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Developmental exposure to manganese induces lasting motor and cognitive impairment in rats

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ABSTRACT

Exposure to high manganese (Mn) levels may damage the basal ganglia, leading to a syndrome analogous to Parkinson's disease, with motor and cognitive impairments. The molecular mechanisms underlying Mn neurotoxicity, particularly during development, still deserve further investigation. Herein, we addressed whether early-life Mn exposure affects motor coordination and cognitive function in adulthood and potential underlying mechanisms. Male Wistar rats were exposed intraperitoneally to saline (control) or $MnCl_2$ (5, 10 or 20 mg/kg/day) from post-natal day (PND) 8–12. Behavioral tests were performed on PND 60–65 and biochemical analysis in the striatum and hippocampus were performed on PND14 or PND70. Rats exposed to Mn (10 and 20 mg/kg) performed significantly worse on the rotarod test than controls indicating motor coordination and balance impairments. The object and social recognition tasks were used to evaluate short-term memory. Rats exposed to the highest Mn dose failed to recognize a familiar object when replaced by a novel object as well as to recognize a familiar juvenile rat after a short period of time. However, Mn did not alter olfactory discrimination ability. In addition, Mn-treated rats displayed decreased levels of non-protein thiols (e.g. glutathione) and increased levels of glial fibrillary acidic protein (GFAP) in the striatum. Moreover, Mn significantly increased hippocampal glutathione peroxidase (GPx) activity. These findings demonstrate that acute low-level exposure to Mn during a critical neurodevelopmental period causes cognitive and motor dysfunctions that last into adulthood, that are accompanied by alterations in antioxidant defense system in both the hippocampus and striatum.

1. Introduction

Despite its essentiality, high levels of manganese (Mn) may accumulate in structures of the basal ganglia, and lead to a neurodegenerative disease called manganism that ultimately results in motor symptoms that include dystonia, bradykinesia and rigidity (Aschner and Aschner, 1991; Erikson et al., 2004; Cersosimo and Koller, 2006). This parkinsonian-like syndrome is characterized by an early stage of neuropsychiatric disorder (*locura manganica*), with behavioral symptoms such as nervousness, hallucinations, memory loss, cognitive deficits, bizarre behaviors

Abbreviations: CNS, central nervous system; ICP-MS, inductively coupled plasma–mass spectrometry; i.p., intraperitoneal; GFAP, glial fibrillary acidic protein; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; GPx, glutathione peroxidase; MAPKs, mitogen activated protein kinases; NPSH, non-protein thiols; PND, post-natal day; NADPH, nicotinamide adenine dinucleotide phosphate.

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and flight of ideas, in addition to the Parkinson's-like effects related to motor dysfunction (Bowler et al., 2006; Bouchard et al., 2007; Guilarte et al., 2008a; Guilarte, 2010).

Sources of Mn intoxication include occupational settings, such as mining or welding, dry battery manufacture and organochemical fungicide use (Bowler et al., 2006). However, it is now recognized that the general population is also at risk of excessive Mn exposure, especially younger individuals. Mn is present ubiquitously in grains, green vegetables, fruits, nuts, spices, and tea and cases of Mn deficiency are uncommon (Roth, 2006). The presence of excessive Mn levels in drinking water or air has been associated with poorer memory and attention (Carvalho et al., 2014; Oulhote et al., 2014) and hyperactive behavior (Bouchard, 2007) in school-aged children. Water containing elevated Mn levels also had adverse effects on 10-year-old children's cognitive function (Wasserman et al., 2006).

Regarding the molecular mechanisms of Mn neurotoxicity, several animal models have been used to mimic the diverse forms of Mn exposure (i.e. environmental, occupational, parenteral nutrition). Additionally, studies using cell culture models have contributed to the description of Mn uptake and cell toxicity mechanisms. It was shown that Mn induces microglial activation and dopaminergic neurodegeneration in the substantia nigra of rats (Zhao et al., 2009). Mn may cause oxidative stress *in vivo* and *in vitro*, which can lead to the activation of intracellular signaling pathways, including mitogen activated protein kinases, MAPKs (Hirata et al., 2004; Ito et al., 2006; Milatovic et al., 2009; Posser et al., 2009; Hernández et al., 2011; Cordova et al., 2012, 2013). It has been shown that the metal causes mitochondrial damage and consequent decrease in ATP production leading to activation of apoptotic pathways and necrosis in PC12 cells (rat pheochromocytoma cell line) (Roth et al., 2002). However, the molecular mechanisms associated with Mn's effects on the basal ganglia, particularly in models of parenteral nutrition during development, require further investigation.

For human neonates, total parenteral nutrition (TPN) represents a risk of developmental Mn exposure due to the presence of Mn as a trace element. Infants and children are particularly vulnerable to excessive supplementation of this metal, which in some cases may lead to hypermanganesemia, dependent upon the duration of the treatment. This occurs because parenteral administration bypasses the regulatory mechanisms of the gastrointestinal tract. The bioavailability of Mn in parenteral fluid is 100%, compared to only 5% for enteral dietary Mn. Of particular importance, the hepatic mechanisms responsible for Mn excretion are not completely developed in newborns. This combined with the high bioavailability of the metal in TPN leads to Mn accumulation (Boggio Bertinet et al., 2000; Dobson et al., 2004; Santos et al., 2014).

The developing central nervous system (CNS) possesses specific periods of high vulnerability to toxic chemicals (Rice and Barone, 2000). During these vulnerable periods, which may encompass either in utero or early postnatal phases, particular toxicants, even at low doses that do not affect adults, may cause significant detrimental effects (Grandjean and Landrigan, 2006, 2014). It has been proposed that environmental exposure to toxic agents during early periods of neural development may increase susceptibility to additional exposure of the CNS or increase the risk of neurological diseases such as Parkinson's disease and schizophrenia (Cory-Slechta et al., 2005; Guilarte et al., 2012; Grandjean and Landrigan, 2014). Mn has been identified as a developmental neurotoxicant associated with hyperactivity, lower intellectual function, impaired motor skills and reduced olfactory function in children (Zoni and Lucchini, 2013; Grandjean and Landrigan, 2014). In animal models, the immature CNS is more susceptible to Mn neurotoxicity compared to the adult (Moreno et al., 2009). Furthermore, experimental evidence suggests that exposure to this metal during development may affect neurological function in adulthood (Kern et al., 2010; Kern and Smith, 2011; Beaudin et al., 2013).

Recently, we demonstrated that developmental intraperitoneal (i.p.) Mn exposure from post-natal day (PND)8–12, a critical period for rodent CNS development, altered the activity of key cell signaling elements in the striatum of rats on PND14 such as ERK1/2 and Akt. Furthermore, increased oxidative stress markers such as isoprostanes and inhibition of respiratory chain complexes were observed. In the same experimental protocol, Mn impaired motor skills in the 3rd, 4th and 5th week of life. Treatment with Trolox, an antioxidant compound derivative of vitamin E prevented these effects (Cordova et al., 2012). Therefore, the present study was designed to investigate whether these adverse effects of Mn exposure on the developing brain might still be present on PND70, and to determine if damage caused by Mn during developmental stages may appear during adulthood. Herein, we demonstrate by using behavioral tasks that there is lasting motor and cognitive impairment induced by early-life Mn acute exposure, accompanied by persistent alterations in oxidative stress parameters and glial fibrillary acidic protein (GFAP) expression in rats.

2. Methodology

2.1. Chemicals

Manganese chloride ($MnCl_2$) and protease inhibitor cocktail were obtained from Sigma (St. Louis, MO, USA). Anti-GFAP antibody and LumiGLO chemiluminescent substrate were purchased from Cell Signaling (Beverly, MA, USA). Goat anti-mouse IgG HRP (horseradish peroxidase) conjugated secondary antibody was purchased from Millipore (Billerica, MA, USA). Mouse anti-β-actin antibody was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Tris and β-mercaptoethanol were obtained from Amresco (Solon, OH, USA). SDS and bis-acrylamide were purchased from USB (Cleveland, OH, USA). HEPES, Triton X-100, Acrylamide and HybondTM nitrocellulose were purchased from GE Healthcare Life Sciences (Piscataway, NJ, USA). All other reagents were of the highest analytical grade.

2.2. Animals

All animal studies were carried out in accordance with the "Guide for the care and use of laboratory animals" (NIH publication, 8th edition, 2011) and approved by the local Ethical Committee for Animal Research. Postnatal day 5 (PND5) Wistar pups, along with the dams, were obtained from our own breeding colony at Universidade Federal de Santa Catarina (UFSC), Brazil. Rats were maintained in an air-conditioned room (21–23 °C) on a 12-h light/dark cycle with water and food *ad libitum*. The total number of litters used was 24. Ten litters were used for ICP-MS, antioxidant enzymes activity analysis on PND70, and performance of open field and olfactory discrimination tests. Eight of those litters were used for object recognition, social recognition and rotarod tests. Six litters were used for western blotting analysis. Another set of 8 litters was treated for tissue collection on PND14. Only the males from each litter were used.

2.3. In vivo Mn treatment

On PND5, the litters were culled to keep only four male pups. These pups were cross-fostered with male pups from another dam in order to keep litter size at eight pups. The two sets of siblings were identified with different color atoxic ink and the pups were marked individually for each treatment. This procedure was carried out in order to maintain the same litter size that was used