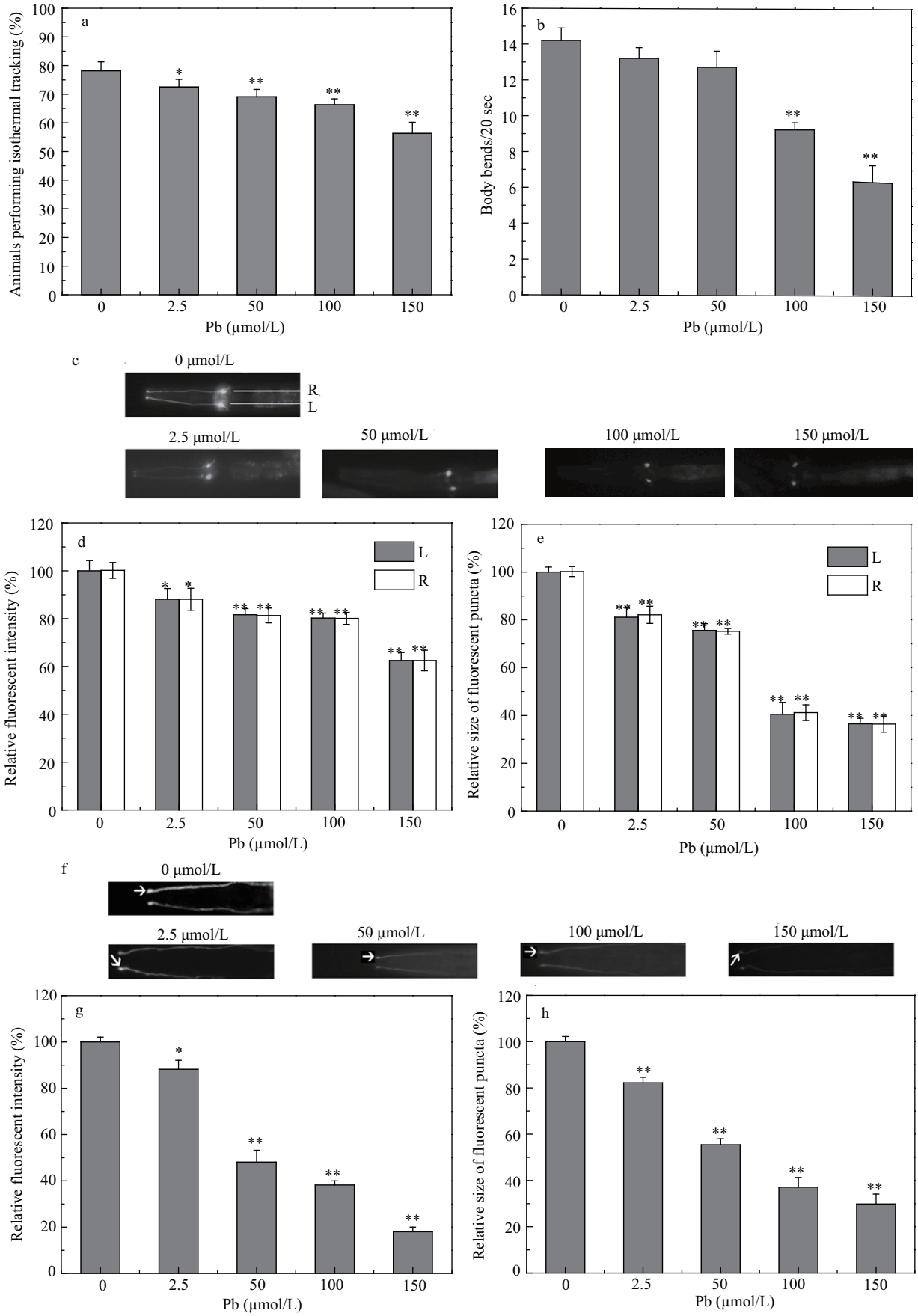

Fig. 1 Effects of Pb exposure on thermotaxis and AFD neuron morphology. (a) on thermotaxis; (b) on body bends; (c) on AFD neuron morphology; "L" or "R" indicates the cell body of left or right AFD neurons labeled by P*gcy-8*::GFP; (d) on fluorescent intensities in cell bodies of AFD neurons; (e) on sizes of fluorescent puncta for cell bodies of AFD neurons; (f) on morphological patterns of sensory endings (indicated by arrowheads) of AFDL neurons; (g) on lengths of sensory endings of AFDL neurons; (h) on sizes of fluorescent puncta for sensory endings of AFDL neurons. Bars represent means ± SEM. * $P < 0.05$; ** $P < 0.01$.

of sensory endings of AFDL neurons (Fig. 1f, h). No cilium loss phenotype, as previously observed in *daf-19* mutants (Swoboda et al., 2000), was found in Pb-exposed nematodes, implying that Pb exposure will not influence cilium formation. Therefore, Pb exposure can alter the development or suppress the extension or growth of sensory endings of AFD cilium.

2.3 Effects of Pb exposure on stress response, oxidative stress, and mitochondrial function in nematodes

We examined the effects of 2.5 and 50 μmol/L Pb exposure on the stress response and oxidative stress in nematodes. The percentage of the population with *hsp-16.2::gfp* expression was increased by 28.6%, and 52.4%, respectively, in Pb-exposed nematodes at concentrations of 2.5 and 50 μmol/L (Fig. 2a). The percentages of the population with *hsp-16.2::gfp* expression in Pb-exposed nematodes at the examined concentrations were above the value of 50%, demonstrating the significant induction of stress response.

Moreover, 50 μmol/L Pb exposure caused the formation of severe oxidative damage, and 2.5 μmol/L Pb exposure induced the formation of moderate oxidative damage in nematodes (Fig. 2b). ROS generation can also be expressed as RFU in animals incubated in CM-H_2DCFDA solution (Wang et al., 2007b). RFUs were increased by 50 μmol/L Pb exposure, and a moderate but significant increase was detected in 2.5 μmol/L Pb-exposed nematodes (Fig. 2c, d).

ROS are byproducts of mitochondrial respiration capa-

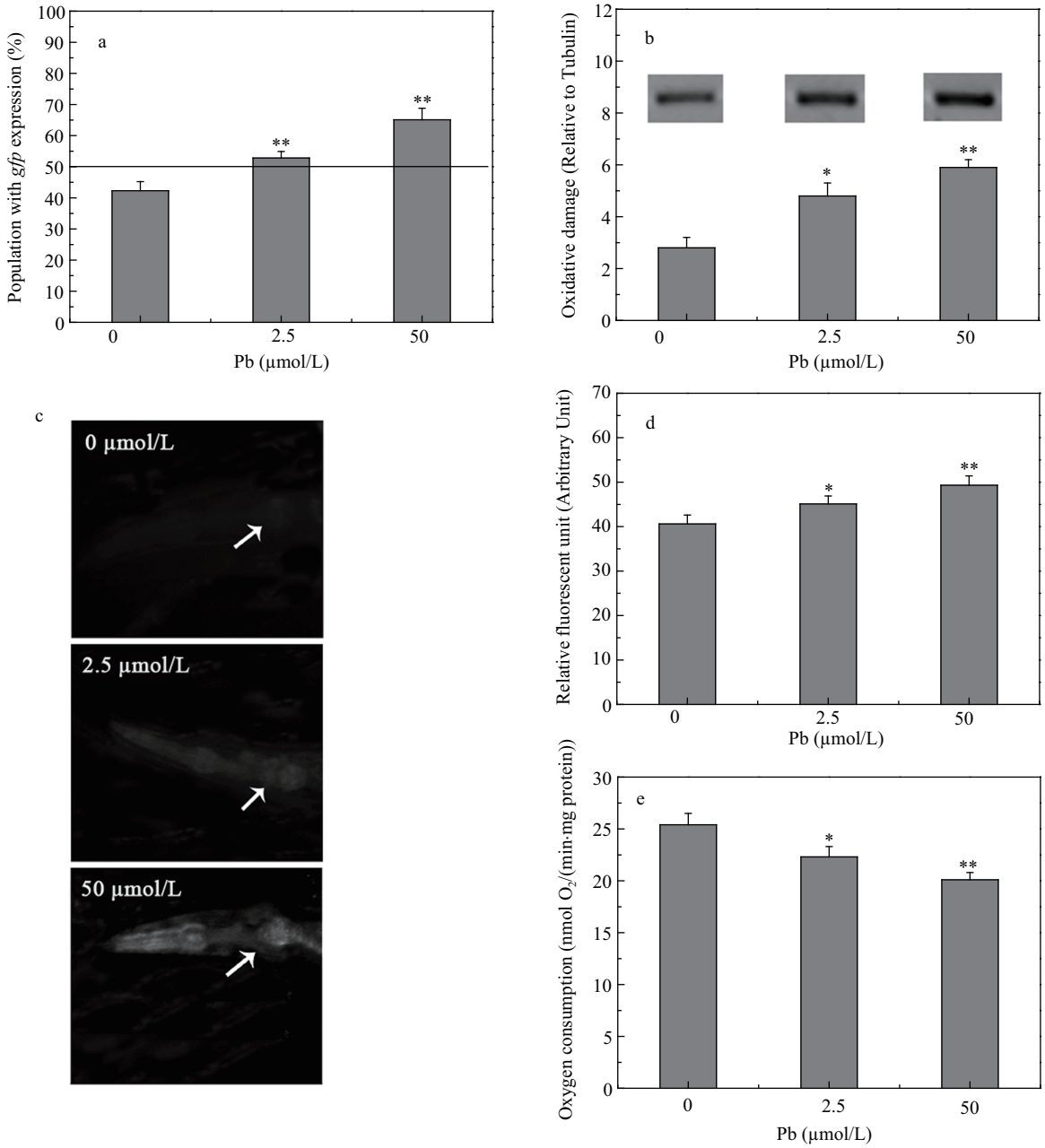

Fig. 2 Effects of Pb exposure on stress response, oxidative stress, and mitochondrial function. (a) on stress response. To evaluate the stress response, significant induction of *hsp-16.2*::*gfp* expression (50% of a population, above the line) was observed; (b) on oxidative damage. Examination of carbonylated proteins reveals increased oxidative damage. The gel data were inserted above each column; (c) typical pictures of ROS production in Pb-exposed nematodes as detected by CM-H_2DCFDA labeling. Arrowheads show position of second pharyngeal bulb; (d) on ROS production. Relative fluorescent intensities are expressed in arbitrary units. (e) on mitochondrial function. Bars represent means ± SEM. * $P < 0.05$; ** $P < 0.01$.

ble of causing oxidative damage within cells. To examine the effects of Pb exposure on mitochondrial function, we further measured whole-worm oxygen consumption. Oxygen consumptions in nematodes exposed to 2.5 and 50 μmol/L Pb were significantly decreased (Fig. 2e), suggesting the alteration of mitochondrial function in Pb-exposed nematodes.

2.4 Effects of antioxidant pre-treatment on oxidative stress and mitochondrial function in Pb-exposed nematodes

DMSO serves as an effective free-radical scavenger in nematodes (Wang et al., 2007b). Pre-treatment with 0.1% DMSO at the L2-larval stage prevented the formation of severe stress response and oxidative damage in nematodes exposed to 50 μmol/L Pb at the young adult stage (Fig. 3a–c), suggesting that DMSO pre-treatment inhibits the formation of severe stress response and oxidative stress in Pb-exposed nematodes.

A variety of antioxidants can be used to treat mitochondrial dysfunction, such as ascorbate and NAC, a precursor for the synthesis of glutathione (Huang and Lemire, 2009). Pre-treatment with 10 mmol/L ascorbate or 5 mmol/L NAC at the L2-larval stage also suppressed the formation of severe stress response and oxidative stress in young adults exposed to 50 μmol/L Pb (Fig. 3a–c). Moreover, pre-treatment with 10 mmol/L ascorbate or 5 mmol/L NAC at the L2-larval stage recovered the mitochondrial function, as revealed by the oxygen consumption in young adults exposed to 50 μmol/L Pb (Fig. 3d), implying that ascorbate or NAC pre-treatment can suppress both the oxidative stress and mitochondrial dysfunction formed in Pb-exposed nematodes.

2.5 Effects of antioxidant pre-treatment on thermotaxis and structural properties of AFD sensory neurons in Pb-exposed nematodes

Compared to the formation of a severe deficit in thermotaxis to cultivation temperature in 50 μmol/L Pb-exposed nematodes, pre-treatment with 0.1% DMSO, 10 mmol/L ascorbate or 5 mmol/L NAC at the L2-larval stage all prevented the occurrence of the thermotaxis defect in young adults exposed to 50 μmol/L Pb (Fig. 4a). Pre-

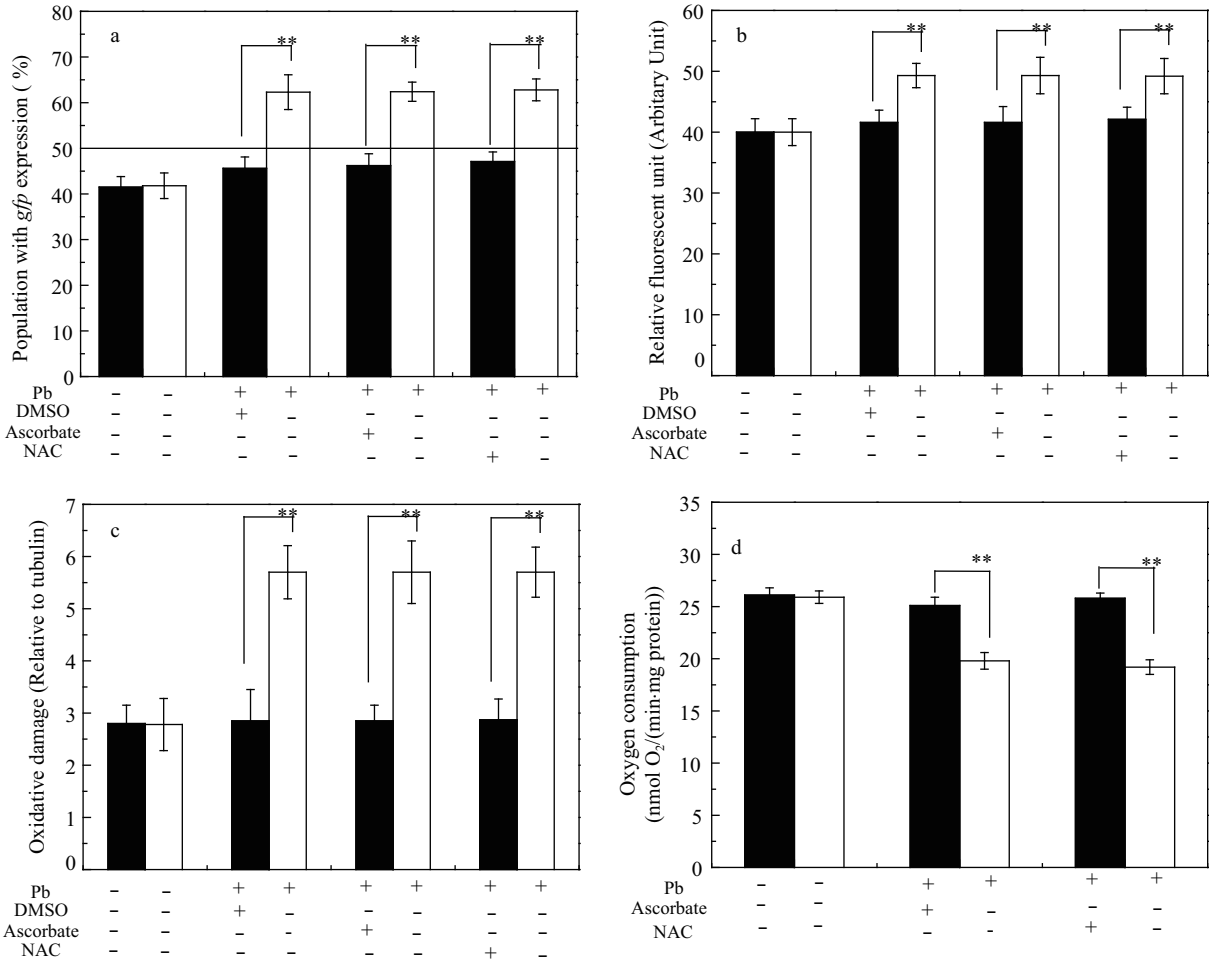

Fig. 3 Effects of antioxidant pretreatment on stress response, oxidative stress, and mitochondrial function in Pb-exposed nematodes. (a) on stress response in Pb-exposed nematodes. To evaluate the stress response, significant induction of *hsp-16.2*::*gfp* expression (50% of a population, above the line) was observed; (b) on ROS production in Pb-exposed nematodes as detected by CM-H_2DCFDA labeling. Relative fluorescent intensities are expressed in arbitrary units; (c) on oxidative damage in Pb-exposed nematodes. Examination of carbonylated proteins reveals increased oxidative damage; (d) on mitochondrial function in Pb-exposed nematodes. Synchronized L2-larvae were treated with 0.1% DMSO for 4 hr, 10 mmol/L ascorbate or 5 mmol/L NAC for 24 hr, then the treated nematodes were exposed to 50 μmol/L Pb for 6 hr when they developed to young adults. Data in Fig. 3d were analyzed using Dunnett's T3 tests because variances were not equal. Bars represent means ± SEM. ** $P < 0.01$.

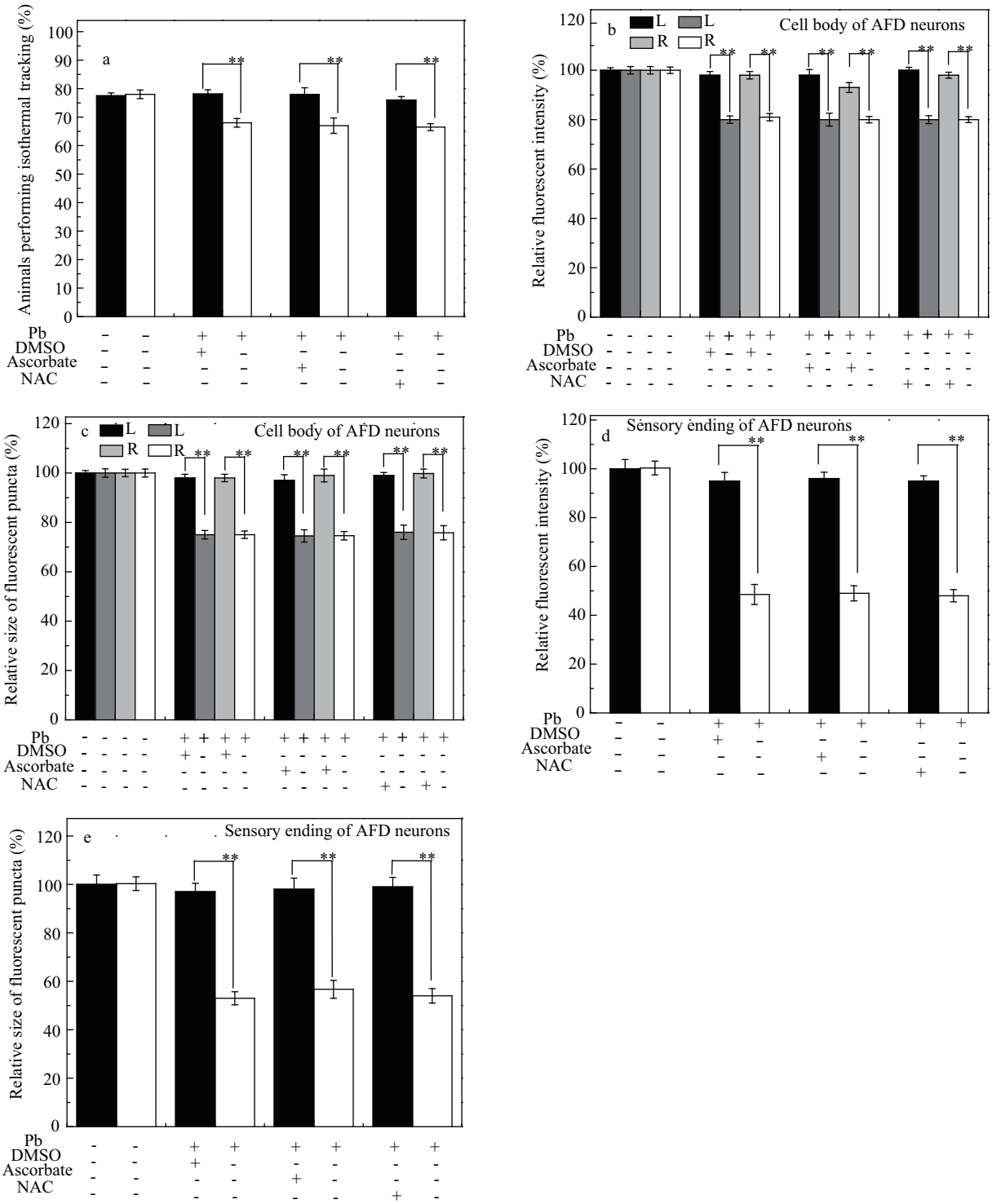

Fig. 4 Effects of antioxidant pretreatment on thermotaxis, morphology and sensory endings of AFD neurons in Pb-exposed nematodes. (a) on thermotaxis in Pb-exposed nematodes; (b) on fluorescent intensities in cell bodies of AFD neurons in Pb-exposed nematodes; (c) on sizes of fluorescent puncta for cell bodies of AFD neurons in Pb-exposed nematodes; (d) on lengths of sensory endings of AFDL neurons in Pb-exposed nematodes; (e) on sizes of fluorescent puncta for sensory endings of AFDL neurons in Pb-exposed nematodes. P*gcy-8*::GFP labels the AFD, and "L" or "R" indicates cell body of the left or right neuron. Synchronized L2-larvae were treated with 0.1% DMSO for 4 hr, 10 mmol/L ascorbate or 5 mmol/L NAC for 24 hr, then the treated nematodes were exposed to 50 μmol/L Pb for 6 hr when they developed to young adults. Bars represent means ± SEM. ** $P < 0.01$.

treatment with 0.1% DMSO, 10 mmol/L ascorbate or 5 mmol/L NAC at the L2-larval stage further suppressed the reductions of relative fluorescent intensities and relative sizes of fluorescent puncta of cell bodies in AFD sensory neurons, and inhibited the decreases of relative fluorescent intensities and relative sizes of fluorescent puncta of sensory endings in AFD sensory neurons in young adults exposed to 50 μmol/L Pb (Fig. 4b–e). Therefore, once the oxidative stress is blocked or suppressed, the reduction of thermotaxis and the formation of structural defects of AFD sensory neurons caused by Pb exposure can be prevented, implying that oxidative stress plays a key role in inducing the formation of severe neurotoxicity from Pb exposure in nematodes.

3 Discussion

Our previous studies have indicated that Pb exposure at certain concentrations could cause severe deficits in the locomotion behaviors, learning and memory behaviors, and structural properties of GABAergic neurons in nematodes (Wang and Yang, 2007; Ye et al., 2008a, 2008b; Du and Wang, 2009; Zhang et al., 2010). In this study, our data further suggest that Pb exposure at certain concentrations induced the decrease of thermotaxis to cultivation temperature of nematodes. In addition, although the percentage of nematodes performing IT behavior after overnight feeding at 20°C was significantly reduced by Pb exposure at all examined concentrations from 2.5 to 150 μmol/L in nematodes, exposure to 100 and 150 μmol/L of Pb also caused a significant decrease of locomotion behavior, implying that Pb exposure at high concentrations may result in multiple neurotoxic effects on the development and behaviors in exposed animals, and the defects of thermotaxis to cultivation temperature induced by Pb exposure at relatively higher concentrations may be largely due to the formation of a severe deficit in locomotion behaviors. These data are consistent with the neurotoxic effects from Pb exposure on the perception behaviors in humans and other animals (Wu et al., 2000; Chuang et al., 2000; Lim et al., 2005).

For *C. elegans*, the thermotaxis behavior is mainly controlled by the AFD sensory neurons, because a significant fraction of the operated animals moved almost randomly on a temperature gradient when the pair of AFD thermosensory neurons was killed (Mori and Ohshima, 1995, 1997; Mori, 1999). Our data suggest that the structural properties of AFD sensory neurons were severely damaged in Pb-exposed nematodes. Exposure to Pb at concentrations of 2.5 and 50 μmol/L caused decreases of relative intensities and relative sizes of fluorescent puncta of cell bodies and relative fluorescent intensities and relative lengths of sensory endings in AFD sensory neurons, suggesting that the observed deficit in thermotaxis to cultivation temperature in Pb exposed nematodes may be largely due to the damage to the structural properties of AFD sensory neurons. Nevertheless, because no cilium loss phenotype could be found in Pb-exposed nematodes, Pb exposure at the examined concentrations will not influence the cilium formation process. The adverse effects of Pb exposure on the structural properties of AFD sensory neurons were similar to those from Hg, Cu, Ag, and Cr exposure (Xing et al., 2009).

It has been reported in other organisms that oxidative stress can be induced by Pb exposure (Wang et al., 2006; Antonio-García and Massó-Gonzalez, 2008). In *C. elegans*, exposure to Pb at concentrations of 2.5 and 50 μmol/L also resulted in the significant induction of oxidative damage, and elevation of ROS production in nematodes. Our data further suggest that the observed induction of oxidative damage and elevation of ROS production in 2.5 and 50 μmol/L Pb-exposed nematodes could be prevented by pre-treatment with DMSO, an effective free-radical scavenger at the L2-larval stage. Moreover, pre-treatment with the antioxidants ascorbate and NAC, used to prevent the formation of mitochondrial dysfunction, also suppressed the induction of oxidative damage and elevation of ROS production in 2.5 and 50 μmol/L Pb-exposed nematodes, suggesting the important roles of normal mitochondrial function in preventing oxidative stress in nematodes. Therefore, our data imply that Pb exposure can induce severe oxidative stress, and the induced oxidative stress observed in Pb-exposed nematodes may be at least partially due to the formation of severe ROS elevation and/or the damage to mitochondrial function. The formation of severe oxidative stress could also be observed in nematodes exposed to other metals (Wang et al., 2007b, 2010a, 2010b; Wang and Xing, 2010; Wu et al., 2011), suggesting that the formation of severe oxidative stress may be a common result from metal exposure in nematodes.

In the present study, especially, we investigated the possible association of oxidative stress with the formation of Pb exposure-induced decrease of thermotaxis behaviors in *C. elegans*. Our data demonstrate that after DMSO, ascorbate, or NAC pre-treatment at the L2-larval stage, the occurrence of a thermotaxis defect in nematodes exposed to 50 μmol/L of Pb could be prevented. Moreover, pre-treatment with DMSO, ascorbate, or NAC at the L2-larval stage suppressed the formation of deficits in relative fluorescent intensity and relative size of fluorescent puncta of cell bodies and relative fluorescent intensities and relative sizes of fluorescent puncta of sensory endings in AFD sensory neurons in nematodes exposed to 50 μmol/L Pb. Therefore, once the oxidative stress is blocked or suppressed in Pb-exposed nematodes, the reduction of thermotaxis to cultivation temperature and the structural defects of AFD sensory neurons caused by Pb exposure in nematodes can be effectively prevented.

To assay the role of oxidative stress in regulating the neurotoxic effects from Pb exposure on thermotaxis behavior, the concentration of 50 μmol/L was selected for Pb exposure among our examined concentrations. One of the reasons is that exposure to 100 and 150 μmol/L of Pb caused significant decreases of locomotion behaviors compared to control. In addition, a severe oxidative stress was formed in nematodes exposed to Pb at the concentration of 50 μmol/L, whereas only a moderate oxidative stress was induced in Pb nematodes at the concentration of 2.5 μmol/L.

4 Conclusions

Pb exposure induced the decrease of thermotaxis behaviors in nematodes. Oxidative stress may play a key role in inducing the Pb-induced neurotoxic effects on the structures and function of AFD sensory neurons. The formation of severe oxidative stress caused by Pb exposure may be due to both the induction of ROS elevation and the damage to mitochondrial function.

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