NeuroToxicology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Manganese exposure exacerbates progressive motor deficits and neurodegeneration in the MitoPark mouse model of Parkinson's disease: Relevance to gene and environment interactions in metal neurotoxicity

Monica R. Langley, Shivani Ghaisas, Muhammet Ay, Jie Luo, Bharathi N. Palanisamy, Huajun Jin, Vellareddy Anantharam, Arthi Kanthasamy, Anumantha G. Kanthasamy*

Parkinson Disorders Research Program, Iowa Center for Advanced Neurotoxicology, Department of Biomedical Sciences, Iowa State University, Ames, IA 50011, United States

ARTICLE INFO

Article history:
Received 31 January 2017
Received in revised form 2 June 2017
Accepted 2 June 2017
Available online xxx

Keywords:
Manganese
Mitochondria
MitoPark
Neuroinflammation
Animal model
TFAM
Dopamine
Parkinson's disease
Gene-environment interaction

ABSTRACT

Parkinson's disease (PD) is now recognized as a neurodegenerative condition caused by a complex interplay of genetic and environmental influences. Chronic manganese (Mn) exposure has been implicated in the development of PD. Since mitochondrial dysfunction is associated with PD pathology as well as Mn neurotoxicity, we investigated whether Mn exposure augments mitochondrial dysfunction and neurodegeneration in the nigrostriatal dopaminergic system using a newly available mitochondrially defective transgenic mouse model of PD, the MitoPark mouse. This unique PD model recapitulates key features of the disease including progressive neurobehavioral changes and neuronal degeneration. We exposed MitoPark mice to a low dose of Mn (10 mg/kg, p.o.) daily for 4 weeks starting at age 8 wks and then determined the behavioral, neurochemical and histological changes. Mn exposure accelerated the rate of progression of motor deficits in MitoPark mice when compared to the untreated MitoPark group. Mn also worsened olfactory function in this model. Most importantly, Mn exposure intensified the depletion of striatal dopamine and nigral TH neuronal loss in MitoPark mice. The neurodegenerative changes were accompanied by enhanced oxidative damage in the striatum and substantia nigra (SN) of MitoPark mice treated with Mn. Furthermore, Mn-treated MitoPark mice had significantly more oligomeric protein and IBA-1-immunoreactive microglia cells, suggesting Mn augments neuroinflammatory processes in the nigrostriatal pathway. To further confirm the direct effect of Mn on impaired mitochondrial function, we also generated a mitochondrially defective dopaminergic cell model by knocking out the TFAM transcription factor by using a CRISPR-Cas9 gene-editing method. Seahorse mitochondrial bioenergetic analysis revealed that Mn decreases mitochondrial basal and ATP-linked respiration in the TFAM KO cells. Collectively, our results reveal that Mn can augment mitochondrial dysfunction to exacerbate nigrostriatal neurodegeneration and PD-related behavioral symptoms. Our study also demonstrates that the MitoPark mouse is an excellent model to study the gene-environment interactions associated with mitochondrial defects in the nigral dopaminergic system as well as to evaluate the contribution of potential environmental toxicant interactions in a slowly progressive model of Parkinsonism.

1. Introduction

Although manganese (Mn) is an essential cofactor needed for normal cellular functions, occupational and environmental exposures to the metal have been linked to increased risk for various neurological disorders including Parkinson's disease (PD) (Haynes et al., 2015; Lucchini et al., 2014; Sanders et al., 2015; Sikk and Taba, 2015). Mn exposure commonly occurs during the production of

* Corresponding author at: Parkinson Disorders Research Laboratory, Department of Biomedical Sciences, 2062 CVM Building, Iowa State University, Ames, IA, United States.
E-mail address: akanthas@iastate.edu (A.G. Kanthasamy).

http://dx.doi.org/10.1016/j.neuro.2017.06.002
0161-813X/

steel, batteries, fuel additives, fireworks, fungicides, welding, and ceramics (Mielke et al., 2002; Yabuuchi and Komaba, 2014). Given excessive exposure to Mn or failure to excrete it, the metal tends to accumulate in the basal ganglia, resulting in a movement disorder somewhat similar to PD called manganism (Bouabid et al., 2015; Peres et al., 2016b). Similar to PD, manganism manifests motor deficits such as rigidity and bradykinesia. However, in manganism patients, a distinctive "cock-walk" gait is observed and neuropsychiatric symptoms often present earlier rather than later in the disease progression (Bowler et al., 2006, 1999; Kwakye et al., 2015). The neurons most affected in manganism occur in the globus pallidus, rather than the SN (Criswell et al., 2015; Perl and Olanow, 2007). Furthermore, manganism patients respond poorly to levodopa therapy, so instead chelation therapy has been used to treat the disorder (Discalzi et al., 2000; Ky et al., 1992).

Mn and other transition metals have long been implicated as risk factors in the etiology of PD, and a recent study suggests that Mn exposure dose-dependently increases UPDRS3 scores in welders (Gorell et al., 1999; Racette et al., 2016). Recent studies showed that welders exposed to Mn fumes display abnormal neurobehavioral changes that correlate with Mn accumulation in the basal ganglia as measured by magnetic resonance imaging (Lee et al., 2016; Lewis et al., 2016). Also, chronic exposure to metals and pesticides is associated with a younger age at onset of sporadic PD (Ratner et al., 2014). Other studies have revealed that genetic defects in Mn transporter proteins cause metal-induced Parkinsonism, including a form of childhood-onset Parkinsonism caused by an autosomal recessive Mn transporter defect. Patients having the homozygous mutation in SLC39A14 had excessive Mn accumulation and responded positively to chelation therapy (Tuschl et al., 2016). Furthermore, several mutations in SLC30A10, a Mn-specific efflux transporter thought to protect cells from Mn-induced toxicity, can cause familial Parkinsonism (Chen et al., 2015a; Leyva-Illades et al., 2014). The pathogenic mechanisms of Mn neurotoxicity are not completely understood, but evidence suggests that, like classical Parkinsonian toxicants, Mn promotes protein aggregation and it also contributes to oxidative stress and mitochondrial dysfunction by inhibiting mitochondrial complexes I and II of the electron transport chain (Aschner et al., 2009; Carboni and Lingor, 2015; Liu et al., 2013; Peres et al., 2016a; Zheng et al., 1998). Furthermore, Mn can indirectly damage neurons by persistently triggering glial activation and neuroinflammation involving both microglia and astrocytes (Filipov and Dodd, 2012; Moreno et al., 2011, 2009a; Streifel et al., 2012). Although striatal dopamine (DA) loss is a hallmark of PD models, contradictory results have been found in Mn-treated rodent studies (Moreno et al., 2009b; Witholt et al., 2000). These neurochemical changes are thought to contribute to the motor and neuropsychiatric symptoms present (Ferrer et al., 2012; Vermeiren and De Deyn, 2017).

Growing evidence has indicated that interactions between environmental exposures and genetic factors play a crucial role in the pathogenesis of PD. Mn and many genetic mutations associated with PD alter common biochemical pathways, allowing for a synergistic effect on the development of PD pathology (Bornhorst et al., 2014; Chen et al., 2015b; Roth, 2014). However, little direct evidence exists on Mn exposure exacerbating Parkinsonism in animal models. Such studies could provide much needed insight into how Mn hastens the pathophysiological processes involved in PD, and thus result in the development of better strategies to prevent or delay disease onset and progression. One study that used a toxin-based model to show effects of subchronic intraperitoneal Mn administration on DA-depleted rats reported that Mn potentiated neurobehavioral deficits but not the DA depletion (Witholt et al., 2000). Additionally, several PD-related genes such as parkin, LRRK2, and ATP13A2 are known to mediate Mn-induced toxicity in cell culture and animal models (Bornhorst et al., 2014; Higashi et al., 2004; Lovitt et al., 2010; Tan et al., 2011). However, none of the neurotoxin-based and genetic PD models faithfully recapitulate the chronic and progressive nature of the disease.

Mitochondrial impairment is well recognized as part of the normal aging process. Moreover, mitochondria serve as a key cellular target for PD pathology as well as for Mn neurotoxicity. However, we lack information on how Mn affects already comprised mitochondrial function as it relates to progression of the neurodegenerative process in PD and aging. Thus, in the present study, we evaluated the effect of a low-dose Mn exposure in the MitoPark mouse, a recently available, mitochondrially defective transgenic (Tg) mouse model of PD. MitoPark mice were generated by conditionally knocking out mitochondrial transcription factor A (TFAM) in DAergic neurons through the Cre-loxP system (Ekstrand et al., 2007). The MitoPark mouse is a unique PD model that recapitulates most of the hallmark behavioral symptoms and neuropathologies associated with PD, including progressive neurodegeneration and protein aggregates, representing a valuable model for studying the neurodegenerative process. Herein, we utilized the MitoPark mouse for a neurotoxicological study of Mn using a similar suite of behavioral, neurochemical, histological, and biochemical analyses routinely adopted in other animal models.

2. Materials and methods

2.1. Chemicals

Dopamine hydrochloride, 3-4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3,3′-diaminobenzidine (DAB), manganese chloride ($MnCl_2$), and hydrogen peroxide were all purchased from Sigma (St Louis, MO). Halt protease and phosphatase inhibitor cocktail was obtained from Thermo Fisher (Waltham, MA). Bradford assay reagent and Western blotting buffers were purchased from Bio-Rad Laboratories (Hercules, CA). Anti-4-hydoxynonenal (4-HNE) antibody was purchased from R&D Systems (Minneapolis, MN). We purchased anti-IBA-1 antibodies from Wako Pure Chemical Industries (Richmond, VA) and Abcam (Cambridge, MA) for immunohistochemistry (IHC) and Western blot, respectively. Anti-tyrosine hydroxylase (TH) antibody was purchased from Millipore (Billerica, MA). Anti-oligomeric antibody (A11) and cell culture reagents were purchased from Invitrogen. The anti-mouse and anti-rabbit secondary antibodies (Alexa Fluor 680 conjugated anti-mouse IgG and IRDye 800 conjugated anti-rabbit IgG) were purchased from Invitrogen and Rockland Inc., respectively.

2.2. Cell lines

For *in vitro* mitochondrial function studies, the rat immortalized mesencephalic DAergic neuronal cell line ($1RB_3AN_{27}$, or N27) was cultured in RPMI 1640 containing 2 mM $_L$-glutamine, 50 U/mL of penicillin and 50 μg/mL streptomycin with 0–10% FBS in incubators at 37 °C and 5% CO_2 as previously described by our lab (Charli et al., 2015). The lentivirus-based CRISPR/Cas9 TFAM knockout plasmid, pLV-U6gRNA-Ef1aPuroCas9GFP-TFAM, with the TFAM gRNA target sequence directed against the exon 1 sequence (CPR555e5e4099bf84.98), was purchased from Sigma-Aldrich. To make lentivirus, the lenti-CRISPR/Cas9 TFAM knockout plasmid was transfected into 293FT cells using the Mission Lentiviral Packaging Mix (Cat#SHP001, Sigma-Aldrich) according to manufacturer's instructions. For negative control

Please cite this article in press as: M.R. Langley, et al., Manganese exposure exacerbates progressive motor deficits and neurodegeneration in the MitoPark mouse model of Parkinson's disease: Relevance to gene and environment interactions in metal neurotoxicity, Neurotoxicology (2017), http://dx.doi.org/10.1016/j.neuro.2017.06.002