NeuroToxicology 32 (2011) 215–226

Contents lists available at ScienceDirect

NeuroToxicology

Manganese exposure induces microglia activation and dystrophy in the substantia nigra of non-human primates

Tatyana Verina [a], Samara F. Kiihl [b], Jay S. Schneider [c], Tomás R. Guilarte [a,*]

[a] Department of Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States
[b] Department of Biostatistics, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States
[c] Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA, United States

ARTICLE INFO

Article history:
Received 27 April 2010
Accepted 19 November 2010
Available online 26 November 2010

Keywords:
Microglia
Dystrophy
Activation
Manganese
Substantia nigra
iNOS
Ferritin
Ferric iron
Neurotoxicity

ABSTRACT

Chronic manganese (Mn) exposure produces neurological deficits including a form of parkinsonism that is different from Parkinson's disease (PD). In chronic Mn exposure, dopamine neurons in the substantia nigra (SN) do not degenerate but they appear to be dysfunctional. Further, previous studies have suggested that the substantia nigra pars reticulata (SNr) is affected by Mn. In the present study, we investigated whether chronic Mn exposure induces microglia activation in the substantia nigra pars compacta (SNc) and SNr in *Cynomolgus macaques*. Animals were exposed to different weekly doses of Mn (3.3–5.0, 5.0–6.7, 8.3–10 mg Mn/kg body weight) and microglia were examined in the substantia nigra using LN3 immunohistochemistry. We observed that in control animals, LN3 labeled microglia were characterized by a resting phenotype. However, in Mn-treated animals, microglia increased in number and displayed reactive changes with increasing Mn exposure. This effect was more prominent in the SNr than in the SNc. In the SNr of animals administered the highest Mn dose, microglia activation was the most advanced and included dystrophic changes. Reactive microglia expressed increased iNOS, L-ferritin, and intracellular ferric iron which were particularly prominent in dystrophic compartments. Our observations indicate that moderate Mn exposure produces structural changes on microglia, which may have significant consequences on their function.

1. Introduction

Chronic exposure to moderate levels of manganese (Mn) from environmental, occupational or in certain clinical conditions such as cirrhosis, leads to cognitive, psychiatric and motor function deficits, with the latter resembling some features of idiopathic Parkinson's disease (PD) (Banta and Markesbery, 1977; Barbeau, 1984; Aschner et al., 2005; Martin, 2006; Perl and Olanow, 2007; Burton and Guilarte, 2009; Guilarte, 2010). Following exposure, Mn accumulates throughout the living brain with some selectivity for basal ganglia nuclei with the highest accumulation occurring in the globus pallidus (Dorman et al., 2006; Guilarte et al., 2006a).

Idiopathic PD is the result of degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) (Braak et al., 2003). However, a loss of DA neurons has not been a common feature in autopsy samples from humans or non-human primates exposed to Mn (Perl and Olanow, 2007; Burton and Guilarte, 2009; Guilarte, 2010). The latter is consistent with neuroimaging studies showing that there is a lack of an effect of Mn exposure on DA transporter levels in the caudate and putamen (striatum) (Guilarte et al., 2008a; Selikhova et al., 2008; Sikk et al., 2010; Guilarte, 2010). Dopamine transporters are a commonly used marker of dopaminergic terminal integrity (Brooks et al., 2003). Neuroimaging data from some occupational exposed cases show reduced levels of DA transporter (Huang et al., 2003; Kim et al., 2002). However, in these cases, one cannot rule out the possibility that these individuals had idiopathic PD with coincidental Mn exposure.

Although chronic Mn exposure does not appear to cause dopamine neuron degeneration, recent Positron Emission Tomography (PET) studies in non-human primates have demonstrated that elevated brain Mn levels result in a marked decrease of *in vivo* dopamine release (Guilarte et al., 2006a, 2008a). These studies suggest that the effect of Mn on dopaminergic neurons is not degeneration or a decrease in tissue dopamine levels but rather it appears to be associated with impairment of dopamine release. These findings are consistent with the fact that L-dopa therapy is

* Corresponding author at: Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 600 Haven Avenue, B1-108, New York, NY 10032, United States. Tel.: +1 212 305 3959; fax: +1 212 305 3857.
E-mail addresses: tverina@jhsph.edu (T. Verina), samara.kiihl@gmail.com (S.F. Kiihl), Jay.Schneider@jefferson.edu (J.S. Schneider), trguilarte@columbia.edu, tguilart@jhsph.edu (T.R. Guilarte).

0161-813X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2010.11.003

Table 1
Dosing regimen of manganese exposed animals.

Animal ID	Dose level ($MnSO_4$, mg/kg)	Dose level (Mn, mg/kg)	Tissue Mn (μg/g tissue)	Dosing interval	Exposure duration (weeks)	Cumulative $MnSO_4$ (mg/kg)	Cumulative Mn (mg/kg)
144T	10–15	3.5–5.0	0.63	1/week	50	515	171.7
107-705	10–15	3.5–5.0	0.56	1/week	42	500	166.7
3154	10–15	3.5–5.0	0.55	1/week	45	515	173.8
9093	15–20	5.0–6.7	0.91	2/week*	59	770	250.8
7469	15–20	5.0–6.7	0.36	2/week*	32	525	170.7
000-8001	15–20	5.0–6.7	1.15	2/week*	34	535	173.9
001-1099	15–20	5.0–6.7	1.08	2/week*	32	535	173.9
7839	25–30	8.3–10.0	1.06	2/week*	38	640	218.3
6697	25–30	8.3–10.0	2.74	Bolus, 2/week*	7	206	68.3
7426	25–30	8.3–10.0	1.61	Bolus, 2/week*	15	340	113.3

Animals exposed to 3.3–5.0 mg Mn/kg BW weekly doses received a single full-dose injection per week. Animals exposed to 5.0–6.7 and 8.3–10.0 mg Mn/kg BW weekly doses received two half-dose injections per week (*). Animals marked with term "bolus" received a single full-dose injection on the first week of dosing, but then were administered two half-dose injections on the subsequent weeks. Animals ID 6697 and 7426 had shorter duration of exposure and received lower cumulative Mn doses because they developed severe movement abnormalities. Additional animals that are not shown in the table were used as controls (ID 6770, 123-193, 001-1167, and 6499).

not effective in Mn-exposed humans with motor abnormalities (Koller et al., 2004; Perl and Olanow, 2007; Guilarte, 2010), a therapy that is effective in idiopathic PD subjects (Lees, 1986).

Exposure to heavy metals is known to alter metal-ion homeostasis in the brain and to induce oxidative stress and neuroinflammation (Riederer et al.,1992; Campbell et al., 2004; Guilarte et al., 2008b). Iron-mediated oxidative damage is known to play a central role in the pathogenesis of neurodegenerative disorders (Riederer et al., 1989; Youdim et al., 1999; Dexter et al., 1991; Connor et al., 1992). Iron brain content is increased in the brains of patients with idiopathic PD and other neurodegenerative diseases (Melega et al., 2007; Riederer et al., 1989; Griffiths and Crossman, 1993; Griffiths et al., 1999; Sofic et al., 1988; Jellinger et al., 1990; Sofic et al., 1991). It is likely that an elevated Mn level may alter iron homeostasis in the brain leading to increased oxidative stress (Verity, 1999), particularly within the SN where the concentrations of iron as well as DA and lipids are high (Riederer et al., 1992).

Microglia participate in the maintenance of brain iron homeostasis and prevent iron-dependent oxidation by sequestering iron within ferritin (Cheepsunthorn et al., 1998; Connor et al., 1994; Han et al., 2002; Zhanget al., 2006). Ferritin consists of functionally distinct heavy (H) and light (L) chain subunits: H-ferritin transforms soluble ferrous (Fe^{2+}) into ferric (Fe^{3+}) iron which in turn is stored by L-ferritin (Levi et al., 1992). The ratio of H/L subunits is brain region- and cell type-specific (Connor and Menzies, 1995, 1996; Connor et al., 1995a). That is, oligodendrocytes contain a mixture of H/L ferritins, while microglia contain mainly L-ferritin, and, therefore, play a major role in iron storage (Connor et al., 1994). Being a highly dynamic cellular population, microglia rapidly respond to any pathological condition by a complex transformation into an activated state and accompanied by the induction of reactive oxygen and nitrogen species (Streit, 1999; Hanisch and Kettenmann, 2007; Colton and Gilbert, 1987; Corradin et al., 1993). It has been suggested that these highly oxidizing radicals may impair the iron binding capacity of ferritin and cause damage to microglia (Agrawal et al., 2001; Biemond et al., 1984).

In the current study, we examined the effect of chronic Mn exposure on microglia in the SNc and SNr of *Cynomolgus macaques*. We report that Mn induces a microglia response manifested by reactive and dystrophic changes and by increased accumulation of intracellular ferric iron, L-ferritin and iNOS expression. These findings indicate that chronic Mn exposure produces microglia activation and dystrophy most prominently in the SNr of non-human primates and these changes may be produced by iron-mediated oxidative stress mechanism.

2. Materials and methods

2.1. Manganese administration and animal care

Young adult male *C. macaques*, 5–6 years of age were used in this study. All animal studies were reviewed and approved by the Johns Hopkins and the Thomas Jefferson University Animal Care and Use Committees. Animals received injections of manganese sulfate into the saphenous vein under 1–3% isoflourane anesthesia once or twice a week. Animals were exposed to different weekly doses of Mn (3.3–5.0, 5.0–6.7, 8.3–10 mg Mn/kg BW) (Table 1) and several of their characteristics have been described in several publications (Guilarte et al., 2006a,b, 2008a,b; Schneider et al., 2006, 2009). All animals were euthanized by ketamine injection (20–30 mg/kg BW) followed by an overdose of pentobarbital (100 mg/kg BW) and the brains were harvested. We should note that brain tissue samples from control and Mn-treated animals has been used extensively in previous studies and thus SN tissue was not available from all of the animals. In the current study, 10 out of a total of 13 Mn-treated animals were available and 4 out of 6 controls.

2.2. Tissue preparation

Brains were removed from the skull, embedded into agarose and cut into 4 mm slabs in the coronal plane. Alternate brain tissue slabs from the right hemisphere were processed for immunohistochemistry in two different ways for different purposes. The first slab (in a rostral to caudal direction) and every other slab was fixed in 4% paraformaldehyde, further embedded in paraffin and cut into 5 μm thick sections which were used in the other experiments not presented in this study. Alternate tissue slabs (starting from the second frontal slab) were immersed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for 28 h, cryoprotected with 20% glycerol, 0.5% DMSO in PB, and frozen at −80°. 50 μm thick sections were cut using a freezing microtome, stored in cryoprotecting solution at −20 °C, and utilized for various immunohistochemical studies as needed. In the current study, two or three sections from anatomically comparable midbrain region were selected from each animal and processed free-floating for detection of microglia.

2.3. Immunohistochemical, immunofluorescent and histochemical labeling

In order to visualize resident microglia in the brain of non-human primates, the following microglia markers were tested: