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Interactive report

Alteration of iron homeostasis following chronic exposure to manganese in rats [1]

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Abstract

Recent studies suggest that manganese-induced neurodegenerative toxicity may be partly due to its action on aconitase, which participates in cellular iron regulation and mitochondrial energy production. This study was performed to investigate whether chronic manganese exposure in rats influenced the homeostasis of iron in blood and cerebrospinal fluid (CSF). Groups of 8–10 rats received intraperitoneal injections of $MnCl_2$ at the dose of 6 mg Mn/kg/day or equal volume of saline for 30 days. Concentrations of manganese and iron in plasma and CSF were determined by atomic absorption spectrophotometry. Rats exposed to manganese showed a greatly elevated manganese concentration in both plasma and CSF. The magnitude of increase in CSF manganese (11-fold) was equivalent to that of plasma (10-fold). Chronic manganese exposure resulted in a 32% decrease in plasma iron ($p < 0.01$) and no changes in plasma total iron binding capacity (TIBC). However, it increased CSF iron by 3-fold as compared to the controls ($p < 0.01$). Northern blot analyses of whole brain homogenates revealed a 34% increase in the expression of glutamine synthetase ($p < 0.05$) with unchanged metallothionein-I in manganese-intoxicated rats. When the cultured choroidal epithelial cells derived from rat choroid plexus were incubated with $MnCl_2$ (100 μM) for four days, the expression of transferrin receptor mRNA appeared to exceed by 50% that of control ($p < 0.002$). The results indicate that chronic manganese exposure alters iron homeostasis possibly by expediting unidirectional influx of iron from the systemic circulation to cerebral compartment. The action appears likely to be mediated by manganese-facilitated iron transport at brain barrier systems.

Keywords: Manganese; Iron; Cerebrospinal fluid; Transferrin receptor; Glutamine synthetase; Choroid plexus

1. Introduction

Abnormal iron homeostasis, both systemically and subcellularly, is believed to be associated with etiology of idiopathic Parkinson's disease (IPD) and chemical-induced Parkinsonism [9,15,20,43]. It has been postulated that cellular Fe overload in the substantia nigra may catalyze the generation of reactive oxygen species and enhance lipid peroxidation. Consequently, Fe-mediated oxidative stress may lead to the degeneration of nigrostriatal dopamine neurons in IPD patients [15,43]. Although a relationship between Mn intoxication and Parkinsonism has long been recognized [3,23,40], the question as to whether manganese-induced neurodegenerative damage results from an unbalanced iron status in blood circulation and/or cerebral compartment remains unexplored, despite the fact that both metals share many similarities in nature [47].

Under normal physiological conditions, the brain stringently regulates iron balance by three well coordinated systems: (a) the influx of iron into brain which is regulated by transferrin receptor-mediated transport at brain barriers; (b) the storage of iron in which the cellular sequestration is largely dependent upon availability of ferritin; and (c) the efflux of iron whose rate is controlled by bulk CSF flow to the blood circulation [6,9,14]. At the brain barriers that separate the systemic circulation from the cerebral compartment (including the interstitial fluid and CSF), the iron–transferrin complex is taken up by endocytosis into cerebral capillary endothelia (and possibly into choroidal epithelia) where the molecules subsequently dissociate.

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Apotransferrin is then recycled to the blood compartment, whereas the released iron crosses the abluminal membrane of the barriers into the cerebral compartment by binding to brain transferrin derived discretely from oligodendrocytes and choroidal epithelia. The cerebral transferrin-bound iron thus becomes available for neurons expressing transferrin receptors [9,26].

The post-translational modulation of transferrin receptor and ferritin synthesis is known to be regulated by a [4Fe–4S] containing protein known as cytoplasmic aconitase (ACO1) or IRP-I. In the absence of iron, ACO1 exhibits a unique affinity to mRNAs that possess an iron responsive element (IRE) stem-loop structure, i.e., to the mRNAs of the major proteins in iron metabolism including ferritin, transferrin receptor, δ-aminolevulinic acid synthase, mitochondrial aconitase (ACO2), and succinate dehydrogenase [5,17].

Our recent studies indicate that manganese exposure substantially inhibits total cellular aconitase activity in the brain [47]. We postulated that the action may be due to the insertion of manganese into one of the four iron sites in aconitase, resulting suppression of ACO1 enzyme's catalytic function. We further postulated that such binding would increase the protein's ability to bind to mRNAs containing IRE's. The net result of these interactions would be a down-regulation of cellular iron utilization and up-regulation of cellular iron uptake. Since the presence of transferrin receptor has been demonstrated in peripheral organs such as in liver and kidney, and in the CNS particularly at the brain barrier systems, any alteration in transferrin receptor and other related iron regulatory proteins would likely change the distribution pattern of iron and disrupt iron homeostasis. It is worth noting also that both manganese and iron, en route to the brain, accumulate in the choroid plexus [13,24,27,47], a tissue where the blood–CSF barrier resides [44].

The purpose of this study was therefore to investigate whether chronic manganese exposure in rats influenced the homeostasis of iron in blood and CSF. By verifying the expression of transferrin receptor mRNA in the choroid plexus, we also examined the steady-state expression of transferrin receptor mRNA in the cultured choroid plexus epithelial cells following manganese exposure. In addition, we examined the status of oxidative stress in brain as demonstrated by over-expression of glutamine synthetase and metallothionein-I in manganese-intoxicated rats.

2. Materials and methods

2.1. Materials

Chemicals were obtained from the following sources: manganese chloride ($MnCl_2 \cdot 4H_2O$), sodium citrate, insulin, transferrin, mouse epidermal growth factor (EGF) and amphotericin B from Sigma Chemical (St. Louis, MO); Dulbecco's modified essential medium (DMEM), fetal bovine serum (FBS), and antibiotic-antimycin from Gibco (Grand Island, NY); RNA Zol B from Tel-Test 'B' (Friendswood, TX); and standard manganese and iron for atomic absorption spectrophotometry from Alfa Products (Danvers, MA). 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 243 ± 10.2 g (Mean $\pm$ SD). Upon arrival, the rats were housed in a temperature-controlled, 12/12 light/dark room, and acclimated for one week prior to experimentation. They were allowed to have free access to pelleted rat chow (Teklad 4% Mouse–Rat Diet, Teklad, Madison, WI) and distilled, deionized water.

2.3. Mn administration and sample collection

$MnCl_2$ dissolved in sterile saline was administered by i.p. injection to rats at a dose of 6 mg of Mn/kg (6 mg Mn/ml) once daily between 9:30 am and 10:30 am for four weeks except for Sundays. This dose regimen was chosen because it was known to be associated with a significant reduction of succinic dehydrogenase (another [4Fe–4S] enzyme) [35–37] and a selective inhibition of aconitase in rat brain [47]. For the control group, the animals received the daily injections of the equivalent volume of sterile saline.

Twenty-four h after the last injection, 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 [45]. Blood samples were collected from the inferior vena cava into heparinized syringes. The blood was centrifuged at $5000 \times g$ for 5 min and the plasma was transferred to an Eppendorf tube. Both CSF plasma samples were stored at -20°C until analyzed. Rat brains were then extricated from the skull and frozen at -70°C for determination of mRNAs of MT-I and glutamine synthesis.

2.4. Atomic absorption spectrophotometry (AAS) analysis

Manganese concentrations in the CSF and plasma were determined by a flameless graphite furnace AAS [28]. Aliquots (50 μl) of CSF and plasma were diluted by 50-fold with an appropriate volume of diluent consisting 8% Triton X-100 and 5% EDTA in distilled, deionized water prior to AAS. Total iron concentrations in plasma and CSF were measured using the method of Yeh and Zee [42]. The plasma and CSF were diluted 10- and 2-fold, respectively, with deionized water prior to AAS.

A diagnostic kit for TIBC from Sigma was used for determining total iron binding capacity (TIBC). Plasma unsaturated iron binding capacity (UIBC) was estimated by adding a known amount of iron to the plasma to fill all available binding sites on transferrin molecules. After centrifugation, the remaining unbound iron was measured by AAS. The difference between the amount of total iron added and remaining unbound iron is the UIBC. The TIBC is then the sum of the total iron and UIBC.

A Perkin–Elmer Model 3030 Zeeman atomic absorption spectrophotometer, equipped with an HGA-600 graphite funace, was used for quantification. The detection limit of these methods was 0.2 ng/ml of assay solution for manganese and 0.5 ng/ml for iron.

2.5. Choroidal epithelial cell culture

Choroidal epithelial cells were cultured using the method established in this laboratory [46]. In short, plexus tissues were collected from Sprague–Dawley rats (4–6 weeks old, both sexes). The plexuses were dissected, washed in DMEM, chopped with scissors, and digested in Hank's buffer containing 0.2% pronase at 37°C for 5 min. The dissociated cells were washed twice in DMEM with 100 units/ml each of penicillin, streptomycin and gentamycin, and 0.25 μg/ml amphotericin B, and resuspended in the same medium supplemented with 10% FBS and 10 ng/ml EGF (growth medium). The cells were plated in 35-mm Petri dishes ($2–3 \times 10^5$ cells per dish) and cultured in a humidified incubator with 95% air–5% CO_2 at 37°C. The growth medium was replaced two days after initial seeding and every other day thereafter. A 6–8 day culture was used in this study.

The cells were exposed to 100 μM Mn (as $MnCl_2$) dissolved in serum-free medium containing 5 μg/ml insulin, transferrin, and 5 ng/ml sodium selenite at 37°C overnight. At the end of exposure, the cells were washed with PBS, and lyzed in RNA Zol B for Northern blot analysis of transferrin receptor mRNA described below.

2.6. Northern blot of transferrin receptor mRNA in cultured choroid plexus cells

Total RNA was extracted from the control and Mn-treated choroidal epithelial cells using RNA Zol B following the instructions of the manufacturer. RNA was normalized by loading equal masses of total RNA as determined by absorbance at 260 nm. Total RNA (20 μg) was electrophoresed on 1% formaldehyde–agarose gels, followed by soaking in 0.05 mM NaOH for 20 min and then 20 × saline–sodium citrate (SSC) for 40 min. After the RNA was transferred to nylon filter in 20 × SSC overnight, the filter was UV-irradiated, prehybridized in 0.5 M phosphate buffer (pH 7.2) containing 7% SDS and 1% BSA at 65°C for 15 min, and then hybridized at 65°C overnight in the same solution containing 2×10^7 cpm/ml of random-primed [^{32}P]-labeled transferrin receptor cDNA probe. The probe was 507 base pair fragment of 5′-end of a full length 3.4 kb rat transferrin receptor cDNA, subcloned to pTZ19U-RTR, which was a gift of Dr. Griswold at Washington State University [31]. The filters were washed three times for 15 min each using 2 × SSC with 0.1% SDS at 65°C, followed by 1 × SSC with 0.1% SDS at 25°C once for 15 min. The filters were then autoradiographed with Kodak Biomax MR film (Eastman Kodak, Rochester, NY) in an intensifying screen at −70°C for 7–14 days. To quantitate transferrin receptor mRNA, the filters were further hybridized with random-primed [^{32}P]-labeled β-actin RNA and autoradiographed for 6–8 h. The X-ray images of the Northern blots were scanned by an Epson Expression 636 scanner into Mac Power PC G3 computer, followed by analysis of the density of mRNA bands corresponding to transferrin receptor and β-actin using NIH Image 1.57 software package. The densities of transferrin receptor mRNA in control and Mn-treated groups were normalized by those of actin mRNA in each corresponding lane.

2.7. Northern blot of mRNAs of MT-I and glutamine synthesis in rat brain

Dr. Robert Andersen (UCLA) generously provided the plasmid containing the cDNA fragment for MT-I. The MT-I gene was cloned into the Pst1 site of pBR322. The cDNA fragments were resolved on a 2% agarose gel [32]. The probes were [32P]dCTP labeled with a random primed DNA labeling kit (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Total RNA from brains was isolated with the RNA Zol B test kit. Hybridization was performed by addition of denatured ^{32}P-labeled probe to prehybridization solution, and incubation for 18 h at 45°C. The membranes were then exposed on Kodak X-Omat AR film with an intensifying screen at −80°C. MT-I mRNA levels were normalized to 28S rRNA [4]. Following hybridization, the 28S rRNA blots were washed, and then exposed on film for autoradiography. The X-ray images of the northern blots were captured with a CCD video camera to a Gateway 2000 computer, and densitometry was performed with the TINA 2.09e program package (Raytest, Straubenhardt, Germany) for the BAS 1500 Fuji Photo Film Image Plate Scanner (Fuji, Tokyo, Japan). The optical density for each MT signal was normalized to the abundance of 28S rRNA in the same lane, and corrected for background. Experiments were performed in three preparations in each group.

The glutamine synthetase (GS) plasmid was obtained from Dr. Steven F. Abcouwer (Harvard Medical School, Boston, MA). The plasmid referred to as pTarGS800 contains a BamHI insert representing approximately 800 bases (5′ end) of rat GS cDNA in pBluescriptKS + . The probes were [α^{32}P]dCTP labeled with a random primed DNA labeling kit. RNA isolation, hybridization, and image analysis were conducted as described above.

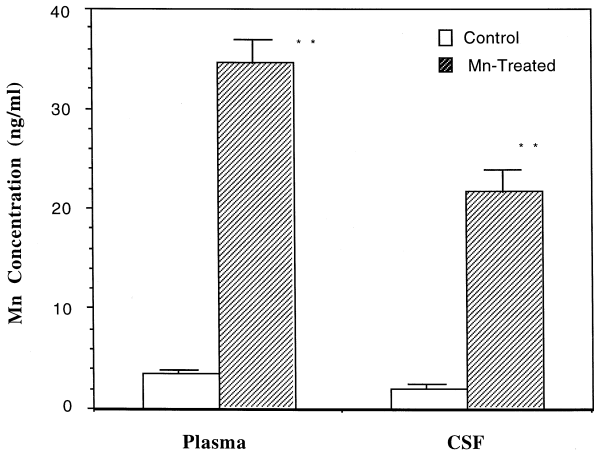

Fig. 1. Increases of manganese concentrations in plasma and CSF following chronic manganese exposure. Rats received i.p. injections of 6 mg/kg Mn as $MnCl_2$ once daily for 30 days. Plasma and CSF samples were collected at day 31 and used for assay of manganese by AAS. Data represent Mean ± SE ($n = 6–8$). **: $p < 0.01$.

2.8. Statistics

Statistical analyses of the differences between groups were performed by using Student's *t* test. The differences between two means were considered significant if *p* values were equal or less than 0.05.

3. Results

Manganese exposure at the dose of 6 mg/kg (i.p.) per day for 30 days resulted in a 10-fold increase in plasma concentration of manganese compared to the controls ($p < 0.01$). The same treatment also increased manganese concentration in the CSF by 11-fold ($p < 0.01$). Thus, the magnitude of increase in CSF manganese was similar to that in plasma (Fig. 1).

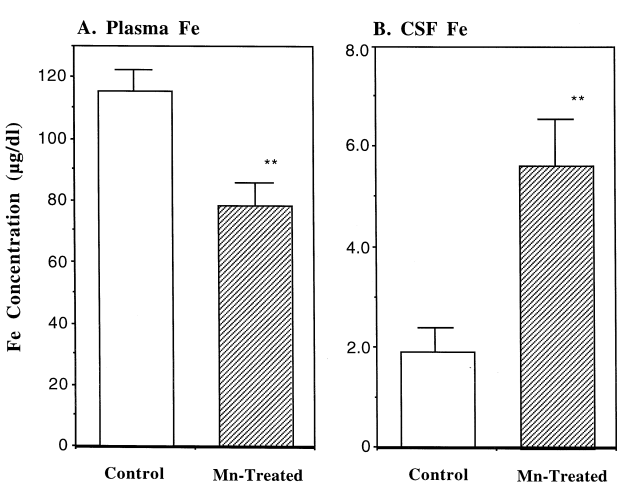

Fig. 2. Decrease of iron concentration in plasma and increase of iron concentration in CSF following chronic manganese exposure. Rats received i.p. injections of 6 mg/kg Mn as $MnCl_2$ once daily for 30 days. Plasma and CSF samples were collected at day 31 and used for assay of iron by AAS. Data represent Mean ± SE ($n = 6–8$). **: $p < 0.01$. (A) Fe in plasma; (B) Fe in CSF.

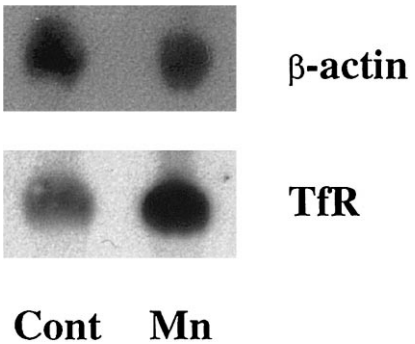

Fig. 3. Manganese exposure promotes the expression of transferrin receptor mRNA in primary culture of choroidal epithelial cells. The cells were incubated with 100 μM Mn (as $MnCl_2$) dissolved in serum-free medium containing 5 ng/ml insulin and transferrin, and 5 μg/ml sodium selenite at 37°C overnight, followed by Northern blot analysis. Cont: control cells; Mn: Mn-treated cells.

When plasma transferrin was measured as total iron binding capacity (TIBC), rats in the manganese-treated group did not show any significant change in TIBC (438 ± 12.4 SD μg/dl in control, vs. 410 ± 11.7 SD μg/dl in Mn group). Chronic exposure to manganese, however, significantly decreased plasma concentration of iron by 32% compared to controls ($p < 0.005$) (Fig. 2A). More surprisingly, the iron concentration in the CSF of the same rats did not decline as did it in plasma. Instead, it markedly increased by 3-fold compared to the controls ($p < 0.01$) (Fig. 2B). The ratio of CSF to plasma iron (FeCSF/plasma) was increased from 0.16 in control rats to 0.72 in the treated rats, reflecting an influx of iron from the systemic circulation to the cerebral compartment following manganese exposure.

We postulated that the increase in CSF iron in the face of diminished plasma iron might be due to the overexpression of transferrin receptor at the brain barrier systems under long-term manganese exposure. Thus, we examined

Table 1
Effect of manganese exposure on the expression of mRNAs of transferrin receptors in cultured choroidal epithelial cells and glutamine synthetase and metallothionein-I in rat brain tissues

	Control	Mn-treated	Increase (%)
Transferrin receptor	0.952 ± 0.006	1.409 ± 0.119**	48.0
Glutamine synthetase	0.918 ± 0.206	1.225 ± 0.267*	33.5
Metallothionein-I	0.867 ± 0.129	0.783 ± 0.280	−9.69

The optic density of bands for transferrin receptor mRNA was normalized to the abundance of β-actin mRNA in the same lane, and data represent the ratio of TfR/β-actin abundance. Similarly, the optic density for GS or MT-I mRNA was normalized to the abundance of 28S rRNA, and data represent the ratio of GS/28S or MT-I/28S abundance. Means ± SD, $n = 3$.

* $p < 0.05$.

** $p < 0.01$ as compared to the control.

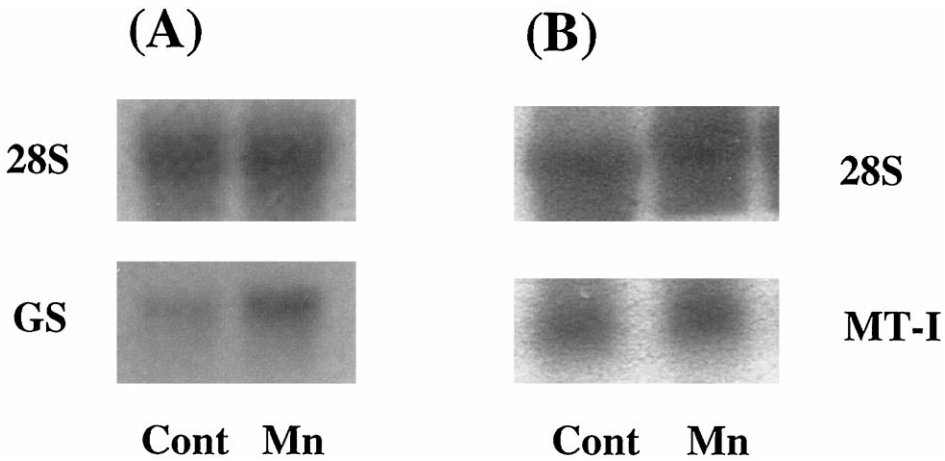

Fig. 4. Manganese exposure promotes the expression of glutamine synthetase mRNA, but not metallothionein-I mRNA in rat brain. Rats received i.p. injections of 6 mg/kg Mn as $MnCl_2$ once daily for 30 days. The brain was removed at day 31 and tissue homogenate used for Northern blot analyses. (A) Glutamine synthetase mRNA; (B) Metallothionein-I mRNA. Cont: control rat brain; Mn: Mn-treated rat brain.

the effect of manganese on the expression of transferrin receptor mRNA in cultured choroidal epithelial cells. Following manganese treatment at 100 μM overnight, the expression of transferrin receptor mRNA appeared to increase on autoradiography by Northern blot (Fig. 3). Quantitative analysis of the optical abundance of the bands corresponding to transferrin receptor mRNA, which was normalized by that of β-actin mRNA in the same lane, revealed that Mn treatment enhanced the expression of transferrin receptor mRNA in choroidal epithelial cells by 50% ($p < 0.05$) (Table 1).

Mn^{2+} can readily catalyze Fenton-like reactions, elicit oxidative stress by reactive oxygen species generated by metal-catalyzed dopamine autooxidation, and potentiate oxidative stress damage within the CNS by sequential activation of glutamate-gated cation channels [1]. Given that glutamine synthetase is highly sensitive to the oxidative stress, we conducted Northern study to analyze the effect of chronic manganese exposure on the expression of mRNAs encoding this protein. In addition, since recent data suggest that MT-I may function as a thiol donor and free radical scavenger, thus providing a defense line against oxidative damage [18], we further examined the expression of MT-I upon a similar treatment. Manganese exposure did not alter the expression of brain MT-I (Table 1); however, it significantly increased the expression of glutamine synthetase by 34% ($p < 0.05$) compared with controls (Fig. 4, Table 1).

4. Discussion

The results of our in vivo experiment, which administered Mn^{2+}, demonstrate that the homeostasis of manganese in the CSF appears to be directly influenced by plasma manganese concentrations. Studies by other investigators have shown that the valent status of manganese ion determines the transport properties of manganese at the brain barrier systems. While Mn^{3+}, a primary form of manganese ions in circulation, enters the brain via a transferrin receptor-mediated mechanism, Mn^{2+} appears to be readily taken up into the CNS, most likely as the free ion or nonspecific protein-bound species [2,30]. By comparing the in vivo rates of metal transfer (Kin) into brain among 13 metal species, Smith et al. [38] suggest that Mn^{2+} is the most permeable species among the metals studied. More recently, studies using transferrin knock-out mice indicate that deficiency in circulating transferrin has no apparent effect on tissue distribution of Mn^{2+} [10,22]. Thus, a non-transferrin-mediated mechanism appears to present for manganese movement across the brain barriers. Our data show parallel increases of manganese in CSF and in plasma when Mn^{2+} was administered. Thus, these results suggest that Mn^{2+} species are poorly restricted by the brain barrier systems.

Chronic Mn exposure produced a significant decrease in plasma iron. The pattern of plasma iron, while unexpected, is consistent with the observations in IPD patients whose circulating iron, ferritin, transferrin as well as total iron binding capacity are all significantly lower than those of control subjects [20]. The explanation for this overall deficiency in iron metabolism in IPD patients remain uncertain; however, manganese-induced diminished plasma iron may be attributed to manganese-enhanced intracellular distribution of iron. Several earlier studies support this assumption. For example, Chua and Morgan [8] observed that manganese supplementation in food led to an increased ^{59}Fe uptake by the brain, liver, and kidneys of rats. The authors concluded that manganese and iron interact during transfer from the plasma to the brain as well as other organs in a synergistic rather than competitive manner. Seligman et al. [34] also report that incubation of cultured human HL60 cells with a manganese–transferrin complex dramatically increased cellular uptake of ^{59}Fe. Moreover, recent studies in our laboratories at Columbia

reveal that manganese promotes the cellular overload of iron in cultured PC12 cells (unpublished data). Thus, it seems highly possible that manganese may interact with iron at cellular iron uptake.

In contrast to the observed diminished plasma iron, manganese injection greatly increased iron concentrations in the CSF. The data suggest a unidirectional influx of iron into the cerebral compartment in manganese-exposed rats. Among the processes involved in brain regulation of iron, the entrance of iron to the cerebral compartment represents a critical step in monitoring cerebral iron homeostasis. It has been generally accepted that transferrin-bound iron gains access to brain via transferrin receptors on brain capillary endothelial cells [14,16]. Evidence also suggests that choroid plexus, a major component of the blood–CSF barrier, by producing transferrin for the CNS, participates in brain iron regulation [11,27]. Although some studies using antibody against transferrin receptor failed to reveal the receptors in choroid plexus capillaries [7,16], others have elucidated their presence [21,26]. The results of the current study corroborate an abundant expression of transferrin receptor mRNA in cultured choroidal epithelial cells. Thus, it appears reasonable that the choroid plexus may co-regulate cerebral iron homeostasis. The degree to which the choroid plexus contributes to the overall brain iron regulation, as compared to the role of the blood–brain barrier, has not yet been established.

The data from our in vitro experiments clearly indicate that Mn treatment elevates the expression of transferrin receptors in the choroid plexus epithelial cells. This could be due to the unique ability of the choroid plexus to sequester manganese following acute or chronic manganese exposure [13,47], and the subsequent interaction of manganese with cellular iron-regulatory protein ACO1. Our previous studies show that manganese alters aconitase enzymatic activity, presumably, by competing with iron for the fourth, highly labile iron binding site of the [4Fe–4S] cube in enzyme's active center [47]. Such action, while suppressing the enzyme's catalytic function, may increase its binding affinity to the mRNAs encoding major proteins in iron metabolism such as ferritin and transferrin receptor [5,17]. The net result of these interactions is an up-regulation of cellular iron uptake. Thus, we postulate that chronic manganese exposure may stimulate the expression of transferrin receptor in the choroid plexus, and possibly at brain capillary endothelia (as well as in other peripheral organs). The overexpression of transferrin receptors at brain barriers and the ensuing facilitated iron transport from blood to the cerebral compartment would explain a compartmental shift of iron from the blood to the CSF. However, it is also possible that the promotive effect of manganese on the expression of transferrin receptor mRNA in cultured epithelial cells may be secondary to manganese-induced impairment in other cellular regulatory processes, such as alteration in mitochondrial energy production.

Excess cellular iron has been linked to the etiology of many neurodegenerative disorders. In experimental animal models, a general increase in the concentrations of iron in the substantia nigra has been found with treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and direct intranigral Fe infusion [12,25,29,33]. Cellular overload of iron in basal ganglia and substantia nigra regions initiates lipid peroxidation and generates iron-mediated reactive oxygen species [15,43]. Several intracellular proteins such as glutamine synthetase and metallothionein-I are reportedly sensitive to oxidative stress [19,39]. For instance, during the oxidation of glutamine synthetase, the reactive H_2O_2 and Fe^{2+} attack enzyme's amino acid residues, followed by a rapid degradation of the protein by intracellular proteases. While the relationship between manganese and oxidative stress in brain is not yet fully understood, it seems reasonable to postulate that the increased expression of glutamine synthetase mRNA may result from manganese-potentiated cellular overload of iron. Increased reactive oxygen species and the resulting oxidative stress may, therefore, inhibit enzyme activity, which, in turn, stimulates the synthesis of the protein. Alternatively, the overexpression of glutamine synthetase may reflect a change in intracellular levels of Mn(II) or the ratio of Mn(II)/Mg(II), since both factors may likely participate in the regulation of cellular glutamine synthetase activity [41]. The failure of manganese to stimulate MT-I mRNA expression is interesting given theories of manganese-induced elevation in free radical levels. In line with this concept, alternative antioxidant systems, such as Mn-SOD activity, glutathione and others, may function more prominently to offset the damage, but such possibilities will have to await further experimentation.

In conclusion, the results of this study indicate, for the first time in the literature, that chronic manganese exposure alters iron homeostasis in systemic circulation by depressing plasma iron and in cerebral compartment by elevating CSF iron. Overexpression of transferrin receptors in the choroid plexus by manganese treatment suggests that manganese, by acting on iron regulatory protein, may promote the expression of transferrin receptor, which partly facilitates the influx of iron from blood to CSF. We infer that manganese-induced Parkinsonism may result from a compartmental shift of iron into the brain, leading to oxidative stress in sensitive brain regions.

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References

[1] M. Aschner, Manganese neurotoxicity and oxidative damage, in: J.R. Connor (Ed.), Metals and Oxidative Damage in Neurological Disorders, Plenum Press, New York, NY, 1997, pp. 77–93.
[2] M. Aschner, M. Gannon, Manganese (Mn) transport across the rat blood–brain barrier: saturable and transferrin-dependent transport mechanisms, Brain Res. Bull. 33 (1994) 345–349.
[3] A. Barbeau, N. Inoué, T. Cloutier, Role of manganese in dystonia, Adv. Neurol. 14 (1976) 339–352.
[4] V. Barbu, F. Dautry, Northern blot normalization with a 28S rRNA oligonucleotide probe, Nucl. Acid Res. 17 (1989) 7115–7118.
[5] H. Beinert, M.C. Kennedy, Aconitase, a two-faced protein: enzyme and iron regulatory factor, FASEB J. 7 (1993) 1442–1449.
[6] M.W. Bradbury, Transport of iron in the blood–brain–cerebrospinal fluid system, J. Neurochem. 69 (1997) 443–454.
[7] R.D. Broadwell, B.J. Baker-Cairns, P.M. Friden, C. Oliver, J.C. Villegas, Transcytosis of protein through the mammalian cerebral epithelium and endothelium. III. Receptor-mediated transcytosis through the blood–brain barrier of blood-borne transferrin and antibody against the transferrin receptor, Exp. Neurol. 142 (1996) 47–65.
[8] A.C. Chua, E.H. Morgan, Effects of iron deficiency and iron overload on manganese uptake and deposition in the brain and other organs of the rat, Biol. Trace Elem. Res. 55 (1996) 39–54.
[9] J.R. Connor, S.A. Benkovic, Iron regulation in the brain: histochemical, biochemical, and molecular considerations, Ann. Neurol. 32 (Sppl) (1992) S51–61.
[10] T.K. Dickinson, A.G. Devenyi, J.R. Connor, Distribution of injected iron-59 and manganese-54 in hypotransferrinemic mice, J. Lab. Clin. Med. 128 (1996) 270–278.
[11] A.J. Dwork, Effects of diet and development upon the uptake and distribution of cerebral iron, J. Neurol. Sci. 134 (Sppl) (1995) 45–51.
[12] Y. He, P.S. Thong, T. Lee, S.K. Leong, C.Y. Shi, P.T. Wong, S.Y. Yuan, F. Watt, Increased iron in the substantia nigra of 6-OHDA induced parkinsonian rats: a nuclear microscopy study, Brain Res. 735 (1996) 149–153.
[13] R.T. Ingersoll, E.B. Montgomery, H.V. Aposhian, Central nervous system toxicity of manganese. I. Inhibition of spontaneous motor activity in rats after intrathecal administration of manganese chloride, Fundam. Appl. Toxicol. 27 (1995) 106–113.
[14] W.A. Jefferies, M.R. Brandon, S.V. Hunt, A.F. Williams, K.C. Gatter, D.Y. Mason, Transferrin receptor on endothelium of brain capillaries, Nature 312 (1984) 162–163.
[15] P. Jenner, A.H. Schapira, C.D. Marsden, New insights into the cause of Parkinson's disease, Neurology 42 (1992) 2241–2250.
[16] K. Kissel, S. Hamm, M. Schulz, A. Vecchi, C. Garlanda, B. Engelhardt, Immunohistochemical localization of the murine transferrin receptor (TfR) on blood–tissue barriers using a novel anti-TfR monoclonal antibody, Histochem. Cell Biol. 110 (1998) 63–72.
[17] R.D. Klausner, T.A. Rouault, J.B. Harford, Regulating the fate of mRNA: The control of cellular iron metabolism, Cell 72 (1993) 19–28.
[18] J.S. Lazo, Y. Kondo, D. Dellapiazza, A.E. Michalska, K.H.A. Choo, B.R. Pitt, Enhanced sensitivity to oxidative stress in cultured embryonic cells from transgenic mice deficient in metallothionein I and II genes, J. Biol. Chem. 270 (1995) 5506–5510.
[19] M. Ledig, J.C. Copin, G. Tholey, M. Leroy, F. Rastegar, F. Wedler, Effect of manganese on the development of glial cells cultured from prenatally alcohol exposed rats, Neurochem. Res. 20 (1995) 435–441.
[20] G. Logroscino, K. Marder, J.H. Graziano, G. Freyer, V. Slavkovich, N. LoIacono, L. Cote, R. Mayeux, Altered systemic iron metabolism in Parkinson's disease, Neurology 49 (1997) 714–717.
[21] J. Lu, C. Kaur, E.A. Ling, Expression and upregulation of transferrin receptors and iron uptake in the epiplexus cells of different aged rats injected with lipopolysaccharide and interferon-gamma, J. Anat. 187 (1995) 603–611.
[22] E.A. Malecki, A.G. Devenyi, J.L. Beard, J.R. Connor, Transferrin response in normal and iron-deficient mice heterozygotic for hypotransferrinemia; effects on iron and manganese accumulation, Biometals 11 (1998) 265–276.
[23] I. Mena, J. Court, S. Fuenzalida, P.S. Papavasiliou, G.C. Cotzias, Modification of chronic manganese poisoning. Treatment with L-dopa or 5-OH tryptophane, New Engl. J. Med. 282 (1970) 5–10.
[24] Y. Michotte, D.L. Massart, A. Lowenthal, L. Knaepen, J. Pelsmaekers, M. Collard, A morphological and chemical study of calcification of the choroid plexus, J. Neurol. 216 (1977) 127–133.
[25] H. Mochizuki, H. Imai, K. Endo, K. Yokomizo, Y. Murata, N. Hattori, Y. Mizuno, Iron accumulation in the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced hemiparkinsonian monkeys, Neurosci. Lett. 168 (1994) 251–253.
[26] T. Moos, Immunohistochemical localization of intraneuronal transferrin receptor immunoreactivity in the adult mouse central nervous system, J. Comp. Neurol. 375 (1996) 675–692.
[27] C.M. Morris, A.B. Keith, J.A. Edwardson, R.G. Pullen, Uptake and distribution of iron and transferrin in the adult rat brain, J. Neurochem. 59 (1992) 300–306.
[28] J. Neve, N. Leclercq, Factors affecting determinations of manganese in serum by atomic absorption spectrometry, Clin. Chem. 37 (1991) 723–728.
[29] C.W. Olanow, P.F. Good, H. Shinotoh, K.A. Hewitt, F. Vingerhoets, B.J. Snow, M.F. Beal, D.B. Calne, D.P. Perl, Manganese intoxication in the rhesus monkey: a clinical, imaging, pathologic, and biochemical study, Neurology 46 (1996) 492–498.
[30] O. Rabin, L. Hegedus, J.M. Bourre, Q.R. Smith, Rapid brain uptake of manganese(II) across the blood–brain barrier, J. Neurochem. 61 (1993) 509–517.
[31] K.P. Roberts, M.D. Griswold, Characterization of rat transferrin receptor cDNA: the regulation of transferrin receptor mRNA in testes and in Sertoli cells in culture, Mol. Endocrinol. 4 (1990) 531–542.
[32] S. Sambrook, E.F. Fritsch, T. Maniatis (Eds.), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, New York, NY, 1989, pp. 8.1–8.86.
[33] S. Sastry, G.W. Arendash, Time-dependent changes in iron levels and associated neuronal loss within the substantia nigra following lesions within the neostriatum/globus pallidus complex, Neuroscience 67 (1995) 649–666.
[34] P.A. Seligman, C. Chitambar, M. Vostrejs, P.L. Moran, The effects of various transferrins on iron utilization by proliferating cells, Ann. N.Y. Acad. Sci. 526 (1988) 136–140.
[35] P.K. Seth, R. Husain, M. Mushtaq, S.V. Chandra, Effect of manganese on neonatal rat: Manganese concentration and enzymatic alterations in brain, Acta Pharmacol. Toxicol. 40 (1977) 553–560.
[36] P.K. Seth, J.S. Hong, C.D. Kilts, S.C. Bondy, Alteration of cerebral neurotransmitter receptor function by exposure of rats to manganese, Toxicol. Lett. 9 (1981) 247–254.
[37] J. Singh, R. Husain, S.K. Tandon, P.R. Seth, S.V. Chandra, Biochemical and histopathological alterations in early manganese toxicity in rats, Environ. Physiol. Biochem. 4 (1974) 16–23.
[38] Q.R. Smith, O. Rabin, E.G. Chikhale, Deliver of metals to brain and the role of the blood–brain barrier, in: J.R. Connor (Ed.), Metals and Oxidative Damage in Neurological Disorders, Plenum Press, New York, NY, 1997, pp. 113–130.
[39] E.R. Stadtman, Protein oxidation and aging, Science 257 (1992) 1220–1224.
[40] L.B. Tepper, Hazards to health: manganese, New Engl. J. Med. 264 (1961) 347–348.

[41] G. Tholey, M. Ledig, P. Mandel, L. Sargentini, A.H. Frivold, M. Leroy, A.A. Grippo, F.C. Wedler, Concentrations of physiologically important metal ions in glial cells cultured from chick cerebral cortex, Neurochem. Res. 13 (1988) 45–50.
[42] Y.Y. Yeh, P. Zee, Micromethod for determining total iron-binding capacity by flameless atomic absorption spectrophotometry, Clin. Chem. 20 (1974) 360–364.
[43] M.B.H. Youdim, D. Ben-Shachar, P. Riederer, The possible role of iron in the etiopathology of Parkinson's disease, Mov. Disord. 8 (1993) 1–12.
[44] W. Zheng, The choroid plexus and metal toxicities, in: L.W. Chang (Ed.), Toxicology of Metals, C.R.C. Press, New York, NY, 1996, pp. 605–622.
[45] W. Zheng, D.F. Perry, D.L. Nelson, H.V. Aposhian, Protection of cerebrospinal fluid against toxic metals by the choroid plexus, FASEB J. 5 (1991) 2188–2193.
[46] W. Zheng, Q. Zhao, J.H. Graziano, Primary culture of rat choroidal epithelial cells: a model for in vitro study of the blood–cerebrospinal fluid barrier, In Vitro Cell Biol. Dev. 34 (1998) 40–45.
[47] W. Zheng, S. Ren, J.H. Graziano, Manganese inhibits mitochondrial aconitase: A mechanism of manganese neurotoxicity, Brain Res. 799 (1998) 334–342.