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Molecular mechanism of distorted iron regulation in the blood–CSF barrier and regional blood–brain barrier following in vivo subchronic manganese exposure

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Abstract

Previous studies in this laboratory indicated that manganese (Mn) exposure in vitro increases the expression of transferrin receptor (TfR) by enhancing the binding of iron regulatory proteins (IRPs) to iron responsive element-containing RNA. The current study further tested the hypothesis that in vivo exposure to Mn increased TfR expression at both blood–brain barrier (BBB) and blood–cerebrospinal fluid (CSF) barrier (BCB), which contributes to altered iron (Fe) homeostasis in the CSF. Groups of rats (10–11 each) received oral gavages at doses of 5 mg Mn/kg or 15 mg Mn/kg as $MnCl_2$ once daily for 30 days. Blood, CSF, and choroid plexus were collected and brain capillary fractions were separated from the regional parenchyma. Metal analyses showed that oral Mn exposure decreased concentrations of Fe in serum (−66%) but increased Fe in the CSF (+167%). Gel shift assay showed that Mn caused a dose-dependent increase of binding of IRP1 to iron responsive element-containing RNA in BCB in the choroid plexus (+70%), in regional BBB of capillaries of striatum (+39%), hippocampus (+56%), frontal cortex (+49%), and in brain parenchyma of striatum (+67%), hippocampus (+39%) and cerebellum (+28%). Real-time RT-PCR demonstrated that Mn exposure significantly increased the expression of TfR mRNA in choroid plexus and striatum with concomitant reduction in the expression of ferritin (Ft) mRNA. Collectively, these data indicate that in vivo Mn exposure results in Fe redistribution in body fluids through regulating the expression of TfR and ferritin at BCB and selected regional BBB. The disrupted Fe transport by brain barriers may underlie the distorted Fe homeostasis in the CSF.

Keywords: Manganese (Mn); Iron (Fe); Brain-Barrier system; Iron regulatory protein (IRP); Iron responsive element (IRE); Transferrin receptor (TfR); Oral administration; Choroid plexus (CP); Blood–CSF barrier (BCB); Blood–brain barrier; Ferritin (Ft)

1. Introduction

Manganese (Mn) exposure induces clinical symptoms resembling the Parkinson's disease (Barbeau, 1985; Gorell et al., 1997; Tepper, 1961). The sources of exposure to Mn are numerous but the most significant contributors are occupational and environmental exposure including inhalation and ingestion. The respiratory and gastrointestinal (GI) tracts are the main portals of entry of Mn in humans. Consumption of drinking water contaminated with Mn has been found to be associated with neurological signs of chronic Mn poisoning (Kondakis et al., 1989; WHO, 1981). However, the mechanism of Mn-induced Parkinsonism has not been completely elucidated.

Earlier studies of Aschner's group (Aschner and Aschner, 1990; Aschner et al., 1999) suggest that iron (Fe) homeostasis may play an important role in the regulation of Mn transport across the blood–brain barrier (BBB). Evidence from this laboratory further indicates that Mn toxicity appears to be associated with altered Fe metabolism at both systemic and cellular levels (Li et al., 2004, 2005; Zheng et al., 1999; Zheng and Zhao, 2001). In vivo Mn exposure via intraperitoneal (i.p.) injection expedites unidirectional influx of Fe from the systemic circulation to cerebral compartment (Zheng et al., 1999); in vitro Mn exposure in a choroidal epithelial Z310 cell line also shows an increased flux of Fe across the blood–cerebrospinal fluid (CSF) barrier (BCB) in an in vitro BCB

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barrier model (Li et al., 2005), all of which support the view that Mn neurotoxicity is associated with a compartment shift of Fe from the blood circulation to the CSF. The interaction of Mn on Fe homeostasis has also been demonstrated among welders who were occupationally exposed to Mn in welding fume (Crossgrove and Zheng, 2004; Li et al., 2004; Lu et al., 2005).

Our previous work indicates that the mimicry between Mn and Fe in their coordination chemistry allows Mn to compete with Fe in the fourth, labile Fe binding site in the active center of iron regulatory proteins (IRPs), alter the expression of Fe uptake-related protein transferrin receptor (TfR), and increase the cellular overload of Fe at the BCB (Li et al., 2005; Zheng et al., 1998; Zheng, 2001). In an in situ brain perfusion model, we have shown that the transport of Tf-bound Fe and free Fe into the CNS is determined by the initial sequestration by the choroid plexus and brain capillaries, and subsequent controlled and slow release from vascular structures into brain interstitial fluid and CSF (Deane et al., 2004). Thus, a distorted regulation of TfR and ferritin (Ft) expression by toxic substances is expectedly to alter Fe transport at both BBB and BCB since both of TfR and ferritin (Ft) are present at brain barriers (Deane et al., 2004; Li and Qian, 2002). Recent study in our group further explored that Mn exposure in vitro increases the expression of TfR mRNA by enhancing the binding of IRPs to RNA which contains iron responsive element (IRE) stem-loop structure and results in alteration of Fe distribution in the immortalized Z310 epithelial cells (Li et al., 2005). However, little is known about whether this scenario would occur in vivo following oral Mn exposure for a relative long period. Verification of this novel observation of Mn interaction with IRPs in intact animals will help understand the important mechanism by which Fe is regulated at BCB and BBB and how Mn may alter these processes.

The current study was designed to use subchronic oral Mn exposure model to test the hypothesis that in vivo exposure to Mn distorted TfR expression at brain barriers, which contributed to an altered Fe homeostasis in the CSF. Experiments were performed to determine Fe status in serum, CSF, choroid plexus, and selected brain regions following subchronic Mn exposure, to determine the effect of oral Mn exposure on the binding activity of IRP1 to IRE-RNA at BCB in the choroid plexus, regional BBB capillaries, and regional brain parenchyma, and to determine cellular mRNA levels of TfR and ferritin in these selected brain regions as affected by in vivo Mn exposure.

2. Materials and methods

2.1. Materials

Chemicals were obtained from the following sources: standard Mn and Fe for atomic absorption spectrophotometry (AAS) from Perkin-Elmer Instruments (Shelton, CT); nitric acid (69.9%, Ultrapure) from J.T. Baker (Phillipsburg, NJ); RNase-free DNase I from Invitrogen Life Technologies (Carlsbad, CA); ATP, GTP, CTP, and UTP (NTPs), [α-^{32}P]UTP from Amersham (Piscataway, NJ); manganese chloride ($MnCl_2{\cdot}4H_2O$), dextran, and all other chemicals from Sigma Chemicals (St. Louis, MO). All reagents were of analytical grade, HPLC grade or the best available pharmaceutical grade.

2.2. Animals

Male Sprague–Dawley rats were purchased from Harlan Sprague–Dawley Inc., Indianapolis, IN. At the time of use the rats were 7–8 weeks old weighing 239 ± 1.8 g (mean $\pm$ S.E.M.). Upon arrival, the rats were housed in a temperature-controlled, 12/12 light/dark room, and acclimated for 1 week prior to experimentation. They were allowed to have free access to chaw Laboratory Rodent Diet 5001 PMI (aka Purina Mills, Inc.) and tap water. The study was conducted in complying with animal rights and approved by Institutional Committee on Animal Uses at Purdue University.

2.3. Mn administration and sample collection

$MnCl_2$ dissolved in sterile saline was administered to rats by oral gavage at a dose of 5 or 15 mg of Mn/kg once daily between 9:00 a.m. and 10:00 a.m. for 30 consecutive days except for weekends. This dose regimen was chosen based on our own previous Mn neurotoxicity studies in humans as well in animals (Crossgrove and Zheng, 2004; Zheng et al., 1998, 2000). For the control group, the animals received the daily oral gavage of the equivalent volume of sterile saline. Twenty-four hour after the last oral gavage, rats were anesthetized with pentobarbital (50 mg/kg, i.p.). CSF samples were obtained through a 26-gauge needle inserted between the protruberance and the spine of the atlas, and were free of the blood (Zheng et al., 1991). Blood samples were collected from the inferior vena cava into syringes. Following standing in room temperature for at least half an hour, the blood was centrifuged at 3400 rpm for 30 min and the serum was transferred to an Eppendorf tube. Both CSF and serum samples were stored at -20 °C until analyzed. Rat brains were dissected from the skull and the choroid plexus collected from lateral and third ventricles. Various brain regions, i.e., striatum (ST), hippocampus (HC), frontal cortex (FC), and cerebellum (CB), were dissected. The capillary fractions from these regions were separated from parenchyma by our method previously reported (Deane et al., 2004) and frozen at -70 °C for further isolation of total RNA and S100 extractions.

2.4. Atomic absorption spectrophotometry (AAS) analysis

Mn and Fe concentrations in the CSF and plasma were determined by an HGA-800 graphite furnace AAS system (Perkin-Elmer Instruments, Norwalk, CT) equipped with a Perkin-Elmer Analyst 100 atomic absorption spectrophotometer. Aliquots (50 μL) of CSF and serum were diluted by 50 μL of distilled, deionized water prior to AAS for Mn measurement and diluted 50-fold for Fe measurement. The detection limit of these methods was 0.5 ng/mL of assay solution for Mn and 0.05 μg/dL for Fe.