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The *Caenorhabditis elegans* model as a reliable tool in neurotoxicology

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Abstract

Caenorhabditis elegans (*C. elegans*) offers an attractive experimental platform as it has a short life cycle, is inexpensive to maintain and most importantly has high degree of evolutionary conservation with higher eukaryotes. Understanding the contribution of inherent genes that regulate neurotoxicity and antioxidant stress responses in the worm provides critical insight into mechanisms of mammalian neurotoxicity. The *C. elegans* model readily enables multi-gene approach, allowing for combinatorial genetic variation to be studied within the context of the influence of multigenic polymorphisms in environmental risk and vulnerability. This review provides a synopsis of recent studies on metal and pesticides toxicity in *C. elegans*, highlighting the utility of the model system in understanding molecular mechanisms that underlie developmental, reproductive and neuronal damage. The continuation of these investigations combining basic toxicological experimentation with novel genetic and high throughput methods will continue to make *C. elegans* an invaluable tool for future research, providing insight into molecular and cellular mechanisms of toxicity.

Keywords

Caenorhabditis elegans; toxicology; metals; neurodegenerative diseases

Introduction

Since Sydney Brenner's Nobel Prize-winning investigations using *Caenorhabditis elegans* to study nervous system development,¹ this nematode has proven to be an invaluable tool in biological research. Its use has prospered due to the multitude of advantages it provides. Some of these include its size (adults are approximately 1 mm in length), short life cycle (approximately 3 days at 20°C), ability to self-fertilize, large brood size (>300 offspring per hermaphrodite) as well as the ability to readily manipulate its gene expression profile.² The

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worms' transparency and the ease of making reporter gene fusions enable visualization of neuronal morphology and protein expression patterns within living worms. Furthermore, the self-fertilizing hermaphrodite allows for quick and easy homozygosity of mutations, and males can be mated to generate multiple mutation strains. *C. elegans* is not only valuable because of these traits but also due to its relevance to mammalian systems, expressing homologues to 60%–80% of human genes, many of which share great sequence identity.³ Gene knockdowns can be generated with relative ease with the use of RNAi techniques, and loss-of-function mutants can be produced by site-directed mutagenesis, thus enabling researches to use the nematode as a powerful genetic tool.

Due to its prolific use in research, there is a substantial amount of information available about the nematode. For example, the genome has been fully sequenced and the nervous system has been studied to such an extent that a wiring diagram is available.^{4,5} *C. elegans* has 302 neurons that signal through 890 electrical junctions, 1410 neuromuscular junctions and 6393 chemical synapses, using the same neurotransmitter systems (cholinergic, gamma-amino butyric acid [GABA]ergic, glutamatergic, dopaminergic [DAergic] and serotonergic) that are expressed in vertebrates.⁶ Many resources are available to *C. elegans* researchers, such as libraries of various strains including the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota, online resources, such as www.wormbase.org and www.wormbook.org.⁷

The use of *C. elegans* as a toxicological model has recently escalated and several recent review articles outline its utility as a model system.^{2,8,9} Chronic and delayed effects of environmental agents are often difficult to evaluate in mammalian models owing to their long life cycles. For this reason, the short lifespan of *C. elegans* has afforded a critical advantage in biomonitoring the consequences of environmental toxicants.^{2,10} As a result of the extensive study of its nervous system, the nematode can be used as a neurotoxicological model. Studies examining neurotoxicants address a vast array of outcomes including measuring endpoints on behavioral, structural, signaling and molecular levels. Various toxicants, including pesticides and metals have been examined with respect to their effects on the *C. elegans* nervous system. Results from these studies have closely emulated those found in mammalian systems, for example, establishing the ability of manganese (Mn) to induce degeneration of dopaminergic (DAergic) neurons and the developmental and behavioral defects caused by metals, such as cadmium (Cd), methylmercury (MeHg), iron (Fe) and organophosphates (OPs). Here, we highlight the response of *C. elegans* to several select toxicants and discuss its utility as a neurotoxicological model in advancing the understanding of mechanism of injury especially within the context of genetic manipulation.

Manganese

Manganese (Mn) is widely introduced into the environment as a pollutant through the manufacture of dry cell batteries, steel,^{11,12} fuel oil additives and antiknock agents.^{13–15} At high concentrations, the brain is highly susceptible to Mn toxicity. Occupational exposure to high levels of Mn can cause extrapyramidal symptoms that resemble idiopathic Parkinson's disease (PD), a disease known as manganism.¹⁶ Mn accumulation in basal ganglia leads to degeneration of DAergic neurons, the main pathological feature of manganism.^{17,18}

Mechanisms that underlie Mn neurotoxicity have been explored in the *C. elegans* model. Our group has found that *C. elegans* recapitulates many of the mammalian features of Mn toxicity.^{19,20} In *C. elegans*, as in mammalian models, high levels of Mn increase mortality, enhance oxidative stress and induce DAergic neurodegeneration. Behavioral alterations, such as decreased head trash and body-bend frequencies were also found after exposure to this metal.²¹

The divalent metal transporter 1 (DMT-1)/NRAMP2 (natural resistance associated macrophage protein 2) is involved in Mn uptake in mammals.¹⁹ The three gene orthologues in worms are called *smf-1*, *smf-2* and *smf-3* and are mostly localized in the intestine¹⁷ but are also present in DAergic neurons.²² Decreased susceptibility to Mn and its accumulation in *smf-1* and *smf-3* knockout worms demonstrate the involvement of SMF-1 and SMF-3 in Mn uptake in *C. elegans*.¹⁷ Furthermore, downregulation of *smf-3* in response to Mn exposure and the higher Mn uptake through SMF-3 compared with other *C. elegans* SMFs suggest that this protein is the counterpart of mammalian DMT-1.¹⁷ Recent work by Settivari et al.²² also indicates a role for *smf* in Mn neurotoxicity, demonstrating that genetic knockdown or deletion of *smf-1* and *smf-2* partially inhibits DAergic neuronal death. These data support analogies between *C. elegans* and mammalian models of Mn uptake and reinforce the importance of *C. elegans* as a research tool for deciphering molecular mechanisms of toxicant-induced neurotoxicity.

Methylmercury

Mercury (Hg) is a global pollutant which knows no environmental boundaries. Even the most stringent control of Hg pollution from anthropogenic sources will not eliminate exposure to potentially toxic quantities, given its ubiquitous environmental distribution.²³ Exposure to methylmercury (MeHg) occurs primarily via the food chain due to its accumulation in fish. The latest statistics in the United States indicate that 46 states have fish consumption advisories covering ~40% of the nation's rivers, lakes and streams. In addition, an estimated 3–4 million children live within 1 mile of the 1300+ active US hazardous waste sites where Hg is a common pollutant.^{4,24} Adults exposed to high levels of this metal experience paresthesias and problems with vision and hearing, caused by lesions to discrete areas of the brain, such as the cerebellum and occipital lobe, while infants and children experience sensory problems and mental retardation, caused by alterations in overall brain architecture.^{25,26} These alterations in brain architecture include microcephaly and inhibition of cell migration leading to distortion of normal cortical layering, cerebellar abnormalities and alterations in glial cells.^{27,28} As alterations in cell migration and in cell number could be much more easily observed in an organism with a small, well-defined nervous system, *C. elegans* was used as a model with the hypothesis that MeHg would induce alterations in cell number, in the placement of neurons or in neuronal outgrowths. Helmcke et al.²⁹ demonstrated that *C. elegans* exposed to MeHg for a short duration does not reveal any overt alterations in the structure of the nervous system of the nematode. Moreover, while a decrease in pharyngeal pumping rate is observed following exposure to MeHg (Figure 1), other behavioral tests, such as thrashing, aimed to assay the overall ability of *C. elegans* to move in its normal sinusoidal pattern do not reveal alterations following MeHg exposure.²⁹ Since levels of Hg in *C. elegans* following short MeHg exposure are comparable to those

levels previously noted in mammalian studies revealing alterations in nervous system architecture, these results suggest that *C. elegans* has a mechanism for coping with short exposure to this toxicant. Several hypotheses for the mechanism of how the nematode is able to protect its nervous system from MeHg insult exist, including that the toxicant is readily metabolized or that repair mechanisms are promptly able to repair damage induced by the metal.³⁰ Elucidation of these pathways could provide insights into prevention or treatment of MeHg poisoning in humans.

Uranium

Depleted uranium (DU) is a byproduct generated in the enrichment process of naturally occurring uranium for the production of its radioactive isotope U²³⁵.³¹ It is extremely toxic, but extensive studies have failed to elucidate molecular mechanisms of toxicity in response to its exposure.³² *C. elegans* studies indicate that even with increased uranium accumulation, there is no significant neuronal degeneration.³³ Studies with reporter gene fusions expressing green fluorescent protein (GFP) established that 1 mM DU exposure, a concentration greater than the LC₅₀, does not result in neurodegeneration in *C. elegans* DAergic neurons, nor neurons expressing other neurotransmitters, such as GABA or glutamate.³⁴ Interestingly, no change is noted in nematode viability in *smf* knockout strains following exposure to DU, suggesting that this transporter likely does not play a critical role in U transport.³⁴

Furthermore, it was demonstrated that small thiol-rich metallothionein (MTL) proteins serve to protect worms from DU toxicity, as evident from increased expression of green fluorescent protein (GFP) in GFP-tagged MTL-1 and MTL-2 worms and from survival data showing that knockout of *mtl-1* and *mtl-2* render worms more sensitive to DU (Figure 2).³⁴ These results are important, as they point to protective mechanisms against DU in *C. elegans*, establishing the basis for exploration of parallel mechanisms in mammalian systems.

Other metals

Among ecotoxicants, in addition to those mentioned above, Ag, Cd, Pb, Fe, Cr, Zn, Ni, Cu, Ba and Al intoxication have been evaluated in the *C. elegans* model. Different exposure paradigms have been applied to extrapolate potential mechanistic effects relevant to mammals, as well as standardizing the model for biomonitoring applications and the assessment of pollution and its remediation.

Low-dose exposure to Cd, Co, Cr, Cu, Hg and Pb for 3 days causes locomotor alterations, reflecting alterations in the nervous system.²¹ Reduced body size, slower development and decreased brood-size and life-span were also noted after exposure to Cd,³⁵ Cu,³⁶ Ba,³⁷ Zn³⁸ and Ag.³⁹ Furthermore, Guo et al.¹⁰ showed that exposure to Hg, Pb, Cr and Cd in the first larval stage leads to impaired development and reproduction, findings that reproduce the impact of these metals in human juvenile population. In a 4-hour exposure paradigm, the toxic effects are particularly severe with Pb and Hg exposure over Cr and Cd; lethality in L1

worms after a 4-hour exposure is comparable to 24-hour exposure in young adult animals. Lethality in the 4-hour assay is ranked as follows: Hg > Pb > Cr > Cd.⁴⁰

Al exposure produces damaging effects to reproductive behavior and development in the nematode, impairing brood size, body size and generation time at low doses. Additionally, with higher Al exposures, the effects are also inherent to the progeny, with generation time defects being much stronger in the progeny compared to their exposed parents.⁴¹ Fe and Ni exposure causes decreased life-span, body-size and behavior alterations, which are also transferred to their progeny.^{42,43} Additionally, it was found that Ba decreases superoxide dismutase (SOD) and catalase activities and also affects DAF-16 expression in the nucleus of intestinal cells.³⁷

As genetic manipulation in *C. elegans* can be carried out with relative ease, the generation of resistant mutant strains provides invaluable information regarding metal toxicity. For instance, MTLs are protective against several metals by increasing their detoxification.^{35,44} Bruinsma et al.⁴⁵ identified mutations that increase the ability of the worm to withstand toxicity that is caused by high levels of Zn, mapping single nucleotide polymorphism markers. Sublethal doses of Cd exposure increase *C. elegans* germline apoptosis, which is dependent of c-Jun-N-terminal kinase (JNK) and p38 MAPK signaling cascades,⁴⁶ findings that demonstrate that the *C. elegans* model can serve as an in vivo model to study mechanisms or chemical toxicity and decipher signal transduction pathways associated with toxic modulation.

Pesticides

Paraquat, rotenone, organophosphates (OPs) and other pesticides have also been assessed in the *C. elegans* model, delineating neurotoxic mechanisms by which these agents exert their effects.² Strains of *C. elegans* have been generated that have either increased⁴⁷ or decreased⁴⁸ sensitivity to paraquat. Although the identities of many of the proteins encoded by the genes that cause these alterations have yet to be determined, researchers have found that alterations in antioxidants, such as superoxide dismutase (SOD), can cause alterations in the response of *C. elegans* to paraquat. Specifically, mutants that SOD display increased sensitivity,⁴⁹ while mutants that overexpress SOD display decreased sensitivity.^{50–51}

Of several OPs tested in *C. elegans*, dichlorvos was shown to be the most toxic, with an LC₅₀ of 0.039 mM. In comparison, acephate and methamidophos have an LC₅₀ in the range of 400–500 mM.⁵² Chlorpyrifos causes developmental delays, with exposure to sublethal doses inducing a dose-dependent decrease in growth, and higher doses (75 µM) completely inhibiting worms from developing past the L2 larval stage.⁵³

C. elegans as a model for neurodegenerative diseases

Formation, trafficking and release of synaptic vesicles in *C. elegans* are highly conserved, employing many of the same proteins as in mammalian neurons.^{4,54} Because *C. elegans* is transparent throughout its life cycle, green fluorescent protein (GFP) fusions have been extensively used to visualize specific neurons and synapses in living animals (Figure 3A and

B), allowing researchers to observe specific neuronal alterations. Specifically, these models permit hypotheses-driven investigations on disease x environmental interactions.

Transgenic expression of human beta-amyloid (A β) peptide in the body wall muscle cells of *C. elegans* was used to better understand aspects of Alzheimer's disease (AD). The *C. elegans* homologue of the human amyloid precursor protein (APP) has a Cu-binding domain that confers resistance to Cu toxicity (compared to wild-type worms).⁵⁵ Furthermore, Minniti et al. found small but significant increase in median and total lifespan of worms upon CuCl₂ exposure.⁵⁶ These studies point out to a general evolutionary trend toward a decreased demand for Cu protection in higher order species and differences in Cu reduction by APP, which can contribute to the neurodegenerative process in AD.

C. elegans models have been constructed to search for genes involved in the etiology of Parkinson's disease (PD) and α -synuclein aggregation. Overall, except for α -synuclein, all the major proteins whose mutations are associated with various forms of PD are strongly conserved in *C. elegans*, especially at the level of their functional domains.⁵⁷ Lakso et al. observed movement deficits in worms with pan-neuronal expression of wild type or A53 α -synuclein.⁵⁸ The Caldwell and Nollen groups both demonstrated that α -synuclein:GFP fusion protein expressed in *C. elegans* muscle cells lead to the formation of visible fluorescent aggregates.^{59,60}

As already mentioned in this review, Mn and PD share similar characteristics and mechanisms of DAergic degeneration. In *C. elegans*, Benedetto et al. have recently demonstrated that Mn causes a dose-dependent DAergic degeneration.²⁰ Furthermore, genetic modifications of proteins inherent to the DAergic system established that accumulation of Mn in the synaptic cleft likely mediates DA oxidation and oxidative stress. The loss of function of the DA reuptake transporter gene (*dat-1*) renders the worms most sensitive to Mn, indicating that a functional DAT-1 is likely required for Mn-induced neurodegeneration.²⁰

Conclusion

These studies highlighted herein demonstrate *C. elegans*' utility as a tool to examine the neurotoxicity of various compounds. Regardless of whether toxicants closely mimic the effects noted in mammalian systems, insights into their mechanisms of action can be obtained through the use of this model organism. *C. elegans* is an ideal model to address the increased interest in new, efficient and highly reproducible approaches to toxicology and for the screening of various chemicals and the understanding of their toxicity. As demonstrated in this brief review, the nematode readily enables genetic approaches (single and multi), thus allowing for combinatorial genetic variation to be studied within the context of the influence of multigenic polymorphisms in environmental risk and vulnerability. With its ease of handling, short lifespan and inexpensive maintenance, it makes for an ideal model for the design and implementation of neurotoxicity studies.

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Figure 1. Pharyngeal pumping rates in *Caenorhabditis elegans* decrease following MeHg exposure. Number of pharyngeal pumps per minute significantly decreased in a dose-dependent manner following 30 minute MeHg exposure of L1 worms 48 hours following treatment at 0.75 and 1 mM MeHg. *Indicates significant difference from untreated group.²⁹

Figure 2. MTs protect *Caenorhabditis elegans* from depleted uranium (DU) toxicity. This figure illustrates the viability of the control N2 Bristol strain and the various *mtl*/knockout strains as the dose of DU, in the form of uranyl acetate, is increased.³⁴

Figure 3.

Examples of green fluorescent protein (GFP) tagged neurons (A) *P_{dat-1}::GFP* (dopaminergic neurons); CEPv: ventral cephalic neuron; CEPd: dorsal cephalic neuron; ADE: deirid neuron; (B) *GLR-1::GFP* (glutamatergic neurons); PVC: posterior ventral cord interneurons; AVG, AVA, AVE: anterior ventral cord interneurons.