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Cadmium-induced serotonergic neuron and reproduction damages conferred lethality in the nematode *Caenorhabditis elegans*

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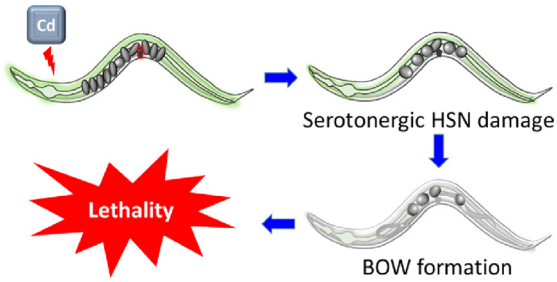
HIGHLIGHTS

- Sterile and male *C. elegans* showed cadmium resistance.
- Cadmium induced BOW contributed to the killing process.
- Cadmium induced serotonergic neuron damage conferred to BOW formation.
- Protection of serotonergic neuron increased cadmium resistance in *C. elegans*.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium is a ubiquitous environmental toxicant. The use of *Caenorhabditis elegans* as a model for monitoring cadmium exposure has revealed several conserved signaling pathways. However, little is known about the killing process during lethality assay. In the present study, we investigated the effects serotonergic neuronal and reproductive damages on cadmium exposure in *C. elegans*. We found that sterile hermaphrodites, males and worms that passed reproduction span presented high cadmium resistance compared to those of young adults. The results demonstrated that reproduction process other than reproduction capacity conferred cadmium sensitivity. Cadmium exposure resulted in high ratio bagging phenotype, which was a severe reproductive deficit with embryos hatched internally that could cause worms to die early. The mechanism of bagging formation was ascribed to cadmium-induced egg laying deficiency that led embryos to retain and hatch in uterus. The addition of serotonin and imipramine promoted egg laying and thereby increased cadmium resistance. The results demonstrated that vulval muscles responsible for egg laying were still functional, while the serotonergic hermaphrodite specific neurons might be dysfunctional under cadmium exposure. Cadmium exposure resulted in shrinkage of serotonergic neuronal body and reduced expressions of tryptophan hydroxylase, the key enzyme for serotonin synthesis. The protection of serotonergic neuron through transient thermal preconditioning improved survival rate. In conclusion, our study demonstrated that damages of serotonergic neurons and reproduction conferred to cadmium-induced lethality.

1. Introduction

The transition heavy metal cadmium is a ubiquitous toxic substance presented in soil, water, air and food. As the consequence of anthropic activity, the environmental concentrations of cadmium

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increased steadily in many areas around the world (ATSDR, 2012). Cadmium is easily absorbed and accumulated in different tissues and organs. Exposure to cadmium may have a potential impacts on human health and wildlife. Acute cadmium exposure may cause the symptoms including fever, myalgias and even death from pulmonary damages (Newman-Taylor, 1998). Chronic cadmium exposure results in diseases of multiple organs such as kidney, liver, blood and bones. Epidemiology studies have linked cadmium exposure to high incidence of cancers in lung, prostate, kidney and bladder (Waalkes, 2003). Because cadmium is among the most difficult heavy metals to remove from the body, exposing to this heavy metal is still a global health problem.

The development of biomonitoring systems provides us with multiple choices to evaluate the impacts of cadmium on human health as well as the wildlife. Traditionally, lethality assay based on rodents was a reliable method to evaluate toxicological effects of many chemicals. However, the use of rodents has cost and ethical concerns. Williams and Dusenbery (1990) developed an alternative *C. elegans* model to predict the toxicological effects of several heavy metal salts. The results for risk assessment are comparable to those of their mammalian counterparts. Moreover, the use of this soil dwelling and genetically tractable animal brings insight into the toxicological effects at the organism level. A bulk collection of mutated strains and characterized RNAi libraries enabled us to reveal the functions of several conserved signaling pathways in response to environmental chemicals. Exposure of *C. elegans* to cadmium caused multiple toxicological effects, such as mortality, reproduction defection, behavior degeneration and neuronal damages, depending on different doses (Moyson et al., 2018; Swain et al., 2010; Williams and Dusenbery, 1990). Based on lethality assay, it was shown that different mutated strains had diverse cadmium sensitivities (Hall et al., 2012; Keshet et al., 2017). These findings have suggested multiple conserved signal transduction pathways in response to cadmium exposure.

Lethality and reproduction are the most frequently used endpoints in this animal model. Based on lethality assay, the American Society for Testing Materials (ATSM) published a standard method for monitoring soil toxicity using *C. elegans* as a model (ATSM, 2014). Based on growth and reproduction of sublethal endpoints, *C. elegans* was also used as a model for assessment toxic effects in sediment and soil samples (ISO, 2010). Cadmium-induced mortality in *C. elegans* has been widely studied, however, data of lethality from different authors showed discrepancy (Moyson et al., 2018; Roh et al., 2009; Williams and Dusenbery, 1990). Moreover, there were very few studies that investigated the mechanisms of the killing process. In fact, most of the studies neglected the fact that adult hermaphrodite might encountered a severe reproduction damage called bag-of-worms (bagging or BOW) during reproduction span. Bagging is defined as larvae hatched and developed inside the uterus and inevitably leads parent worms to die early (Trent et al., 1983). Thus, the formation of matricidal hatching would exaggerate mortality rate. Genetic screen found that high rates of bagging phenotype presented in worms with deficiencies either in serotonergic system or in egg laying system (Trent et al., 1983; Weinshenker et al., 1995). Recent year studies have linked high rates of bagging formation with age-related degeneration in reproductive or nervous system (Pickett and Kornfeld, 2013). In metazoans, nervous system plays critical roles in integration information and stress responses. Cadmium exposure affects the functions of cholinergic, dopaminergic and serotonergic neurons. Epidemiological studies found that cadmium exposure resulted in cognitive dysfunctions and emotional disruptions through disturbance of serotonergic nervous system (Wang and Du, 2013). In *C. elegans*, serotonergic nerve system modulates a number of distinct behaviors. The serotonergic hermaphrodite specific neuron (HSN) and its innerved vulval muscles modulate egg laying behavior during reproduction span (Sze et al., 2000). Because egg laying deficiency resulted in mortality (Trent et al., 1983), we assumed that the damage in hermaphrodite specific neurons played important roles in mediating the lethal effect caused by cadmium. The aim of the present study was to investigate the effects of serotonergic neuronal and reproductive damage on the killing process under cadmium exposure.

2. Materials and methods

2.1. Worm strains and reagents

Strains used in this study were Bristol N2 wild type, *fem-1(hc17)* IV, *him-5(e1490)* V, *myo-3(st386)* V; P_{myo-3}::GFP, lin-15B&lin-15A(n765) X; P_{tph-1}::dsRed2, *egl-1(n1084)* V, *egl-1(n1084n3082)* V. All of the strains were obtained from the Caenorhabditis Genetics Center (CGC) (University of Minnesota, MN, USA), which is funded by the NIH National Center for Research Resources. *fem-1(hc17)* IV; *him-5(e1490)* V, which produced about 50% of males, was obtained by standard genetic crossing (Fay, 2006). Unless specified otherwise, worms were cultured at 20 °C in Petri dishes on nematode growth medium (NGM) seeded with *Escherichia coli* OP50 as food. Synchronized growth worms were obtained by standard methods (Sulston and Hodgkin, 1988). Briefly, gravid worms were collected and lysed in a solution containing 1.0 M of NaOH and 20% of sodium hypochlorite. Eggs were collected and hatched at 20 °C overnight. The new hatchers arrested at L1 stage in the absence of OP50. Synchronized L1 stage worms were inoculated onto NGM until young adult. *fem-1(hc17)* temperature sterile worms were obtained by culturing synchronized L1 worms at 25 °C until young adult and subsequently transferred to 20 °C before use (Doniach and Hodgkin, 1984). To obtain worms of post reproduction stage, the young adults were cultured at 20 °C and picked daily to remove newborn until day 5. Male worms were picked at late L4 stage and cultured until young adult. For thermal treatment, worms were treated at 35 °C for 1 h in a water bath and then recovered at 20 °C for 1 h before cadmium exposure. $CdCl_2$ (Sigma-Aldrich, St Louis, MO) was dissolved and diluted with K-medium (containing 52 mM NaCl and 32 mM KCl) before use. Serotonin and imipramine were products of TCI Chemicals (Tokyo, Japan).

2.2. Lethality assay

Lethality assay was performed at 20 °C for all strains. To estimate lethality rate, aliquots of 0.9 ml K-medium containing 0.25 mM of cadmium were dispensed into each of a 12-well plate, and 0.1 ml of OP50 suspension was added so that the final OD_{550} was 0.5. Twenty worms at indicated developmental stages were put into each well. Dead worms were checked and piped out every 12 h under a stereomicroscope until all worms were killed. Survivors were transferred to fresh plate every two days. Worms lacking any response to platinum wire probe were defined as dead.

2.3. Bagging formation assay

For bagging formation assay, worms were exposed to 0.25 mM of $CaCl_2$. The number of viable worms with internally hatched eggs were counted and removed under a dissecting microscope at 24, 48 and 72 h, respectively. The bagging phenotype was defined by the presence of hatched progeny in the uterus. The ratio of bagging was calculated by counting the number of worms with internally hatched eggs divided by the number of viable worms. The cumulative ratio of bagging was calculated by counting the cumulative number of worms with internally hatched eggs divided by the