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Relationship between brain accumulation of manganese and aberration of hippocampal adult neurogenesis after oral exposure to manganese chloride in mice

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ABSTRACT

We previously found persistent aberration of hippocampal adult neurogenesis, along with brain manganese (Mn) accumulation, in mouse offspring after developmental exposure to 800-ppm dietary Mn. Reduction of parvalbumin (Pvalb)$^+$ γ-aminobutyric acid (GABA)-ergic interneurons in the hilus of the dentate gyrus along with promoter region hypermethylation are thought to be responsible for this aberrant neurogenesis. The present study was conducted to examine the relationship between the induction of aberrant neurogenesis and brain Mn accumulation after oral Mn exposure as well as the responsible mechanism in young adult animals. We used two groups of mice with 28- or 56-day exposure periods to oral $MnCl_2 \cdot xH_2O$ at 800 ppm as Mn, a dose sufficient to lead to aberrant neurogenesis after developmental exposure. A third group of mice received intravenous injections of Mn at 5-mg/kg body weight once weekly for 28 days. The 28-day oral Mn exposure did not cause aberrations in neurogenesis. In contrast, 56-day oral exposure caused aberrations in neurogenesis suggestive of reductions in type 2b and type 3 progenitor cells and immature granule cells in the dentate subgranular zone. Brain Mn accumulation in 56-day exposed cases, as well as in directly Mn-injected cases occurred in parallel with reduction of Pvalb$^+$ GABAergic interneurons in the dentate hilus, suggesting that this may be responsible for aberrant neurogenesis. For reduction of Pvalb$^+$ interneurons, suppression of brain-derived neurotrophic factor-mediated signaling of mature granule cells may occur via suppression of c-Fos-mediated neuronal plasticity due to direct Mn-toxicity rather than promoter region hypermethylation of *Pvalb*.

1. Introduction

Manganese (Mn) is a trace essential metal for living organisms. It is important for protein and energy metabolism, bone mineralization, metabolic regulation (Aschner et al., 2009), and, as manganese superoxide dismutase, antioxidation (Borgstahl et al., 1992). However, excessive airborne Mn exposure causes multiple aberrations of the neuronal system, called manganism in humans, with features similar to Parkinson's disease (Dobson et al., 2004). Histologically, abnormalities of the nigrostriatal

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Arc, activity-regulated cytoskeleton associated protein; BDNF, brain -derived neurotrophic factor; c-Fos, FBJ osteosarcoma oncogene; Chrna4, cholinergic receptor, nicotinic, alpha polypeptide 4; Chrna7, cholinergic receptor, nicotinic, alpha polypeptide 7; Ct, threshold cycle; Dcx, doublecortin; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GCL, granule cell layer; GFAP, glial fibrillary acidic protein; Gria1, glutamate receptor, ionotropic, AMPA1; Gria2, glutamate receptor, ionotropic, AMPA2 (alpha 2); Gria3, glutamate receptor, ionotropic, AMPA3 (alpha 3); Grin2, aglutamate receptor, ionotropic, NMDA2A (epsilon 1); Grin2d, glutamate receptor, ionotropic, NMDA2D (epsilon 4); Grm1, glutamate receptor, metabotropic 1; Hprt, hypoxanthine-guanine phosphoribosyltransferase; Mn, manganese; NeuN, neuron-specific nuclear protein; NMDA, N-methyl-D-aspartate glutamate; Ntrk2, neurotrophic tyrosine kinase, receptor, type 2; Pax6, paired box 6; PCNA, proliferating cell nuclear antigen; Pvalb, parvalbumin; SGZ, subgranular zone; Tbr2, T-box brain protein 2; TrkB, tropomyosin-related kinase B receptor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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dopaminergic neuron system are found in manganism as well as Parkinson's disease in humans (Kitazawa et al., 2005). Additionally, Mn exposure may cause developmental neurotoxicity; we and others have reported that oral Mn exposure during development causes neurotoxicity in rats and mice (Reichel et al., 2006; Ohishi et al., 2012; Wang et al., 2012).

The dentate gyrus in the hippocampal formation of the mammalian brain is crucial for higher brain functions such as learning, memory, and motivation (Montaron et al., 2004). During postnatal life, neurogenesis continues in the subgranular zone (SGZ) of the dentate gyrus (Zhao et al., 2008). In the SGZ, type 1 stem cells differentiate into type 2a, type 2b and type 3 progenitor cells. The type 3 cells undergo final mitosis to differentiate into immature granule cells, and then to mature granule cells (Hodge et al., 2008). It is reported that γ-aminobutyric acid (GABA)-ergic interneurons in the hilus of the dentate gyrus support the development and maintenance of granule cells (Lussier et al., 2009). GABAergic interneurons have been classified into several subpopulations based on their expression of parvalbumin (Pvalb), calretinin, neuropeptide Y, vasoactive intestinal peptide and somatostatin (Kubota and Kawaguchi, 1994; Ascoli et al., 2008; Batista-Brito et al., 2008), and changes in interneuron subpopulations occur in association with disruption of neurogenesis (Akane et al., 2013; Wang et al., 2013).

In mice, we have found that maternal Mn exposure affects developmental neurogenesis in the SGZ of the dentate gyrus, even at the adult stage after the cessation of Mn exposure (Wang et al., 2012). We also observed a sustained increase in the population of immature GABAergic interneurons synthesizing reelin, a secreted extracellular matrix glycoprotein that plays a critical role in neuronal migration and positioning during brain development, throughout the adult stage in mice developmentally exposed to Mn (Wang et al., 2012). We also found epigenetic modification of *Pvalb* gene regulation after developmental Mn exposure in subpopulations of GABAergic interneurons in mice (Wang et al., 2013). Because GABAergic interneurons provide direct neural inputs to type 2 progenitor cells in the SGZ to promote neural differentiation (Tozuka et al., 2005), reduction of $Pvalb^+$ cells may inhibit neuronal differentiation after Mn exposure.

In the developmental exposure studies of Mn in rats and mice, we found an increase in brain Mn concentration in animals showing aberrations in hippocampal neurogenesis (Ohishi et al., 2012; Wang et al., 2012). There are some differences in in vivo kinetics of ingested Mn between neonates and adults. It is reported that the biliary excretion system, the main excretion pathway of Mn influencing Mn body burden, does not function during the neonatal period in humans (Lönnerdal, 1994). In addition, juvenile rats have higher capability for intestinal Mn absorption than adult rats (Lönnerdal et al., 1987). Moreover, Mn is more efficiently distributed into the brain through the blood-brain barrier in neonatal rats than adult rats (Mena, 1974). While adult-stage oral $MnCl_2$ exposure for 28 days by gavage only slightly increases the Mn concentration in the cerebral cortex of rats (Roels et al., 1997), behavioral aberrations can be induced by exposure to high doses of oral $MnCl_2$ for 19 weeks in adult rats (Torrente et al., 2005). These results suggest that Mn exposure even at the adult stage can affect hippocampal neurogenesis.

Because there is a possibility that high doses of orally administered Mn lead to Mn accumulation in the brain, even adult-stage exposure may affect neurogenesis. The present study was performed to investigate the relationship between the brain accumulation of Mn and its effect on hippocampal neurogenesis by oral Mn exposure in the framework of a regular toxicity study using mice. Young adult mice were subjected to repeated oral doses of $MnCl_2$ through their diet. We used a direct administration model of Mn through intravenous injections as a positive control for Mn accumulation in the brain. Because Mn accumulation in the brain is apparent after long-term exposure by oral doses (Torrente et al., 2005), we set two exposure periods of 28 and 56 days. We also examined the mechanism of the disruption of neurogenesis in terms of the contribution of interneuron populations.

2. Materials and methods

2.1. Chemicals and animals

Manganese chloride hydrate ($MnCl_2 \cdot xH_2O$; CAS No. 73913-06-1) containing 27.8% Mn was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

A total of hundred-sixty-four 5-week-old Slc:ICR mice, purchased from Japan SLC Inc. (Shizuoka, Japan), were acclimated to laboratory conditions for 1 week. Animals were given free access to powdered diets and were kept in standard conditions (room temperature, 24 ± 1 °C; relative humidity, 56 ± 11%; 12-h light/dark cycle). A regular basal diet (MF; Oriental Yeast Co. Ltd. Tokyo, Japan) (Mn concentration: 4.84 mg/100-g basal diet) and water (Mn concentration: 0.00 mg/L) were given ad libitum throughout the experimental period.

2.2. Experimental design

All procedures in this study were conducted in compliance with the "Guidelines for Proper Conduct of Animal Experiments" (Science Council of Japan, June 1, 2006) and according to the protocol approved by the Animal Care and Use Committee of the Tokyo University of Agriculture and Technology.

For the 28-day oral dose experiment, mice were treated with 0 ($n = 26$) or 800 ppm ($n = 26$) of Mn in the form of $MnCl_2 \cdot xH_2O$ mixed in the powdered basal diet. For 56-day oral dose experiment, 52 mice were also treated with the same amounts of Mn. For direct intravenous exposure experiment, 26 mice per group were injected through the caudal vein with 5-mg/kg body weight of Mn in the form of $MnCl_2 \cdot xH_2O$ or saline as a vehicle control once a week for 4 weeks. In the oral dose experiment, the dietary exposure level at 800 ppm was selected, based on our previous study results to show persistent aberration of hippocampal neurogenesis and brain Mn accumulation through the adult stage after developmental exposure to Mn in mice (Wang et al., 2012). With regard to the direct intravenous exposure experiment, once weekly dosing regimen at 5 mg/kg body weight was selected according to the previous study that have shown brain Mn accumulation and evidence for affection of dopaminergic neuron system after 6 weeks treatment in rats (Williams et al., 2010). At the end of the animal study, animals were sacrificed by exsanguination from the abdominal aorta under deep CO_2/O_2 anesthesia. The body weights of animals were determined once a week and food consumption was measured once every 3 days. Brains were collected at autopsy in all animals.

For pyrosequencing analyses, additional mice were orally treated with 0 ($n = 4$) or 800 ppm ($n = 4$) of Mn in diet for 56-day.

2.3. Determination of cerebral metal concentrations

To measure concentrations of Mn, Fe, Cu and Zn in the brain tissue, frozen cerebral hemisphere tissue of animals ($n = 10$/group in oral dose experiment, $n = 9$/group in intravenous injection experiment) were digested using a microwave oven (MARS5, CEM Corp., Matthews, NC, USA); the digested samples were analyzed using inductively-coupled plasma mass spectrometry (HP-7500; Hewlett-Packard Co., Palo Alto, CA, USA) with the monitoring mass