Organotellurium and Organoselenium compounds attenuate Mn-induced toxicity in C. elegans by preventing oxidative stress.

By: Daiana Silva Avilaa, Alexandre Benedetto, Catherine Au, Flávia Manarin, Keith Erikson, Felix Antunes Soares, João Batista Teixeira Rocha and Michael Aschner,

Abstract:

Organochalcogens have been widely studied given their antioxidant activity, which confers neuroprotection, antiulcer, and antidiabetic properties. Given the complexity of mammalian models, understanding the cellular and molecular effects of organochalcogens has been hampered. The nematode worm Caenorhabditis elegans is an alternative experimental model that affords easy genetic manipulations, green fluorescent protein tagging, and in vivo live analysis of toxicity. We previously showed that manganese (Mn)-exposed worms exhibit oxidative-stress-induced neurodegeneration and life-span reduction. Here we use Mn-exposed worms as a model for an oxidatively challenged organism to investigate the underlying mechanisms of organochalcogen antioxidant properties. First, we recapitulate in C. elegans the effects of organochalcogens formerly observed in mice, including their antioxidant activity. This is followed by studies on the ability of these compounds to afford protection against Mn-induced toxicity. Diethyl-2-phenyl-2-tellurophenyl vinyl phosphonate (DPTVP) was the most efficacious compound, fully reversing the Mn-induced reduction in survival and life span. Ebselen was also effective, reversing the Mn-induced reduction in survival and life span, but to a lesser extent compared with DPTVP. DPTVP also lowered Mn-induced increases in oxidant levels, indicating that the increased survival associated with exposure to this compound is secondary to a decrease in oxidative stress. Furthermore, DPTVP induced nuclear translocation of the transcriptional factor DAF-16/FOXO, which regulates stress responsiveness and aging in worms. Our findings establish that the organochalcogens DPTVP and ebselen act as antiaging agents in a model of Mn-induced toxicity and aging by regulating DAF-16/FOXO signaling and attenuating oxidative stress.

Article:

The reactivity of organoselenium and organotellurium compounds [1], [2] and [3], characterized by high nucleophilicity and antioxidant potential, provides the basis for their pharmacological activities in mammalian models. This class of compounds possesses anti-inflammatory, antiulcer, anticancer, and hepato- and neuroprotective properties [1], [4], [5], [6] and [7]. These pharmacological properties inherent to ebselen, diphenyl diselenide ((PhSe)2), and diethyl-2-phenyl-2-tellurophenyl vinyl phosphonate (DPTVP) are attributed to their ability to scavenge reactive oxygen species (ROS) and reactive nitrogen species [1], [5], [8] and [9]. It has been also shown that toxicity in cancer and neuroblastoma cell lines, caused by high doses of these compounds, is modulated by activation of apoptotic pathways [9], [10] and [11]. Nevertheless, because of the complexity of mammalian models, it has been difficult to determine the molecular pathways and specific proteins that are modulated in response to treatments with these compounds.

Accordingly, we used a simpler genetic model, the nematode worm Caenorhabditis elegans. C. elegans is a useful experimental model given its high degree of orthology between the worm and the human genomes, its ease of handling, and the relatively straightforward generation of knockout strains for genes of interest as well as transgenic worms expressing green fluorescent protein (GFP)-tagged genetic reporters for tissue expression or protein localization studies [12] and [13]. Thanks to the large brood size and short reproductive cycle of C. elegans, low- and high-throughput screens for toxicants have been established [14] and [15]. The evaluation of several metals in this model has contributed to novel information on their toxic mechanisms. For instance, manganese (Mn) toxicity in C. elegans has uncovered the role of dopamine and ROS in mediating Mn-induced dopaminergic (DAergic) neurodegeneration [16] and [17].

Mn-induced neurodegeneration is modulated by activation of several transcription factors. For example, Benedetto et al. demonstrated that the expression of SKN-1, the worm's orthologue of the human transcription factor nuclear erythroid-derived 2-like 2, is altered by Mn exposure [16]. SKN-1 activation, in turn, leads to transcription of antioxidant genes, conferring protection against ROS. In parallel, DAF-16, a transcription factor and the worm's orthologue of mammalian FOXO (forkhead box O), activates genes that codify antioxidant enzymes, such as superoxide dismutase (SOD) and E3 ubiquitin ligase [18]. These antioxidants neutralize reactive oxygen species, which are also involved in the aging process. Consequently, this pathway has been implicated in the regulation of stress resistance and life span [18] and [19]. Notably, DAF-

16 belongs to the DAF-2 insulin-like cascade, which is negatively regulated by phosphorylation. Inhibition of this phosphorylation cascade causes the translocation of DAF-16 into the nucleus, where it binds to a DAF-16 binding element (a core sequence TTGTTTAC of the DNA), thus increasing transcriptional activation of antioxidants and, in turn, reducing oxidative stress and slowing down the aging process [20].

To address the antioxidant mechanisms of organotellurium and organoselenium, we characterized the effects of four organochalcogens (ebselen, (PhSe)2, and diphenyl ditelluride ((PhTe)2)) and DPTVP (see Fig. 1) on C. elegans survival and life span. We hypothesized that these compounds (1) act as antioxidants, decreasing the levels of ROS and carbonylated proteins, or (2) attenuate Mn uptake in the worms, thus diminishing its toxic effects. Our results establish that DPTVP has the greatest antioxidant activity vs the other tested compounds. The attenuation of Mn toxicity by DPTVP is associated with modulation of the DAF-16 pathway, absent changes in Mn uptake in the worms.

Figure 1 has been omitted from this formatted document.

Materials and methods

Chemicals

Ebselen, (PhSe)$_2$, PhTe)$_2$, and DPTVP (Figs. 1A‑D) were synthesized according to previously described methods [21], [22] and [23]. OxyBlot protein oxidation analysis kits were purchased from Millipore (S7150; Billerica, MA, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA).

C. elegans strains and handling of the worms

C. elegans Bristol N2 (wild type) and TJ356 (DAF-16::GFP) were handled and maintained at 20 ° C on*Escherichia coli* OP50/NGM (nematode growth medium) plates as previously described [24]. All strains were provided by the *Caenorhabditis* Genetics Center (University of Minnesota, Twin Cities, MN, USA). Synchronous L1 populations were obtained by isolating embryos from gravid hermaphrodites using bleaching solution (1% NaOCl; 0.25 M NaOH), followed by flotation on a sucrose gradient to segregate eggs from dissolved worms and bacterial debris, accordingly to standard procedures previously described [25]. All experiments were carried out at 22 ° C in a humidified-controlled environment.

Dose‑response curves and acute Mn exposure treatments

The lethal dose 50% (LD$_{50}$) of organotellurium and organoselenium compounds in *C. elegans* was determined with doses ranging from 0.01 to 5 mM. Five thousand synchronized L1 worms per dose were treated at 22 ° C for 30 min by constant agitation in a rotator with each of

the compounds, followed by three washes with 85 mM NaCl solution at the end of the incubation. Worms were placed on OP50-seeded NGM plates and the dose‑response curves were plotted from scoring the number of surviving worms on each dish at 24 h postexposure. LD_{50} values were obtained from those curves. We chose doses of organochalcogens below the LD_{05} (5% lethality) for subsequent experiments. To assess the effect of organochalcogens on Mn-exposed animals, we pretreated worms for 30 min at 22 °C with nonlethal doses of each of the compounds and subsequently washed the worms three times in 85 mM NaCl. The worms were then exposed for 30 min at 22 °C to 35 mM manganese chloride ($MnCl_2$), which corresponds to the LD_{25} for $MnCl_2$ as previously reported by Benedetto et al. [16]. Next, the worms were washed and placed on OP50-seeded NGM plates. Scoring of surviving worms was performed 24 h after $MnCl_2$ exposure. For all dose‑response curves, scores were normalized to percentage control (0 mM organochalcogens/0 mM $MnCl_2$ exposure).

Life-span experiments

Synchronized L1 worms were acutely exposed to the organochalcogen compounds and/or $MnCl_2$ as described earlier. Live and healthy-looking worms (around 30 per condition; in duplicates) were collected on the same day at the late L4 stage and transferred every 4 days to new OP50-seeded NGM plates. Survival was assessed each day until all the worms died. All tested *C. elegans* strains were assessed in parallel and each experiment was performed three times. Plotted curves represent averages of those three independent experiments.

ROS measurement

Synchronized L1 were acutely pretreated for 30 min with ebselen, $(PhSe)_2$, or DPTVP followed by 30 min exposure to $MnCl_2$ (0 or 35 mM) as earlier described. Next, the worms were washed two additional times in M9 buffer. $2'$,$7'$-Dichlorodihydrofluorescein diacetate (DCF-DA) at a final concentration of 1 mM was added for 1 h and the worms were maintained in the dark. The worms were then washed four times in M9 buffer. The worms were frozen and thawed twice and homogenized by sonication and then centrifuged. The supernatants were transferred to a 96-well plate and their fluorescence levels (excitation 485 nm; emission 535 nm) were detected with a FLEXstation III (Molecular Devices, Sunnyvale, CA, USA) preheated to 37 °C. The fluorescence from each well was measured for 40 min (at 10-min intervals). Fluorescence measurements were normalized to time 0 values and rates of increased fluorescence (reflecting ROS levels) were expressed as percentage control. The experiments were repeated three times in triplicate independent worm preparations [16].

Protein oxidation determination

Twenty-thousand worms were pretreated for 30 min with DPTVP (0, 1, or 10 μM) and then exposed for 30 min to $MnCl_2$ (35 mM), as previously described. Next, the worms were homogenized by sonication in a lysis buffer containing 85 mM sodium chloride, 1% Triton X-

100, 10 mM Tris buffer (pH 6.8), 1 $\times$ protease inhibitor, and 50 mM dithiothreitol. After centrifugation (11,000 g for 1 min), the supernatant was isolated and the protein concentration was determined with the Bradford method [26]. One-hundred micrograms of proteins was derivatized with 2,4-dinitrophenylhydrazine (DNPH), which is converted to 2,4-dinitrophenylhydrazone (DNP) in the presence of carbonyls from oxidized proteins. The carbonyls were detected by Western blotting with a commercial antibody directed against derivatized carbonyl groups (anti-2,4-DNP, rabbit IgG) and visualized by horseradish peroxidase-conjugated secondary antibody following the manufacturer's instructions (OxyBlot analysis kit; Millipore). Purified β-actin (A1978; Sigma) was used as a control and the bands' density was acquired with ImageJ (W.S. Rasband, U.S. National Institutes of Health, Bethesda, MD, USA; http://imagej.nih.gov/ij/, 1997‑2011).

Mn content measurement by atomic absorption spectrophotometry

Triplicates of 10,000 L1 worms per condition were treated with $MnCl_2$ as previously described. The samples were washed eight times with NaCl and subsequently dehydrated in a vacuum oven at 65 $^{\circ}$ C for 2 h and further digested in 200 μl ultrapure nitric acid for 24 h in a sand bath (60 $^{\circ}$ C). A 20-μl aliquot was brought to a 1-ml total volume with 2% nitric acid and analyzed for Mn content using graphite furnace atomic absorption spectroscopy (Varian AA240; Varian, Palo Alto, CA, USA). Bovine liver digested in ultrapure nitric acid was used as internal standard for analysis (NBS Standard Reference Material; U.S. Department of Commerce, Washington, DC, USA; diluted at 5 μg Mn/L).

Epifluorescence microscopy

For each slide, a minimum of 20 worms were mounted on 4% agarose pads in M9 and anesthetized with 0.2% tricaine/0.02% tetramisole in M9 medium. Fluorescence was acquired with an epifluorescence microscope (Nikon Eclipse 80i; Nikon Corp., Tokyo, Japan) equipped with a Lambda LS xenon lamp (Sutter Instrument Co.) and Nikon Plan Fluor 20 $\times$ dry and Nikon Plan Apo 60 $\times$ 1.3 oil objectives. Microscopes were housed in air-conditioned rooms (20‑22 $^{\circ}$ C).

Statistics

Dose‑response lethality curves, longevity curves, and ROS content and OxyBlot analysis were generated with GraphPad Prism (GraphPad Software, Inc.). We used a sigmoidal dose‑response model with a top constraint at 100% to draw the curves and determine the LD_{50} or the average life-span values. Statistical analysis of significance was carried out using one-way analysis of variance for the dose‑response curves, longevity curves, and ROS content, followed by post hoc Bonferroni test when the overall p value was < 0.05. Unpaired two-tailed t test was used to

assess statistical differences in mean values. In all figures, error bars represent the standard error of the mean.

Results

Survival rate and life span after organochalcogen exposure

As shown in Fig. 2, the LD_{50} for $(PhSe)_2$ and ebselen corresponded to 0.05 and 0.21 mM, respectively. Worms showed higher tolerance to organotellurium vs organoselenium compounds, with LD_{50} of 2.69 and ~ 10 mM for $(PhTe)_2$ and DPTVP, respectively. Because of its high toxicity in rodent models, $(PhTe)_2$ was used in this study only to obtain the LD_{50} for comparison with DPTVP. Analogous to a previous rodent study, $(PhTe)_2$ was significantly more toxic than DPTVP [27].

Figure 2 has been omitted from this formatted document.

As shown in Fig. 3, life span after 48 h exposure to ebselen (Fig. 3A), $(PhSe)_2$ (Fig. 3B), or DPTVP (Fig. 3C) treatment, at all tested doses, was indistinguishable from controls, indicating that these compounds do not increase worms' mortality.

Figure 3 has been omitted from this formatted document.

Effects of sublethal doses of ebselen and DPTVP on Mn-induced toxicity

Mn (35 mM) caused ~ 25% decrease in worm survival. Pretreatment with sublethal doses of DPTVP (10 μM) protected the worms, increasing the survival rate to levels indistinguishable from those of the control group (Fig. 4C, $p < 0.05$ compared to the Mn-treated group). Ebselen (10 μM) also protected against Mn-induced toxicity (Fig. 4A, $p < 0.05$ compared to the Mn-treated group). Higher doses of ebselen caused an increase in Mn-induced toxicity, whereas higher DPTVP doses did not affect the worms' survival (data not shown). In contrast, $(PhSe)2$ (10 μM) failed to protect against Mn-induced toxicity, causing a significant increase in the worms' mortality (Fig. 4B, $p < 0.05$). Based on these data, for the next series of assays we chose to use the lower Mn doses, which did not cause any toxic effects.

Figure 4 has been omitted from this formatted document.

Effects of ebselen, $(PhSe)_2$, and DPTVP on Mn-induced aging

As previously noted [16], high Mn doses decreased the life span of exposed worms vs controls (Fig. 5,$p < 0.05$). Ebselen, $(PhSe)_2$, or DPTVP pretreatment prolonged the life span in Mn-exposed worms (Fig. 5,$p < 0.05$ vs the 35 mM Mn group).

Figure 5 has been omitted from this formatted document.

Effects of ebselen and DPTVP on Mn-induced oxidative stress

Because oxidative stress is associated with aging, next we characterized parameters of oxidative stress. ROS levels were determined with the DCF-DA dye, which is oxidized to the DCF fluorophore in the presence of free radicals. Mn exposure caused a significant increase in DCF-DA oxidation from $t = 20$ min, reflecting the generation of ROS (Fig. 6, $p < 0.05$). Pretreatment with ebselen, (PhSe)$_2$, or DPTVP significantly decreased ROS levels even in the control, Mn-untreated animals (Fig. 6, $p < 0.01$), resulting in a per se effect. Because DPTVP showed reduced toxic effects vs ebselen and (PhSe)$_2$ (Fig. 2), we further characterized the antioxidant efficacy of DPTVP in Mn-exposed animals by determining protein oxidation as a biochemical parameter. In Mn-treated worms, carbonyl levels were significantly increased compared to untreated worms (Fig. 7, $p < 0.05$). This increase was abolished by pretreatment of the worms with 1 or 10 μM DPTVP (Fig. 7, $p < 0.05$ vs the 35 mM Mn group). DPTVP alone showed a per se effect, given that the DPTVP-alone groups (1 and 10 μM) showed significantly lower carbonyl levels compared to the control group (Fig. 7, $p < 0.05$).

Figures 6-7 have been omitted from this formatted document.

Effects of DPTVP on DAF-16 translocation

Next, to better understand the mechanisms by which DPTVP mediates its antioxidant and antiaging mechanisms, we investigated DAF-16::GFP subcellular localization upon DPTVP pretreatment. The worm DAF-16 is orthologous to the FOXO transcription factor in humans and plays a key role in regulating longevity. As shown in Fig. 8A, DPTVP pretreatment (1 or 10 μM) led to DAF-16::GFP translocation from the cytoplasm to the nucleus (diffuse staining in Fig. 8A), consistent with the well-defined circular staining observed in treated worms [28] (Figs. 8B and C).

Fig. 8. DPTVP causes nuclear migration of transcriptional factor DAF-16 in *C. elegans.* (A) Control; (B) DPTVP 1 μM; (C) DPTVP 10 μM.
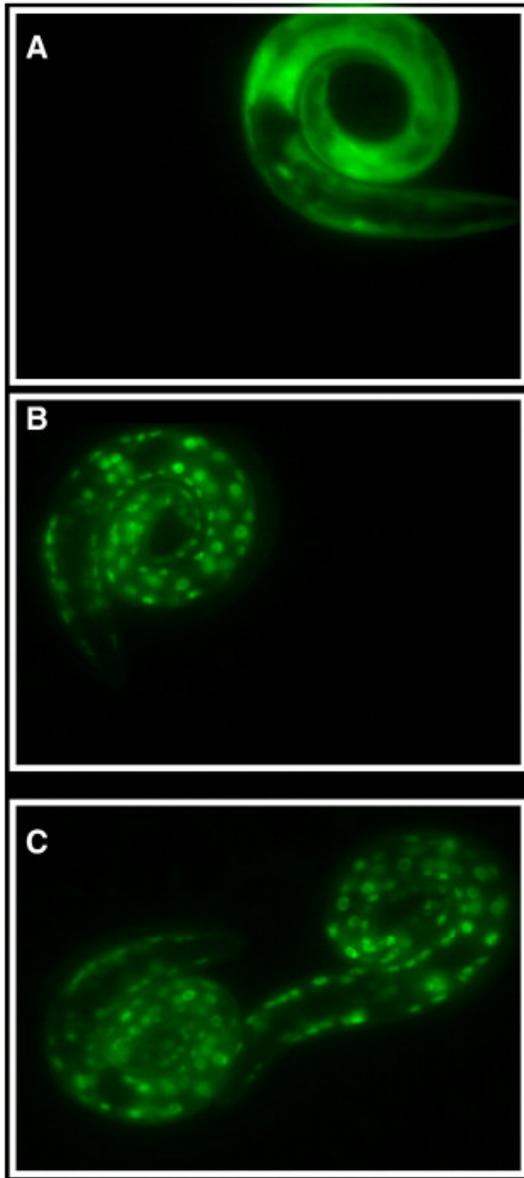
Effects of organotellurium compounds on Mn levels

To determine whether DPTVP affects Mn levels in wild-type worms, we measured Mn concentrations in whole animals. We observed no change in Mn levels upon DPTVP pretreatment compared to worms treated with Mn alone, indicating that DPTVP does not affect Mn uptake or release (Fig. 9).

Figure 9 has been omitted from this formatted document.

Discussion

In this study, we used the C. elegans model to investigate the antioxidant properties of organochalcogens within the context of a known environmental insult, Mn. We established that

ebselen, and to a greater extent DPTVP, confers protection against Mn-induced aging and toxicity. Our results suggest that these effects are due to reduction in oxidative stress associated with the nuclear translocation of the transcription factor FOXO/DAF-16. Furthermore, they occur in the absence of significant changes in whole worm Mn concentrations.

Oxidative stress is associated with the pathogenesis of several diseases, is a sequela of multiple chemical exposures, and is known to modulate longevity in several experimental models [29], [30] and [31]. Using mammalian models, researchers have studied the molecular basis of ROS generation, its cellular effects, and the efficacy of various antioxidants in mitigating ROS-induced cellular damage. The potential for organochalcogens to offer efficacious treatment for disease models associated with oxidative stress has been of interest to several research groups [1], [2], [23], [32], [33] and [34]. The ROS-scavenging activity and glutathione peroxidase mimetic property of the organochalcogens probably account for their efficacy in attenuating oxidative stress both in vitro and in in vivo rodent models [8], [32], [34], [35] and [36]. Notably, ebselen was deemed a promising molecule and it entered clinical trials [37]. Its failure in these trials has led to the design and synthesis of novel organoselenium compounds with antioxidant properties for the development of potential therapeutic agents [38] and [39]. Among several of the newly tested molecules, DPTVP has been characterized as the most potent antioxidant, as well as a hepato- and neuroprotective compound [4], [27], [40] and [41].

Previous studies have indicated that mice and rats show distinct responses to organochalcogens, with rats appearing more sensitive to organotellurium compounds [1]. Notably, diphenyl ditelluride afforded protection in adult mice (but not rats) against 4-aminopyridine-induced neurotoxicity and oxidative stress [42]. Our studies suggest that, analogous to mice, C. elegans exhibited relatively low sensitivity to the toxic effects of organotellurium compounds (Fig. 1). Furthermore, diphenyl diselenide was more toxic to C. elegans than its tellurium analogue. The rationale for the differential species sensitivity to diphenyl diselenide has yet to be determined; however, differences in the rates of metabolism and formation of toxic metabolites probably underlie this differential sensitivity [1].

In worms surviving the acute high-dose exposure to ebselen and (PhSe)2, the toxic effects were short-lasting (Figs. 3A and B), with life span in treated and untreated worms revealing no significant differences, even when ebselen and (PhSe)2 were applied at doses as high as 100 μM. The same trend was also observed for the DPTVP curve (Fig. 3C). Given these observations and the lack of chronic sequelae, we opted to study the efficacy of these compounds in attenuating Mn-induced toxicity in C. elegans.

Mn is an essential metal and important for brain development and the functioning of multiple enzymes, such as Mn-SOD and glutamine synthase [43] and [44]. Nevertheless, exposure to high levels of this metal leads to its preferential accumulation in basal ganglia [43] and [44], resulting in parkinsonian-like symptoms [44]. Mn can oxidize dopamine and lead to mitochondrial dysfunction, causing excessive ROS generation [45]. Accordingly, oxidative stress is one of the main features of Mn-induced toxicity. The C. elegans model replicates features inherent to mammalian Mn exposure [16], [17] and [46], including specificity to DAergic neurons and increased generation of oxidative stress, as well as reduced life span and morphological alterations in dopamine-containing neurons [16], [17], [46] and [47].

In this study, treatment with DPTVP or ebselen failed to extend the worms' longevity per se, although both protected against Mn-induced decrease in life span (Fig. 5). This finding is in agreement with Zhang et al. [48], who demonstrated that the antioxidant epigallocatechin gallate failed to alter life span in N2 (wild-type) worms under normal conditions (20 °C), yet increased the redox potential and survival rate in response to environmental stress [48]. Notably, DPTVP and ebselen also decreased the Mn-induced mortality (observed after 24 h exposure, Fig. 4), providing additional evidence for the ability of these compounds to confer protection against Mn-induced toxicity.

DPTVP and ebselen afford this protection by decreasing oxidative stress in response to Mn exposure. ROS generation, assayed by means of DCF-DA dye fluorescence, was significantly decreased when worms were pretreated with these compounds, in the presence or in the absence of Mn (Fig. 6). In agreement, protein oxidation levels were restored to control levels in worms treated with DPTVP (Fig. 7). This reduction in protein oxidation levels and in ROS production suggests that DPTVP may act as a scavenging agent, as other antioxidants do in worms [48] and [49].

In addition, DPTVP may also act by modulating intracellular processes that modulate antioxidant defenses. Accordingly, we further investigated whether DPTVP caused the translocation of the transcription factor DAF-16 into the nucleus. DAF-16 is the worm homologue of human FOXO belonging to the insulin/IGF-like signaling pathway, in which it is the central determinant of the endocrine control of stress response, aging, fat metabolism, fertility, and diapause [18] and [50]. Upstream of DAF-16 is the surface receptor DAF-2; its activity (upon ligand binding) triggers a phosphorylation cascade. AKT is downstream of DAF-2 and when phosphorylated, AKT, in turn, phosphorylates DAF-16, preventing its nuclear translocation. When the DAF-2 receptor is

inactivated or deleted, DAF-16 remains nonphosphorylated and therefore translocates into the nucleus, where it activates the transcription of prolongevity genes [18] and [50]. In addition, other pathways interfere with proteins upstream of DAF-16, thus affecting the translocation of this transcription factor [51]. Herein, we demonstrated for the first time that an organotellurium compound caused DAF-16 translocation from the cytosol to the nucleus. This finding indicates that organotellurium can modulate this pathway, probably by inducing the production of enzymatic antioxidants that are dependent upon DAF-16, such as SOD and glutathione peroxidase [18]. In turn, these antioxidants can neutralize ROS generated in response to Mn, thus attenuating protein oxidation.

A previous study in rats exposed to Mn for 4 months and thereafter cotreated with DPTVP for 2 weeks revealed a decrease in Mn levels in the striatum of these animals compared with controls [4]. Analogous to the present findings in C. elegans, there was decreased oxidative stress in rats treated with DPTVP. Accordingly, we investigated whether Mn levels would be decreased in worms in response to DPTVP pretreatment. The results showed no change upon such treatment, with levels indistinguishable from those in Mn-treated wild-type worms. Accordingly, tellurium, the metal inherent to DPTVP, does not seem to compete with Mn for the same transporters (Fig. 9). In fact, Wetli et al. demonstrated that ebselen decreased iron uptake in HEK293 cells; however, this decrease was not mediated through DMT1, which also transports Mn [52]. Thus, that study and ours indicate that uptake of Se and Te ions is not mediated via DMT1.

Taken together, our results show that in addition to a direct antioxidant effect [4] and [41], pretreatment with DPTVP caused DAF-16 nuclear translocation, protecting the worms from Mn-induced oxidative stress. Because aging is modulated by oxidative stress and the DAF-16 pathway [50], we posit that DPTVP possesses antioxidant and antiaging properties, thus modulating the insulin/IGF-like signaling in C. elegans. Our findings illustrate the utility of the worm model in elucidating protective and toxic mechanisms as well as for the identification of pharmacological targets for antioxidant drug development. Future studies could be profitably directed at identifying additional targets for the organochalcogens and potential pathways that reduce oxidative damage and delay the aging process.

References

C.W. Nogueira, G. Zeni, J.B. Rocha. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem. Rev., 104 (2004), pp. 6255–6285

L.A. Ba, M. Doring, V. Jamier, C. Jacob. Tellurium: an element with great biological potency and potential. Org. Biomol. Chem., 8 (2010), pp. 4203–4216

G. Zeni, D.S. Ludtke, R.B. Panatieri, A.L. Braga. Vinylic tellurides: from preparation to their applicability in organic synthesis. Chem. Rev., 106 (2006), pp. 1032–1076

D.S. Avila, D. Colle, P. Gubert, A.S. Palma, G. Puntel, F. Manarin, S. Noremberg, P.C. Nascimento, M. Aschner, J.B. Rocha, F.A. Soares. A possible neuroprotective action of a vinylic telluride against Mn-induced neurotoxicity. Toxicol. Sci., 115 (2010), pp. 194–201

H. Imai, D.I. Graham, H. Masayasu, I.M. Macrae. Antioxidant ebselen reduces oxidative damage in focal cerebral ischemia. Free Radic. Biol. Med., 34 (2003), pp. 56–63

R.P. Ineu, M.E. Pereira, M. Aschner, C.W. Nogueira, G. Zeni, J.B. Rocha. Diphenyl diselenide reverses gastric lesions in rats: involvement of oxidative stress. Food Chem. Toxicol., 46 (2008), pp. 3023–3029

C.R. Jesse, L. Savegnago, C.W. Nogueira. Mechanisms involved in the antinociceptive and anti-inflammatory effects of bis selenide in mice. J. Pharm. Pharmacol., 61 (2009), pp. 623–630

K. Briviba, R. Tamler, L.O. Klotz, L. Engman, I.A. Cotgreave, H. Sies. Protection by organotellurium compounds against peroxynitrite-mediated oxidation and nitration reactions. Biochem. Pharmacol., 55 (1998), pp. 817–823

L. Engman, T. Kandra, A. Gallegos, R. Williams, G. Powis. Water-soluble organotellurium compounds inhibit thioredoxin reductase and the growth of human cancer cells. Anticancer Drug Des., 15 (2000), pp. 323–330

G. Scambia, P.B. Panici, F. Battaglia, G. Ferrandina, C. Gaggini, S. Mancuso. Growth inhibitory effect of diheptyl diselenide on various human cancer cell lines. Anticancer Res., 9 (1989), pp. 1697–1699

T. Posser, M.T. de Paula, J.L. Franco, R.B. Leal, J.B. da Rocha. Diphenyl diselenide induces apoptotic cell death and modulates ERK1/2 phosphorylation in human neuroblastoma SH-SY5Y cells. Arch. Toxicol., 85 (2011), pp. 645–651

M. Chalfie, Y. Tu, G. Euskirchen, W.W. Ward, D.C. Prasher. Green fluorescent protein as a marker for gene expression. Science, 263 (1994), pp. 802–805

M.B. Gerstein, Z.J. Lu, E.L. Van Nostrand, C. Cheng, B.I. Arshinoff, T. Liu, K.Y. Yip, R. Robilotto, A. Rechtsteiner, K. Ikegami, P. Alves, A. Chateigner, M. Perry, M. Morris, R.K. Auerbach, X. Feng, J. Leng, A. Vielle, W. Niu, K. Rhrissorrakrai, A. Agarwal, R.P. Alexander, G. Barber, C.M. Brdlik, J. Brennan, J.J. Brouillet, A. Carr, M.S. Cheung, H. Clawson, S. Contrino, L.O. Dannenberg, A.F. Dernburg, A. Desai, L. Dick, A.C. Dose, J. Du, T. Egelhofer, S. Ercan, G. Euskirchen, B. Ewing, E.A. Feingold, R. Gassmann, P.J. Good, P. Green, F. Gullier, M. Gutwein, M.S. Guyer, L. Habegger, T. Han, J.G. Henikoff, S.R. Henz, A. Hinrichs, H. Holster, T. Hyman, A.L. Iniguez, J. Janette, M. Jensen, M. Kato, W.J. Kent, E. Kephart, V. Khivansara, E. Khurana, J.K. Kim, P. Kolasinska-Zwierz, E.C. Lai, I. Latorre, A. Leahey, S. Lewis, P. Lloyd, L. Lochovsky, R.F. Lowdon, Y. Lubling, R. Lyne, M. MacCoss, S.D. Mackowiak, M. Mangone, S. McKay, D. Mecenas, G. Merrihew, D.M. Miller III, A. Muroyama, J.I. Murray, S.L. Ooi, H. Pham, T. Phippen, E.A. Preston, N. Rajewsky, G. Ratsch, H. Rosenbaum, J. Rozowsky, K. Rutherford, P. Ruzanov, M. Sarov, R. Sasidharan, A. Sboner, P. Scheid, E. Segal, H. Shin, C. Shou, F.J. Slack, C. Slightam, R. Smith, W.C. Spencer, E.O. Stinson, S. Taing, T. Takasaki, D. Vafeados, K. Voronina, G. Wang, N.L. Washington, C.M. Whittle, B. Wu, K.K. Yan, G. Zeller, Z. Zha, M. Zhong, X. Zhou, J. Ahringer, S. Strome, K.C. Gunsalus, G. Micklem, X.S. Liu, V. Reinke, S.K. Kim, L.W. Hillier, S. Henikoff, F. Piano, M. Snyder, L. Stein, J.D. Lieb, R.H. Waterston. Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. Science, 330 (2010), pp. 1775–1787

K.J. Helmcke, D.S. Avila, M. Aschner. Utility of Caenorhabditis elegans in high throughput neurotoxicological research. Neurotoxicol. Teratol., 32 (2010), pp. 62–67

R.T. Peterson, R. Nass, W.A. Boyd, J.H. Freedman, K. Dong, T. Narahashi. Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology, 29 (2008), pp. 546–555

A. Benedetto, C. Au, D.S. Avila, D. Milatovic, M. Aschner. Extracellular dopamine potentiates Mn-induced oxidative stress, lifespan reduction, and dopaminergic neurodegeneration in a BLI-3-dependent manner in Caenorhabditis elegans. PLoS Genet., 6 (2010)

R. Settivari, J. Levora, R. Nass. The divalent metal transporter homologues SMF-1/2 mediate dopamine neuron sensitivity in Caenorhabditis elegans models of manganism and Parkinson disease. J. Biol. Chem., 284 (2009), pp. 35758–35768

R. Baumeister, E. Schaffitzel, M. Hertweck. Endocrine signaling in Caenorhabditis elegans controls stress response and longevity. J. Endocrinol., 190 (2006), pp. 191–202

S.S. Lee, S. Kennedy, A.C. Tolonen, G. Ruvkun. DAF-16 target genes that control C. elegans life-span and metabolism. Science, 300 (2003), pp. 644–647

C.T. Murphy. The search for DAF-16/FOXO transcriptional targets: approaches and discoveries. Exp. Gerontol., 41 (2006), pp. 910–921

A.L. Braga, E.F. Alves, C.C. Silveira, L.H. Andrade. Stereoselective addition of sodium organyl chalcogenolates to alkynylphosphonates: synthesis of diethyl 2-(organyl)-2-(organochalcogenyl)vinylphosphonates. Tetrahedron Lett., 41 (2000), pp. 161–163

W.S. Haller, K.J. Irgolic. Diaryl ditellurides from Grignard-reagents and elemental tellurium. J. Organomet. Chem., 39 (1972), pp. 97–101

C. Paulmier. Selenoorganic functional groups. C. Paulmier (Ed.), Selenium Reagents and Intermediates in Organic Synthesis, Pergamon Press, Oxford (1986)

S. Brenner. The genetics of Caenorhabditis elegans. Genetics, 77 (1974), pp. 71–94

T. Stiernagle. Maintenance of C. elegans. I.A. Hope (Ed.), C. elegans: a Practical Approach, Oxford Univ. Press, New York (1999)

M.M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem., 72 (1976), pp. 248–254

D.S. de Avila, M.C. Beque, V. Folmer, A.L. Braga, G. Zeni, C.W. Nogueira, F.A. Soares, J.B. Rocha. Diethyl 2-phenyl-2 tellurophenyl vinylphosphonate: an organotellurium compound with low toxicity. Toxicology, 224 (2006), pp. 100–107

J.C. Schafer, M.E. Winkelbauer, C.L. Williams, C.J. Haycraft, R.A. Desmond, B.K. Yoder. IFTA-2 is a conserved cilia protein involved in pathways regulating longevity and dauer formation in Caenorhabditis elegans. J. Cell Sci., 119 (2006), pp. 4088–4100

B. Halliwell, J.M. Gutteridge, C.E. Cross. Free radicals, antioxidants, and human disease: where are we now? J. Lab. Clin. Med., 119 (1992), pp. 598–620

T. Toda, M. Nakamura, H. Morisawa, M. Hirota, R. Nishigaki, Y. Yoshimi. Proteomic approaches to oxidative protein modifications implicated in the mechanism of aging. Geriatr. Gerontol. Int., 10 (Suppl. 1) (2010), pp. S25–31

S.O. Simmons, C.Y. Fan, R. Ramabhadran. Cellular stress response pathway system as a sentinel ensemble in toxicological screening. Toxicol. Sci., 111 (2009), pp. 202–225

L. Engman, N. Al-Maharik, M. McNaughton, A. Birmingham, G. Powis. Thioredoxin reductase and cancer cell growth inhibition by organotellurium compounds that could be selectively incorporated into tumor cells. Bioorg. Med. Chem., 11 (2003), pp. 5091–5100

M. Parnham, H. Sies. Ebselen: prospective therapy for cerebral ischaemia. Expert Opin. Investig. Drugs, 9 (2000), pp. 607–619

H. Sies, G.E. Arteel. Interaction of peroxynitrite with selenoproteins and glutathione peroxidase mimics. Free Radic. Biol. Med., 28 (2000), pp. 1451–1455

B.K. Sarma, G. Mugesh. Antioxidant activity of the anti-inflammatory compound ebselen: a reversible cyclization pathway via selenenic and seleninic acid intermediates. Chemistry, 14 (2008), pp. 10603–10614

A. Sausen de Freitas, A. de Souza Prestes, C. Wagner, J. Haigert Sudati, D. Alves, L. Oliveira Porciuncula, I.J. Kade, J.B. Teixeira Rocha. Reduction of diphenyl =diselenide and analogs by mammalian thioredoxin reductase is independent of their glutathione peroxidase-like activity: a possible novel pathway for their antioxidant activity. Molecules, 15 (2010), pp. 7699–7714

T. Yamaguchi, K. Sano, K. Takakura, I. Saito, Y. Shinohara, T. Asano, H. Yasuhara. Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial: Ebselen Study Group. Stroke, 29 (1998), pp. 12–17

A. Ogawa, T. Yoshimoto, H. Kikuchi, K. Sano, I. Saito, T. Yamaguchi, H. Yasuhara. Ebselen in acute middle cerebral artery occlusion: a placebo-controlled, double-blind clinical trial. Cerebrovasc. Dis., 9 (1999), pp. 112–118

I. Saito, T. Asano, K. Sano, K. Takakura, H. Abe, T. Yoshimoto, H. Kikuchi, T. Ohta, S. Ishibashi. Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. Neurosurgery, 42 (1998), pp. 269–277 discussion 277-268

D.S. Avila, P. Gubert, C.L. Dalla Corte, D. Alves, C.W. Nogueira, J.B. Rocha, F.A. Soares. A biochemical and toxicological study with diethyl 2-phenyl-2-tellurophenyl vinylphosphonate in a sub-chronic intraperitoneal treatment in mice. Life Sci., 80 (2007), pp. 1865–1872

D.S. Avila, P. Gubert, A. Palma, D. Colle, D. Alves, C.W. Nogueira, J.B. Rocha, F.A. Soares. An organotellurium compound with antioxidant activity against excitotoxic agents without neurotoxic effects in brain of rats. Brain Res. Bull., 76 (2008), pp. 114–123

V.B. Brito, J.B. Rocha, V. Folmer, F. Erthal. Diphenyl diselenide and diphenyl ditelluride increase the latency for 4-aminopyridine-induced chemical seizure and prevent death in mice. Acta Biochim. Pol., 56 (2009), pp. 125–134

]M. Aschner, K.M. Erikson, E. Herrero Hernandez, R. Tjalkens. Manganese and its role in Parkinson's disease: from transport to neuropathology. Neuromolecular Med., 11 (2009), pp. 252–266

A. Benedetto, C. Au, M. Aschner. Manganese-induced dopaminergic neurodegeneration: insights into mechanisms and genetics shared with Parkinson's disease. Chem. Rev., 109 (2009), pp. 4862–4884

R. Graumann, I. Paris, P. Martinez-Alvarado, P. Rumanque, C. Perez-Pastene, S.P. Cardenas, P. Marin, F. Diaz-Grez, R. Caviedes, P. Caviedes, J. Segura-Aguilar. Oxidation of dopamine to

aminochrome as a mechanism for neurodegeneration of dopaminergic systems in Parkinson's disease: possible neuroprotective role of DT-diaphorase. Pol. J. Pharmacol., 54 (2002), pp. 573–579

C. Au, A. Benedetto, J. Anderson, A. Labrousse, K. Erikson, J.J. Ewbank, M. Aschner. SMF-1, SMF-2 and SMF-3 DMT1 orthologues regulate and are regulated differentially by manganese levels in C. elegans. PLoS One, 4 (2009), p. e7792

J. Xiao, Q. Rui, Y. Guo, X. Chang, D. Wang. Prolonged manganese exposure induces severe deficits in lifespan, development and reproduction possibly by altering oxidative stress response in Caenorhabditis elegans. J. Environ. Sci. (China), 21 (2009), pp. 842–848

L. Zhang, G. Jie, J. Zhang, B. Zhao. Significant longevity-extending effects of EGCG on Caenorhabditis elegans under stress. Free Radic. Biol. Med., 46 (2009), pp. 414–421

T.M. Bass, D. Weinkove, K. Houthoofd, D. Gems, L. Partridge. Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. Mech. Ageing Dev., 128 (2007), pp. 546–552

M. Tatar, A. Bartke, A. Antebi. The endocrine regulation of aging by insulin-like signals. Science, 299 (2003), pp. 1346–1351

J.M. Tullet, M. Hertweck, J.H. An, J. Baker, J.Y. Hwang, S. Liu, R.P. Oliveira, R. Baumeister, T.K. Blackwell. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell, 132 (2008), pp. 1025–1038

H.A. Wetli, P.D. Buckett, M. Wessling-Resnick. Small-molecule screening identifies the selanazal drug ebselen as a potent inhibitor of DMT1-mediated iron uptake. Chem. Biol., 13 (2006), pp. 965–972