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Null allele mutants of *trt-1*, the catalytic subunit of telomerase in *Caenorhabditis elegans*, are less sensitive to Mn-induced toxicity and DAergic degeneration

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ABSTRACT

Exposure to manganese (Mn) represents an environmental risk factor for Parkinson's disease (PD). Recent evidence suggests that telomerase reverse transcriptase (TERT), the catalytic subunit of mammalian telomerase participates in non-telomeric functions and may play a role in cellular protection from oxidative stress and DNA damage. *trt-1* is the catalytic subunit of telomerase in *Caenorhabditis elegans* (*C. elegans*). The present study investigated the relationship between *trt-1* mutation and Mn-induced neurotoxicity. Wild-type (*wt*) and *trt-1* worms were subjected to an acute Mn treatment of 1 h at the first larval (L1) stage. Survival assay and behavior (Basal slowing response, chemotaxis) were assessed. Dopaminergic (DAergic) neurodegeneration was evaluated in successful crosses of *trt-1* worms expressing green fluorescent protein (GFP) (*dat-1*:GFP worms).

trt-1 worms were less sensitive to Mn-induced lethality compared to *wt* worms. Mn induced DAergic degeneration in *wt* worms, but not in *trt-1* worms. Basal slowing was altered in both *wt* and *trt-1* worms; however *trt-1* worms were significantly less affected in their basal slowing behavior compared to *wt* worms. Mn treatment did not affect chemotaxis by NaCl in either *wt* or *trt-1* mutants worms. Combined, the results establish that null mutation in *trt-1* improves survival and attenuates damage to the DAergic system.

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1. Introduction

Manganese (Mn) is an abundant trace element that exists naturally in food, water, air and soil. It is present in body tissues in human, where it is necessary for important biological functions at physiological levels. Mn is needed for normal metabolism of carbohydrates, lipids, proteins and amino acids. It is involved in regulating growth and development, as well as homeostasis of vital cellular processes (Karki et al., 2013; Erikson et al., 2007; Aschner et al., 2005; Aschner and Aschner 2005). Mn is also vital for peak brain development and formation of skeletal structures. Ingested Mn is highly regulated and so toxicity via this route is rare. However, environmental and occupational exposures to high concentration of Mn are serious public health concerns. Despite its role in vital metabolic functions, toxic concentrations of Mn are associated with neurological deficits that result in altered cognitive functions and motor coordination. Neurotoxicity from Mn exposure results in a Parkinsonian-like syndrome, known as manganism. The syndrome presents clinical manifestations that are akin, but not identical to Parkinson's disease (PD) (Benedetto et al., 2010; Au et al., 2009).

Genetic factors are known to contribute to development of PD. However, considering that they account for only 10–12% of PD occurrences, environmental factors may play a major role in the origin of idiopathic PD. It is now well documented that Mn exposures may well be one of such environmental risk factors for PD. Occupational exposure to chronic low levels or acute high levels of Mn is the common source of Mn intoxication. However, several reports also suggest that accumulation of high levels of Mn in drinking water is a possible source of Mn intoxication. (Chen

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et al., 2015; Wasserman et al., 2011). Mn is known to accumulate in neurons, oligodendrocytes and astrocytes. Within the cell, Mn accumulates in the mitochondria, where it disrupts ATP synthesis. This causes a fall in ATP levels in the cell and an increase in oxidative stress, contributing to Mn toxicity. In addition, Mn oxidizes extracellular dopamine (DA) to reactive oxygen species. Furthermore, Mn exposure results in decreased levels of free thiol and hydroxyl groups in antioxidant proteins within the cell. The combined rise in oxidative species and fall in reductive capability can lead to dendritic disintegration and cellular toxicity (Neal and Guilarte, 2012).

Telomerase is a reverse transcriptase best known for its role in maintaining telomere in dividing cells. Two subunits are necessary for this function; the enzyme TERT (telomerase reverse transcriptase) and the RNA component TERC (telomerase RNA component) (Singhapol et al., 2013). Telomerase, and especially its catalytic subunit telomerase reverse transcriptase (TERT), participate in non-telomeric functions including control of gene expression, growth factors and cellular proliferation (Hoffmeyer et al., 2012; Park et al., 2009; Li et al., 2005). In addition, TERT translocates to the mitochondria from the nucleus upon external stress (Singhapol et al., 2013), protecting nuclear and mitochondrial DNA from oxidative stress and DNA damage (Singhapol et al., 2013; Haendeler et al., 2009). TERT expression has afforded protection in rodents (Iannilli et al., 2013) and human (Spilsbury et al., 2015) brain cells. *trt-1* is the catalytic subunit of telomerase in *C. elegans* (Meier et al., 2006). The TERT protein in humans and *C. elegans* show similarity in structure (Fig. 1).

C. elegans is an established model of Mn neurotoxicity (Avila et al., 2012; Benedetto et al., 2010; Au et al., 2009). *C. elegans* is a genetically tractable organism, is simple to grow, demonstrate life stages with a short lifespan that are predictable and well characterized, and also has a nervous system that is simple and can be visualized with ease. Thus *C. elegans* provides a complimentary research model where the DAergic system may be easily assessed (Chen et al., 2015).

The present study investigated the effects of Mn toxicity in *trt-1* mutation of *C. elegans*, with a view to assess the role of the protein TRT-1 in Mn-induced toxicity and DAergic neurodegeneration. Considering the aforementioned non-telomeric protective ability of TERT, we hypothesized that lack of *trt-1* in *C. elegans* will lead to hypersensitivity to Mn and worsen Mn-induced DAergic damage.

2. Methods

2.1. C. elegans *strains and handling*

C. elegans strains were handled and maintained at 20 °C as previously described (Benedetto et al., 2010). The following strains were used: N2, wild-type (*wt*); YA1059, *trt*-1 (*ok410*) *I*; MT15620, *cat-2* (*n4547*) *II*; BY200, *dat-1*:GFP (*vtIs1*) *V*. The *ok410* variant of *trt-1* gene used in the present study has been extensively described (Wormbase, 2016; Meier et al., 2006). The mutation is an indel with a 13 bp in-frame insertion occurring at the breakpoint of *trt-1* (*ok410*). The *ok410* variant eliminate coding sequence for several essential reverse transcriptase motifs of DY3.4 protein, and thus is predicted a null allele. All strains were obtained from the Caenorhabditis Genetics Center, CGC (US).

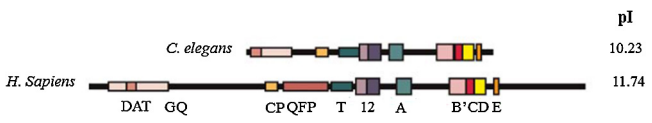

Fig. 1. Structure of TERT protein in *C. elegans* and Humans with isoelectric point (pI) indicated (modified from Meier et al., 2006).

2.2. Acute Mn exposure

Stock solution containing 2 M $MnCl_2$ (Sigma-Aldrich) were prepared in 85 mM NaCl. Acute Mn exposure was performed as previously described (Chakraborty et al., 2015). In brief, worms were synchronized by alkaline hypochlorite treatment of gravid adults and collected egg populations were placed on unseeded nematode growth media (NGM) plates to grow to the first larval stage (L1). About 2500 synchronized L1 worms were treated with $MnCl_2$ for 1 h. After Mn treatment, worms were placed on nematode growth media (NGM) plates for lethality or survival assay, evaluation of DAergic degeneration, and behavioral assays (basal slowing response and plasticity in chemotaxis by NaCl).

2.3. Survival assay

Survival assay was performed as previously described (Chakraborty et al., 2015). Wild type and trt-1 mutant worms were acutely treated as described with $MnCl_2$ (at concentrations of 0, 2.5, 5, 7.5, 10, 20, 25, 50, or 100 mM) in siliconized tubes for 1 h. Tubes containing treated worms were centrifuged at 7000 rpm for 2 min and washed 4 times with 85 mM NaCl. 30–60 worms were then plated onto OP50-seeded NGM plates in triplicates. Forty eight hours after treatment, the total number of surviving worms was scored as a percentage of the initial plated worm count. Assay was repeated in six independent worm preparations for each tested strain.

2.4. Evaluation of DAergic degeneration

BY200 (*dat-1*:GFP) worms express green fluorescent protein (GFP) in DAergic neurons. Successful crosses of dat-1:GFP worms with *trt-1* mutants were obtained to evaluate DAergic neurons. 2500 synchronized L1 worms were treated with $MnCl_2$ (0, 5 or 10 mM) as described earlier. After washing, worms were placed onto OP50-seeded NGM plates to recover. At 2 h post treatment, 20 worms per condition were mounted onto 4% agar pads (in M9 buffer) and anesthetized with 3 mM levamisole. Worms were observed under an Olympus BX41 fluorescent microscope and scored for DAergic degeneration. Each strain was scored for absence ("intact") or presence of any of the following morphological changes representing degeneration; dendritic puncta formation, shrunken soma, and loss of dendrites or soma. Representative confocal images (using the PerkinElmer Spinning Disk Confocal Microscope) of each morphological phenotype were taken and processed as previously described (Chakraborty et al., 2015). Each experiment was repeated independently four times in each strain.

2.5. Behavioral assays

2.5.1. Basal slowing response

This assay which assesses DAergic integrity is performed using protocols developed by Sawin et al., 2000. In brief, 2500 synchronized worms were acutely treated with $MnCl_2$ (0 or 5 mM) as described above, and transferred to OP50-seeded NGM plates. Twenty hours following treatment assay plates were prepared as follows; seeded assay plates were prepared by adding ~20 μL OP50 bacteria onto 60 mm plates and spread in a circle with the bottom of a small glass culture tube to give a ring-shaped bacterial lawn (see Fig. 2a). Seeded and unseeded plates were prepared per group and kept at 37° C overnight and cooled to room temperature prior to usage for the experiment the following day. Once treated worms reached young adult stage (~63 h post-synchronization), animals were washed 3 times with S-Basal buffer and then transferred to the central clear zone of the ring-

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