A Possible Neuroprotective Action of a Vinylic Telluride against Mn-Induced Neurotoxicity

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Manganese (Mn) is a metal required by biological systems. However, environmental or occupational exposure to high levels of Mn can produce a neurological disorder called manganism, which has similarities to Parkinson's disease. Diethyl-2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) is an organotellurium compound with a high antioxidant activity, especially in the brain. The present study was designed to investigate the effects of long-term low-dose exposure to Mn in drinking water on behavioral and biochemical parameters in rats and to determine the effectiveness of vinylic telluride in attenuating the effects of Mn. After 4 months of treatment with MnCl₂ (13.7 mg/kg), rats exhibited clear signs of neurobehavioral toxicity, including a decrease in the number of rearings in the open field and altered motor performance in rotarod. The administration of DPTVP (0.150 μmol/kg, ip, 2 weeks) improved the motor performance of Mn-treated rats, indicating that the compound could be reverting Mn neurotoxicity. Ex vivo, we observed that Mn concentrations in the Mn-treated group were highest in the striatum, consistent with a statistically significant decrease in mitochondrial viability and [³H]glutamate uptake, and increased lipid peroxidation. Mn levels in the hippocampus and cortex were indistinguishable from controls, and no significant differences were noted in the ex vivo assays in these areas. Treatment with DPTVP fully reversed the biochemical parameters altered by Mn. Furthermore, DPTVP treatment was also associated with a reduction in striatal Mn levels. Our results demonstrate that DPTVP has neuroprotective activity against Mn-induced neurotoxicity, which may be attributed to its antioxidant activity and/or its effect on striatal Mn transport.

Key Words: tellurium; manganese neurotoxicity; oxidative stress; glutamate; mitochondria; neuroprotector.

Chronic environmental or occupational exposure to Mn in mammals can lead to the development of a parkinsonian-like or dystonic state, referred to as manganism (Barbeau, 1984; Wang et al., 1989). Special concern has been raised regarding the sequelae of chronic exposure to Mn not only in occupationally exposed individuals but also within the population at large, given the increased usage of Mn as a gasoline additive (methylcyclopentadienyl manganese tricarbonyl) (Hinderer, 1979). Mn exposure can cause motor deficits in mammals that are characterized by extrapyramidal motor abnormalities, such as rigidity, hypokinesia, tremor, and gait disturbances (Newland, 1999). Such effects have been attributed to a selective interaction of Mn with the basal ganglia downstream of the nigrostriatal dopaminergic projection and, in particular, with the globus pallidus and striatum, which are areas that have a propensity to preferentially accumulate Mn (Shinotoh et al., 1997). Nevertheless, it has also been reported that Mn neurotoxicity may also be due to an indirect excitotoxic event caused by altered glutamate metabolism, preceding the changes in the dopaminergic system (Brouillet et al., 1993; Erikson and Aschner, 2002; Lee et al., 2009a; Sidoryk-Wegrzynowicz et al., 2009).

Studies have demonstrated that astrocytes accumulate Mn through a high affinity, high capacity, and specific transport system (Aschner et al., 1992), with the ensuing development of Alzheimer type II astrocytosis (Pentschew et al., 1963). In this condition, astrocytes show morphological and metabolic alterations, leading to altered function (Cavanagh and Kyu, 1971). As synaptic glutamate reuptake predominantly occurs in astrocytes (Danbolt, 2001), this function is impaired by Mn exposure, as already demonstrated in vitro (Hazell and Norembarg, 1997). Furthermore, Erikson and Aschner (2002) have shown that the Mn-induced decrease in glutamate uptake is associated with decreased messenger RNA expression of the glutamate/aspartate transporter (GLAST), the major glutamate transporter in astrocytes. This causes an abnormal increase in glutamate levels in the synaptic cleft, which is excitotoxic to neurons (Choi, 1988).

It remains unknown how Mn exposure can lead to altered glutamatergic transmission; however, the current hypothesis considers the role of impaired energy metabolism resulting from mitochondrial dysfunction and free radical production...
(Erikson et al., 2004). At the cellular level, Mn accumulates in mitochondria (Morello et al., 2008) and inhibits the complexes of the electron transport chain (Zhang et al., 2004), which impairs oxidative phosphorylation (Gavin et al., 1992) and ATP production (Brouillet et al., 1993). The resulting decreased energy production alters mitochondrial permeability transition, leading to excessive Ca\(^{2+}\) influx and massive reactive oxygen species (ROS) production (Gavin et al., 1992). ROS, in turn, cause oxidation of important cellular components, such as lipids (Halliwell and Chirico, 1993), as well as alterations in excitatory transmission by causing abnormal glutamate release and defective glutamate uptake (Danbolt, 2001).

Given that oxidative stress plays a central role in Mn-induced neurotoxicity, several authors have investigated the potential neuroprotective effect of antioxidants against Mn-induced toxicity. In mitochondrial preparations, N-acetylcysteine (NAC), glutathione, and vitamin C prevented ROS production caused by high concentrations of Mn (Zhang et al., 2004). In cultured astrocytes, Chen and Liao (2002) observed that NAC attenuated the prooxidant effects of Mn. Ex vivo, Hazell et al. (2006) reported that NAC blocked the astrocytosis caused by acute exposure to Mn. These studies demonstrate that antioxidant treatment is effective against the toxic effects of Mn in the nervous tissue.

Organotellurium compounds are potent in vitro antioxidants (Engman et al., 1995; Souza et al., 2009). Nevertheless, these compounds can also be extremely toxic following in vivo exposure (Laden and Porter, 2001; Nogueira et al., 2004). It appears that the nature of the organic moiety can considerably influence the toxicity of organotellurides in rodents (Borges et al., 2008; Savegnago et al., 2006). Nevertheless, diethyl-2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) is a vinyl telluride compound with very low toxicity (LD\(_{50}\) to mice > 500 \(\mu\)mol/kg and to rats ~4 \(\mu\)mol/kg), as well no neurotoxic effects, as it does not alter oxidant/prooxidant balance, mitochondrial viability nor behavior. (Avila et al., 2007, 2008). Furthermore, this compound has high antioxidant activity (Avila et al., 2007, 2008; de Avila et al., 2006) and was reported to be an efficient hepatoprotector in a model involving marked oxidative stress (Avila, Palma, Colle, Scolari, Manarin, Silveira, Nogueira, Rocha, and Soares, unpublished date). Given the ability of Mn to increase ROS generation in the brain (Aschner et al., 2007; Erikson et al., 2004), we hypothesize that the chronic effects of Mn could be attenuated by treatment with a vinyllic telluride compound. Thus, the objectives of the study were to investigate in a rat model the effects of long-term low-dose Mn exposure on (1) in vivo motor activity, (2) biochemical parameters in various brain areas ex vivo, and (3) whether the antioxidant activity of vinyllic telluride provides an efficacious treatment modality in attenuating both the behavior and the biochemical alterations caused by Mn in a rat model. Animals were evaluated for motor performance in the open-field and rotarod tasks, and biochemical measures included rates of lipid peroxidation; mitochondrial viability; ROS production activity; and \(^{3}H\)glutamate uptake in cortex, hippocampus, and striatum of rats, which permits assessment of regional brain sensitivity in response to in vivo Mn exposure.

**MATERIALS AND METHODS**

**Chemicals**

DPTVP was synthesized by the addition of alkynylphosphonates to a solution of sodium organyl tellurolate, prepared by the reduction of diorganyl ditellurides with sodium borohydride in ethanol at room temperature (Braga et al., 2000). Manganese chloride (MnCl\(_{2}\)·H\(_{2}\)O –99% of purity) and all other chemicals were of analytical grade and obtained from standard commercial suppliers.

**Animals**

Adult Wistar male rats (200–250 g) from our own breeding colony were maintained in an air-conditioned room (20°C–25°C) under natural lighting conditions with water and food (Guabi-RS, Brazil) ad libitum. All experiments were conducted in accordance with the Guiding Principles of the Animal Care and Wellness Committee of the Universidade Federal de Santa Maria.

**Animal Treatments**

Twenty animals were randomly divided into two groups containing 10 animals each. Group 1 received drinking water daily for 4 months and group 2 received drinking water containing 13.7 mg/kg of MnCl\(_{2}\) daily for 4 months. The Mn dosage was selected based on previous pilot experiments in which the progression of behavioral alterations was characterized (data not shown), which corresponded to a dose of 4.11 mg/day of Mn to a rat weighting 300 mg.

Water consumption was monitored every 2 days in order to correct Mn dosage if necessary, gain weight was monitored every 2 weeks, and behavioral alterations were determined monthly. At the onset of behavioral alterations in group 2 animals, both groups (1 and 2) were randomly divided into four groups containing five animals each as follows:

- 1-Control (water/canola oil, ip)
- 2-DPTVP control (water/DPTVP 0.150 \(\mu\)mol/kg, ip)
- 3-Mn (MnCl\(_{2}\)/canola oil, ip)
- 4-Mn + DPTVP (MnCl\(_{2}\)/DPTVP 0.150 \(\mu\)mol/kg, ip)

Rats were injected ip with vehicle control or DPTVP daily for 2 weeks with continuous treatment in the drinking water (control or Mn).

**Behavioral Evaluations**

**Open field.** Rats were individually placed at the center of a clean open-field apparatus (45 × 45 × 30 cm, divided into nine squares). Prior to the evaluation, animals were habituated to the box for 1 min within the box. Spontaneous ambulation (number of segments crossed with the four paws) and exploratory activity (expressed by the number of rearings on the hind limbs) were recorded for 6 min by two blind observers (Burger et al., 2005).

**Rotarod task.** The integrity of the motor system was evaluated with the rotarod test. Briefly, the rotarod apparatus consists of a rod 30-cm long and 3 cm in diameter that is subdivided into three compartments by discs 24 cm in diameter. The rod rotates at a constant speed of 10 rpm. The animals were selected 24 h previously to the beginning of the Mn treatment by eliminating those rats that did not remain on the bar for two consecutive periods of 60 s. For rats already treated with Mn and/or DPTVP, animals were only habituated to the rotarod 24 h previously the test. On the day of the evaluation, rats were subjected to four trials by two different blinded observers. The latency for first fall off from the apparatus and number of falls were scored. The cut-off time was 120 s.

**Biochemical Analysis**

**Tissue preparation.** At the end of the treatment, rats were sacrificed by decapitation; the brain was removed; and the cortices, the two hippocampi, and the striata were dissected. One cortex, one hippocampus, and one striatum were randomly chosen and homogenized (1:10) in 10mM Tris buffer (pH 7.4) and
which was remained on the samples overnight. In the following day, aliquots (10 µl) were centrifuged (3000 × g, 15 min) and thiobarbituric acid reactive substances (TBARS) levels were measured at 532 nm (Rios and Santamaria, 1991). An aliquot of the homogenate was used for protein determination, as described below.

Mitochondrial viability. Mitochondrial function in the cortex, hippocampus, and striatum was quantified by measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a dark violet formazan product by mitochondrial dehydrogenases (Mosmann, 1983). Slices (0.4 mm) of the brain areas were preincubated at 37°C for 15 min in 500 µl of oxygenated buffer, containing (in millimolar): 118 NaCl, 1.2 KH2PO4, 4.7 KCl, 2.5 CaCl2, and 1.17 MgSO4. Then, the MTT reduction assay was started by adding 0.5 mg/ml MTT. After 60 min of incubation, medium was removed and the formazan in the slices was solubilized in 1.5 ml dimethyl sulfoxide. The rate of MTT reduction was measured spectrophotometrically in the supernatant at a test wavelength of 570 nm and a reference wavelength of 630 nm. The slices were solubilized in SDS 1%, and an aliquot was used for protein determination, as described below.

3H-glutamate uptake. Slices were preincubated at 35°C for 15 min and then washed with a Hank’s buffered salt solution (HBSS) containing: 137mM NaCl, 1.26mM CaCl2, 0.41mM MgSO4, 0.49mM MgCl2, and 5.55mM glucose, adjusted to pH 7.2. The glutamate uptake assay was performed according to Frizzo et al. (2002) with some modifications. Briefly, uptake was carried out at 35°C by adding 100µM of unlabeled glutamate and 0.33 µCi/ml [3H]glutamate (specific activity 49.0 Ci/mmol; Amersham Pharmacia Biotech). The reaction was stopped after 7 (cortex), 5 (hippocampus), and 3 (striatum) min by washing two times with 1 ml cold HBSS, immediately followed by addition of 0.5N NaOH, which was remained on the samples overnight. In the following day, aliquots (10 µl) were collected for protein measurement, as described below. Na+-independent uptake was determined by using choline chloride instead of NaCl, which was subtracted from the total uptake to obtain the Na+-dependent uptake. Incorporated radioactivity was determined with a Packard scintillator (TRI-CARB 2100 TR). All experiments were performed in triplicate and corrected by protein content.

Measurement of Mn levels. Total Mn content was determined in an atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn in the samples was determined using an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn content was determined in an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn content was determined in an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn content was determined in an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn content was determined in an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added. Total Mn content was determined in an air-acetylene flame atomic absorption spectrometer (navAA 300; Analytik Jena), using 1 g/l of Mn peroxide added.

Protein determination. Aliquots from the S1 and homogenized slices were separated to protein measurements that were assessed according to the method of Lowry et al. (1951).

Statistical Analysis

Statistical significance was assessed by multivariate analysis of variance (for repeated measures of body weight) or by one-way ANOVA, followed by Student-Newman-Keuls test for post hoc comparison. Results were considered statistically significant when p < 0.05.

RESULTS

Behavioral Evaluations

After 4 months of treatment with MnCl2, rats showed no statistically significant changes in their body weight (Table 1).

Nevertheless, Mn treatment was associated with significant alterations in the behavioral tests, characterized by a decrease in the number of rearings in the open field, a decrease in the latency to the first fall, and increase in the number of falls in the rotarod task (p < 0.05; Table 2). DPTVP coadministration for 14 days (0.150 µmol/kg, ip) improved the performance of the Mn-exposed rats in the open-field task, once their number of rearings was indistinguishable from control group (Fig. 1B). No statistically significant effect of DPTVP was observed in the number of crossings (Fig. 1A). Furthermore, the vinyltelluride administration in Mn-exposed rats also lead to an amelioration of the latency to the first fall in the rotarod task, once the values were statistically identical from controls (p < 0.05; Table 2, Fig. 1C). Indicating that vinyltelluride treatment reversed the effect of Mn to levels statistically indistinguishable from controls. No improvement was observed in the number of falls (data not shown).

Biochemical Analysis

As shown in Figure 2, Mn exposure caused a significant increase in TBARS production in the striatum (p < 0.05; Fig. 2C) but not in the cortex and hippocampus (Figs. 2A and 2B). Subchronic treatment with DPTVP led to a decrease in striatal lipid peroxidation to levels indistinguishable from controls (Fig. 2C). Additionally, mitochondrial viability was significantly

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TABLE 1

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<th>Body Weight Values (grams) of Control and Mn-Treated Rats after 4 Months of Treatment and after DPTVP Posttreatment</th>
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<td>Mn + DPTVP</td>
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Note. Data are expressed as mean ± SEM for each group (n = 5). BW 0, body weight before Mn treatment; BW 4 mo, body weight after 4 months of Mn treatment; BW end, body weight after DPTVP cotreatment.

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TABLE 2

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<th>Behavioral Alterations in Rats Caused by 4 Months of Mn Treatment in the Drinking Water</th>
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Note. Data are expressed as mean ± SEM for each group (n = 5). *Statistical difference from control group.
decreased by Mn exposure, and this effect was inherent only to the striatum ($p < 0.05$; Fig. 3C). Cortical and hippocampal MTT reduction were not significantly affected by Mn treatment. Treatment with telluride reversed the striatal Mn-induced mitochondria effect to levels indistinguishable from controls ($p < 0.05$; Fig. 3C). Similarly, [$^3$H]glutamate uptake was significantly decreased by Mn treatment in the striatum ($p < 0.05$; Fig. 4C), but no alterations were observed in the cortex and hippocampus (Figs. 4A and 4B). DPTVP administration effectively attenuate the Mn-induced [$^3$H]glutamate uptake inhibition to control levels ($p < 0.05$; Fig. 4C).

**Mn Levels**

Mn levels were significantly increased in the striatum of Mn-treated animals compared with the controls ($p < 0.05$; Fig. 5). Striatum from control animals contained $0.26 \pm 0.06 \mu g$ Mn/g, while Mn-treated ones contained $0.52 \pm 0.16 \mu g$ g. In animals
treated with DPTVP Mn levels were similar to those in controls (0.204 ± 0.027 μg/g) (p < 0.05; Fig. 5).

DISCUSSION

The present study shows for the first time the neuroprotective efficacy of DPTVP against Mn-induced neurotoxicity in a rat model of physiologically relevant chronic low-dose drinking water exposure. Mn exposure was associated with significant behavioral alterations, concomitant with increased oxidative stress, and alterations in the glutamatergic neurotransmission in rat striatum. Notably, 14-day vinylic telluride cotreatment fully restored all measured parameters to control levels, establishing the efficacy of this compound as a potential treatment modality for Mn exposure.

Exposure to Mn levels higher than the adequate intake (2.3 mg/day for men and 1.8 mg/day for women) may lead to manganism, a disorder characterized by dopaminergic degeneration and a complex behavioral syndrome, which includes motor deficits (Barbeau, 1984). Behavioral tests, such as the...
open-field task, permit the evaluation of primary motor activity. Previous studies have shown that Mn exposure can increase (St-Pierre et al., 2001), decrease (Vezer et al., 2007), or result in unchanged activity levels in animals, dependent upon the route of administration, the dose, and the exposure period (Dorman et al., 2000). The present study establishes decreased exploratory activities in Mn-treated rats in a chronic low-dose drinking water exposure paradigm. Furthermore, when motor coordination was appraised in the rotarod apparatus, animals treated with Mn showed lower latencies to the first fall in comparison to controls, corroborating Mn-induced impaired motor activity (Vezer et al., 2007).

The Mn-induced neurobehavioral alterations were associated with increased oxidative stress and impairment of glutamatergic homeostasis. The biochemical analysis showed increased lipid peroxidation (Fig. 2C), decreased mitochondrial viability (Fig. 3C), and attenuated [3H]glutamate uptake (Fig. 4C) in the striatum concomitant with significantly elevated Mn levels in this brain area. These results are consistent with previous reports in the literature, which show the preferred Mn accumulation in basal ganglia in several rats and nonhuman primate models (Aschner et al., 2005, 2007; Newland, 1999). Considering that striatum is an important brain region for integration of sensorimotor function by central dopaminergic-glutamatergic interactions (Calabresi et al., 1997), the damage caused by Mn is fully consistent with impairment of motor performance.

Compared to several other tellurium compounds, DPTVP has been shown to possess low toxicity and to have an effective antioxidant action in cerebral tissue in vitro (Avila et al., 2008; de Avila et al., 2006). Consistent with these observations, DPTVP has also been shown to protect against SNP-induced decrease in mitochondrial viability in several brain regions in vitro, reinforcing its potent antioxidant capacity (Avila et al., 2008). Nevertheless, there were no previous studies establishing a possible neuroprotective efficacy of DPTVP in an in vivo model.

We observed that attenuated Mn-induced generation of ROS upon DPTVP treatment led to behavioral recovery and restoration of the biochemical parameters that were altered by Mn exposure in striatum. It has been reported in the literature that organochalcogens compounds can prevent or revert altered behavior in rodents (Burger et al., 2005; Fachinetto et al., 2007), effects that have been attributed to their antioxidant action. The DPTVP effects depicted here can be related to the findings of Hazell et al. (2006), who demonstrated that cotreatment with NAC in rats protected from Mn-induced astrocytosis in the brain. Furthermore, oxidative stress caused by Mn in different models can be protected by antioxidants in vitro (Lee et al., 2009a,b; Marreilha dos Santos et al., 2008), supporting that oxidative stress is a key mechanism for Mn-induced neurotoxicity.

Intracellularly, high levels of Mn are commonly noted within mitochondria (Gavin et al., 1992; Malecki, 2001), leading to the collapse of the mitochondrial membrane potential and resulting in bioenergetic deficits and enhanced generation of free radicals (Gavin et al., 1992). Thus, our findings of compromised mitochondrial viability are in agreement with previous findings (Malecki, 2001; Sidoryk-Wegrzynowicz et al., 2009; Yin et al., 2008). Furthermore, the overproduction of ROS causes oxidation of proteins, DNA, and, in particular, lipids (Halliwell and Chirico, 1993), as observed in the present study as increased lipid peroxidation in the striatum. DPTVP treatment restored lipid peroxidation to control levels and increased the mitochondrial viability that had been altered by Mn exposure.

Consistent with its ability to attenuate ROS generation, DPTVP reversed the Mn-induced inhibition of [3H]glutamate uptake in striatum. It has been reported that Mn can affect the glutamatergic system due to its accumulation in astrocytes, impairing astrocytic glutamate uptake (Lee et al., 2009a; Morrello et al., 2008; Pentschew et al., 1963). Consequently, there is overactivation of NMDA receptors, massive...
demonstrated that TeO$_2$ literature regarding cellular mechanisms of Te$^{2+}$ levels were similar to those in control rats. There is no report in the literature regarding cellular mechanisms of Te$^{2+}$ transport but its 2$^+$ valence, it is possible that Te$^{2+}$ competes with Mn$^{2+}$ for transport on DMT-1. In addition, several studies with oxygenion tellurite (TeO$_2^-$) have shown that it can be taken up via a monocarboxylate transport system (Borghese et al., 2008), which also transports Mn in the 3$^+$ valence state (Aschner et al., 2008). Furthermore, Borghese et al. (2003) demonstrated that TeO$_2^-$ can be also transported through a process which is pH dependent and very similar to the proton-dependent transport mediated by DMT-1, suggesting that this transporter could be also involved in the transport of tellurium molecules. These results suggest a possible competition between Mn and Te for transport sites on several nonspecific metal transporters; however, further studies will be necessary to establish whether the efficacy of DPTVP in attenuating Mn-induced neurotoxicity resides in a direct transport competition between Mn and Te, in addition to its well-described antioxidant properties (Avila et al., 2008; de Avila et al., 2006).

In summary, the present study establishes the sensitivity of striatal tissue in a physiologically relevant model of Mn exposure in drinking water. Increased striatal Mn levels were associated with motor deficits reflected by increased oxidative stress, mitochondrial dysfunction, and attenuated glutamate uptake (Fig. 6). These effects were fully reversed by DPTVP, most likely as a result of its antioxidative properties. Nevertheless, future considerations should be directed at defining the ability of DPTVP to directly compete with Mn for shared transport sites, such as DMT-1, the monocarboxylate transport system, and/or others. In conclusion, this study establishes that DPTVP could be an effective therapy to treat Mn intoxication and that antioxidant therapy should be considered as an alternative therapeutic treatment of Mn toxicity.

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