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Manganese Neurotoxicity: A Focus on the Neonate

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Abstract

Manganese (Mn) is an essential trace metal found in all tissues, and it is required for normal amino acid, lipid, protein, and carbohydrate metabolism. While Mn deficiency is extremely rare in humans, toxicity due to overexposure of Mn is more prevalent. The brain appears to be especially vulnerable. Mn neurotoxicity is most commonly associated with occupational exposure to aerosols or dusts that contain extremely high levels (> 1-5 mg Mn/m^3) of Mn, consumption of contaminated well water, or parenteral nutrition therapy in patients with liver disease or immature hepatic functioning such as the neonate.

This review will focus primarily on the neurotoxicity of Mn in the neonate. We will discuss putative transporters of the metal in the neonatal brain and then focus on the implications of high Mn exposure to the neonate focusing on typical exposure modes (e.g., dietary and parenteral). Although Mn exposure via parenteral nutrition is uncommon in adults, in premature infants, it is more prevalent, so this mode of exposure becomes salient in this population. We will briefly review some of the mechanisms of Mn neurotoxicity and conclude with a discussion of ripe areas for research in this underreported area of neurotoxicity.

Keywords

manganese; neurotoxicity; neonate; brain; blood-brain barrier

Introduction

Manganese (Mn), a common naturally-occurring element, is second only to iron in terms of prevalence in the environment. It is considered an essential nutrient and is crucial for maintaining the proper function and regulation of many biological processes such as producing ATP and blood clotting. More prominently, Mn is a constituent of many enzymes involved in carbohydrate (pyruvate carboxylase) and protein (arginase) metabolism, is utilized by various antioxidant enzymes such as superoxide dismutase (MnSOD), and activates the glycosyltransferase necessary for the mucopolysaccharides utilized by cartilage, bone and

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other connective tissues (For a more detailed review, see: Hurley and Keen). The first reported cases of Mn deficiency were in chickens suffering from perosis, which later was discovered to be due to inadequate glycosyltransferase activity causing the malformed bones. This symptom of Mn deficiency manifests itself during the formation and growth of bones and connective tissues during development. In adult animals, including humans, Mn deficiency, which is extremely rare, is characterized by weight loss and blood clotting problems. Therefore, in humans pathologies associated with abnormal Mn biology revolve around exposure to excessive Mn and not its deficiency.

Mn is used in numerous industries including steel production, formulating gasoline anti-knock additives (methylcyclopentadienyl manganese tricarbonyl; MMT), mining, welding, battery assembly and glass and ceramics manufacturing. Considering this wide-spread use of Mn, it is important to identify populations that may be vulnerable to Mn intoxication, particularly since chronic Mn overexposure results in the onset of a neurological phenotype, known as manganism, which present with motor symptoms resembling those of Parkinson's disease (Aschner and Aschner, 1991; Pal et al., 1999; Lee, 2000). Generally, airborne Mn is considered to be the most relevant route of exposure in occupational settings (Dorman et al., 2002; Dobson et al., 2003; Erikson et al., 2004a; Aschner et al., 2005; Erikson et al., 2005). However, dystonia and movement disorders have been described in case reports of adults and children receiving prolonged total parenteral nutrition (TPN) and have been associated with magnetic resonance imaging (MRI) abnormalities suggesting Mn-associated changes in the basal ganglia.

Mn deposition in the brain has potentially important implications for long-term neurodevelopmental outcome in exposed infants. In monkeys and rats, a correlation exists between the severity of central nervous system (CNS) symptoms and Mn brain concentrations, with both the rate and extent of Mn transport into the CNS influencing the clinical outcome (Suzuki et al., 1975; Roels et al., 1997). In neonatal rats, high dietary Mn intake resulted in developmental deficits (Tran et al, 2002). It has been observed that infants who require prolonged parenteral nutrition during their early neonatal course have worse developmental outcomes than gestational age-matched control infants, even after correcting for confounders such as respiratory complications and socioeconomic status (Morris et al., 1999). While the explanation for this discrepancy in developmental outcomes is undoubtedly complex and multi-factorial, the potential contribution of Mn toxicity to the poor outcomes of infants dependent for an extended time on parenteral nutrition has not been fully acknowledged or studied. Neither is information available on the contribution of co-morbidities such as iron deficiency and cholestasis to the ability of the neonatal brain to regulate Mn uptake. It is known that increases in blood and brain Mn levels have been reported in persons with liver disease (Spahr et al., 1996; Rose et al., 1999); and data suggest that iron (Fe) deficiency may be a risk factor for Mn neurotoxicity (Ellingsen et al., 2003; Erikson et al., 2002a; Erikson et al., 2004a). This last point is especially relevant considering the prevalency of Fe deficiency throughout the world (approximately 2 billion people are affected).

This review will focus primarily on the neurotoxicity of Mn in the neonate. We will discuss putative transporters of the metal in the neonatal brain and then focus on the implications of high Mn exposure to the neonate focusing on typical exposure modes (e.g., dietary and parenteral). Although Mn-exposure via parenteral nutrition is uncommon in adults, in premature infants, it is more prevalent, so this mode of exposure becomes salient in this population. We will conclude with a discussion of ripe areas for research in this underreported area.

Manganese Transport into Brain

Transferrin/transferrin receptor in Mn transport

Little is also known about transport mechanisms of Mn during the developmental period. Putative transport mechanisms in the adult include the divalent metal transporter 1 (DMT-1) and the transferrin receptor (TfR). Protein expression of DMT-1 and TfR is seen as early as postnatal day 5 (PN5) and increases through PN15 in all regions examined (cortex, hippocampus, striatum) (Siddappa et al., 2002; Garcia et al., 2006), verifying that DMT-1 and TfR are present in the developing brain; however, whether functional, remains unknown.

A series of studies has shown that Mn influx to the brain, but not efflux, is carrier mediated (Crossgrove et al., 2003; Yokel et al., 2003). In plasma, Mn is bound to small molecular weight carriers such as albumin and citrate or proteins like transferrin. Transferrin (Tf) is the primary iron binding/transport protein in plasma and is also known to bind Mn. Mn does not avidly complex with sulfhydryl (-SH) groups or amines, and it shows little variation in its stability constants for endogenous complexing ligands such as glycine, cysteine, riboflavin, and guanosine. In the absence of Fe, the binding sites of Tf can accommodate a number of other metals raising the possibility that Tf functions *in vivo* as a transport agent for many of these metals. Mn binding to Tf is time-dependent (Keefer et al., 1970; Scheuhammer and Cherian, 1985; Aschner and Aschner, 1990). When complexed with Tf, Mn is exclusively present in the trivalent oxidation state, with 2 metal ions tightly bound to each Tf molecule (Aisen et al., 1969). At normal plasma Fe concentrations (0.9-2.8 μg/ml), normal iron binding capacity (2.5-4 μg/ml), and at normal Tf concentration in plasma, 3 mg/ml, with 2 metal-ion-binding sites per molecule (Mr 77000) of which only 30% are occupied by Fe^{+3}, Tf has available 50 μ mole of unoccupied Mn^{3+} binding sites per liter (Aschner and Aschner, 1990).

Since Tf receptors are present on the surface of the cerebral capillaries (Fishman et al., 1985; Jeffries et al., 1984; Partridge et al., 1987) and endocytosis of Tf is known to occur in these capillaries (Partridge et al., 1987), it has been suggested that Mn (in the trivalent oxidation state) enters the endothelial cells complexed with Tf. Mn is then released from the complex in the endothelial cell interior by endosomal acidification and the apo-Tf Tf complex is returned to the luminal surface (Morris et al., 1992a; Morris et al., 1992b) without the assistance of DMT1 (Divalent Metal Transporter-1) (Moos et al., 2006). Mn released within the endothelial cells is subsequently transferred to the abluminal cell surface for release into the extracellular fluid. The endothelial Mn is delivered to brain-derived Tf for extracellular transport and the manganese subsequently taken up by neurons that posses both transferrin receptors and DMT1 (Moos and Morgan, 2002). A recent study by Moos et al. (2006) indicates that Tf-bound metal gains access to the brain through the blood-CSF barrier where the metal then diffuses into neurons form the ventricles and subarachnoid space. Support for receptor-mediated endocytosis of a Mn-Tf complex in cultured neuroblastoma cells (SHSY5Y) was recently demonstrated by Suarez and Eriksson (1993). Sloot and Gramsbergen (1994) have demonstrated anterograde axonal transport of ^{54}Mn in both nigrostriatal and striatonigral pathways. Furthermore, *in vivo*, intravenous administration of ferric-hydroxide dextran complex significantly inhibits Mn brain uptake, and high Fe intake reduces CNS Mn concentrations, corroborating a relationship between Fe and Mn transport (Aschner and Aschner, 1990; Diez-Ewald et al., 1968).

It is noteworthy to mention the distribution of Tf receptors in relationship to CNS Mn accumulation. Pallidum, thalamic nuclei, and substantia nigra contain the highest Mn concentrations (Barbeau et al., 1976). Interestingly, Fe concentrations in these structures are the highest as well (Hill and Switzer, 1984). Although the areas with dense Tf distribution (Hill et al., 1985) do not correspond to the distribution of Mn (or Fe), the fact that Mn-accumulating areas are efferent to areas of high Tf receptor density suggests that these sites

may accumulate Mn through neuronal transport (Sloot and Gramsbergen, 1994). For example, the Mn rich areas of the ventral-pallidum, globus pallidus, and substantia nigra receive input from the nucleus accumbens and the caudate-putamen (Walaas and Fonnum, 1979; Nagy et al., 1978) - two areas abundantly rich in Tf receptors.

Experiments examining Tf's role in Mn transport are often conducted with Mn-bound Tf complexes. However, experiments using the hypotransferrinemic mouse, which lacks functional Tf, has shown that Mn transport to the brain is not affected indicating that transferrin is not necessary for brain Mn acquisition across the blood-brain barrier (Malecki et al., 1999). Tf gains access to the cell by binding to its receptor, the TfR, where then the complex is endocytosed. Divalent metal transporter (DMT1) is a component of the endosome and functions to acidify the endosome to allow for release of Tf bound metal and then functions to pump the metal into the cytosol. Presumed is that Mn also is released from Tf-Mn complexes by the same mechanism (Roth and Garrick, 2003; Gunter et al., 2006) however more studies are needed. This mechanism was elucidated in reticulocytes where TfR is abundant and Tf-Fe complexes (and perhaps Tf-Mn complexes) were able to enter cells but the metal could not be released into the cytosol (Garrick et al., 1993). Several *in vitro* studies have shown that Mn^{3+} does not accumulate intracellularly to any significant amount to lead to neurotoxicity. Gunter et al., (2005) found no evidence of stabilization of Mn^{3+} complexes in human neuroteratocarcinoma (NT2) cells and primarly rat astrocyte cultures or in nerve growth factor treated PC12 cells (Gunter et al., 2005). The cause of manganese neurotoxicity is suggested to by due to Mn^{2+} inhibition of Ca^{2+} activation and control of ATP production (Gunter et al., 2006).

Divalent Metal Transporter (DMT1) in Mn transport

The plasma and transmembrane transport of Fe and Mn are thought to share common mechanisms. Divalent Metal Transporter-1 (DMT1/Nramp2/SLC11A2) has been suggested to be the Mn transporter in the duodenum in the adult. Conflicting arguments exist over whether Tf receptors are present or absent in the entrerocyte (Parmley et al., 1985; Pietrangelo et al., 1992 (Oates et al., 2000). Studies by Conrad et al. indicate that Mn shares the ferrous iron transport mechanism (DMT1) but no the ferric pathway (β_3-integran and mobilferrin) (Conrad et al., 2000).

DMT1 is a divalent-metal transporter that functions in a pH-dependent fashion stimulated by acidic pH, which is suggestive of a proton/metal-symport mechanism. DMT1 is expressed at the duodenum brush border in mice, where it is responsible for transferrin-independent uptake of dietary iron from the intestinal lumen. DMT1 also colocalizes with transferrin in the recycling endosomes of many cell types, including reticulocytes, where it transports iron from the acidified lumen of the endosomes into the cytoplasm. Two animal models exist, *mk* (microcytic anemic) mouse and the Belgrade rat (*b*/*b*) (Canonne-Hergaux et al., 2001; Fleming et al., 1998). Both animals are anemic from birth and require iron supplementation to survive. The Belgrade rat has also been shown to have impaired Mn metabolism (Chua & Morgan, 1997) and has been the model used in several Mn transport studies. Interestingly, it is also known that mutations in NRAMP2 homologues in bacteria and yeast disrupt Mn transport (Kehres & Maguire, 2003; Portnoy et al., 2002; Rosakis & Koster, 2004; Rosakis & Koster, 2005).

Chua and Morgan found that Mn and iron shared similar transport mechanisms in the erythroid tissue, duodenal mucosa, kidney and blood-brain barrier where Mn and iron transport were reduced in these tissues in the Belgrade homozygous rat (*b*/*b*) (Chua & Morgan, 1997). Transport of Mn to reticulocyes was altered only when Tf-Mn was the form of Mn however and not under low-affinity conditions. Interestingly, Mn liver levels were higher in the b/b rat suggesting an alternate uptake pathway for non-Tf bound Mn. Tf mediate process seems to

decline as rats mature due to changes in the rate of growth and proliferation. Crossgrove and Yokel have studies the transport of Mn across the BBB using the Belgrade rat. They found no difference between *b*/*b* rats and control rats suggesting that DMT1 does not play a role in uptake of Mn in the adult animal. Neuronal expression of transferrin receptors and DMT1 in adult rats implies that neurons at this age acquire iron by receptor-mediated endocytosis of transferrin followed by iron transport out of endosomes mediated by DMT1. The existence of the mutated DMT1 molecule in neurons suggests that the low cerebral iron uptake in b/b rats derives from a reduced neuronal uptake rather than an impaired iron transport through the blood-brain barrier (Moos & Morgan, 2004). Kidney studies remain to show the importance of DMT1 as well as different isoforms (Abouhamed et al., 2006; Canonne-Hergaux & Gros, 2002; Wareing et al., 2003; Ferguson et al., 2003). Pharmacokinetic studies show that iron status can alter lung Mn transport to circulation (Brain et al., 2006; Heilig et al., 2005; Thompson et al., 2006) DMT1 is not the transporter involved (Heilig et al., 2006). Recently is has been verified that the *b*/*b* Belgrade rat lacks transport mechanisms for divalent metals in its small intestine (Knopfel et al., 2005). It seems clear that duodenal absorption of metals is DMT1 regulated. However, while present early in postnatal development, it does not appear to be iron-regulated until adulthood (Leong, et al., 2003a; 2003b).

Other potential transporters of brain Mn

As mentioned the mechanism in which Mn is transported across the BBB has recently come to light. It appears that facilitated diffusion (Rabin et al., 1993), active transport (Murphy et al., 1991; Aschner and Gannon, 1993; Rabin et al., 1993), DMT-1-mediated transport (Erikson et al., 2004a; Garrick et al., 2003), ZIP8-, store-operated calcium channels as well as transferrin (Tf)-dependent transport (Aschner and Gannon, 1993) mechanisms are all involved in shuttling Mn across the BBB. Although non-protein-bound Mn enters the brain more rapidly than Tf-bound Mn (Murphy et al., 1991; Rabin et al., 1993), it is unclear as to which form represents the predominant mechanism of transport *in situ*.

A small fraction of Mn is found in plasma as Mn-citrate (Crossgrove et al., 2003; Yokel and Crossgrove, 2004). The same authors have suggested that a Mn citrate tridentate complex with a non-coordinated central carboxylate recognition moiety is likely a substrate for the organic anion transporter or a monocarboxylate transporter (MCT). Candidates for transport of Mn citrate may include MCT and/or members of the organic anion transporter polypeptide (OATP) or ATP-binding cassette (ABC) superfamilies.

A couple of recent studies suggest that calcium channels may be another potential transport mode for cellular Mn transport. The first study showed that Mn transport into the brain was not dependent on DMT-1 but rather another transport mode such as calcium channels {Crossgrove et al., 2005) (Yokel et al., 2003). The other study looked at ^{54}Mn uptake in the human alveolar cell (A549) and found that calcium channel blockers significantly inhibited Mn uptake (Heilig et al., 2006). Pharmacological studies of ^{54}Mn uptake by A549 cells, a human alveolar cell line, suggest that metal uptake by type II alveolar epithelial cells is associated with activities of both L-type Ca(2+) channels and TRPM7, a member of the transient receptor potential melastatin subfamily. Their results demonstrate that Fe and Mn are absorbed by the pulmonary epithelium through different pathways and reveal the potential role for calcium channels in lung metal clearance. The difference in transport between Fe and Mn has also been noted in the olfactory pathway. Where inhaled Mn was transported to the brain, Fe was not readily transported by the olfactory tract (Rao et al., 2003). In other studies using Mn enhanced MRI (MEMRI) to trace odor pathways in the brain, Pautler & Koretsky were able to inhibit the transport of Mn transport via the olfactory neurons using the calcium channel blocker diltiazem in conjunctions with $MnCl_2$ administered directly into the naris and verified using MRI (Pautler & Koretsky, 2002).

Finally, recent evidence implies a role for the ZIP transporter proteins in Mn transport, though whether functional at the BBB has yet to be determined. The ZIP transporter proteins, members of the solute-carrier-39 (SLC39) metal-transporter family, having 14 members-highly conserved orthologs-between mouse and human (Eide et al., 2004). Zip proteins have been originally divided into two subfamilies, referred to as subfamily I, consisting mostly of fungal and plant sequences, and subfamily II, which consists of insect, nematode, and mammalian sequences. More recently, PSI-BLAST(position-specific iterative-basic local alignment search tool) analysis uncovered two additional subfamilies, referred to as gufA and the LIV-1 or LIV-1 subfamily of ZIP transporters (LZT). In plants, several ZIP proteins have been implicated in divalent metal transport, including zinc (Zn), Fe, and Mn. A recent in vitro study (He et al., 2006) in mouse fetal fibroblast cultures established that ZIP8 is a high affinity for Mn. The Km of 2.2 μM for Mn2+ is close to physiological concentrations and within the same range determined in many cell lines or tissues. However, whether Slc39 and organic transporters function in Mn transport remains to be examined under physiologically relevant conditions. Definitive studies to assess other protein functions (Slc39, MCT) in physiological roles are needed.

Modes of Manganese Exposure

Diet and water

The most important source of Mn for the population at large is diet, with most daily intakes falling below 5 mg Mn/kg. Adult dietary intake of Mn is estimated to be between 0.9 to 10 mg Mn/day (ATSDR, 2000; Finley and Davis, 1999). Based on the Total Diet Study, grains, beverages (tea), and vegetables provide approximately 33, 20, and 18% of dietary Mn in adult males, respectively (Pennington and Young, 1991).

Levels of Mn in excess of 30 mg/kg can be found in certain foods, such as grain, rice and nuts. Levels of Mn are also high in tea; a cup of tea may contain as much as 0.4 to 1.3 mg Mn (ATSDR, 2000). Another important source of dietary Mn intake is Mn-containing dietary supplements. Many of these contain Mn levels of 5–20 mg (NAS, 2001). Water concentrations of Mn typically range from 1 to 100 μg/L with most values below 10 μg/L (Keen and Zidenberg-Cherr, 1994). Greater than 60% of the nation's drinking water has been systematically assessed since 1991 to determine the quality of sources of drinking water in the US. The analysis, conducted by the National Water-Quality Assessment Program (NAWQA) of the U.S. Geological Survey (USGS, 2005), suggests that roughly 6% of domestic wells contain high levels of Mn in drinking water in the range of 300 μg/L. This level of Mn exposure has recently been shown to be associated with reduced Full-Scale, Performance, and Verbal raw scores in children in Bangladesh (Wasserman et al., 2006).

Milk and infant formulas

Human milk is generally low in Mn content (1.8-27.5 μg/L); however, Mn concentrations in infant formulas can vary dramatically (33-300 μg/L) (Keen and Zidenberg-Cherr, 1994; Murthy et al., 1971; Stastny et al., 1984; Vaughan et al., 1979). In human (and animal) milk Mn concentrations are associated with the stage of lactation (Casey et al., 1985; Stastny et al., 1984; Vaughan et al., 1979; Krachler and Rossipal, 2000; Table 1). For example Stastny and coworkers (1984) reported that mean (± SD) human milk Mn concentrations in the fourth week of lactation were 6.6 ± 4.7 μg/L and these levels were significantly higher than those collected during the 12^{th} week of lactation (3.5 ± 1.4 μg/L). Notably, in human milk Mn is in the trivalent oxidation state where it is bound to lactoferrin, the major iron-binding protein in milk. Receptors for this protein are abundant in the brush border membranes of epithelial cells throughout the length of the small intestine, thus allowing for regulation of the uptake of Mn across the gastrointestinal tract. Since in formula Mn is in the divalent oxidation state,

absorption through the GI tract cannot be regulated by lactoferrin receptors. Accordingly, Mn transport of infant formula-derived Mn is likely governed by mechanisms different from those from Mn in human breast milk.

In general, infant formulas contain much higher Mn concentrations compared to those observed in human milk (Table 1). Given the risk posed by high Mn concentrations in infant formulas (Golub et al., 2005) their composition has significantly changed over the last 20 years. For example, Enfamil (Mead Johnson and Company) contained 1289 μg Mn/L in 1983; levels dropped to 105 μg/L shortly thereafter (Stastny et al., 1984). It has been suggested that consumption of soy-based infant formulas is a potential area of concern for human infants as levels of 200-300 μgMn/L are common (Lonnerdal 1994).

Parenteral exposure

Risk for Mn neurotoxicity is associated with total parenteral nutrition (TPN), as these nutritional solutions are commonly formulated to include Mn along with other essential trace metals and they can contain Mn as a contaminant (Hambidge et al., 1989; Kurkus et al., 1984). A report by Wilson et al. (1992) found that Mn concentrations in TPN solutions ranged from 5.6 to 8.9 μg/L, in the absence of any supplementation.

Few studies have addressed Mn kinetics upon TPN administration (Takagi et al., 2002). Furthermore, no clear standard has been recommended for the daily dose of parenteral Mn, with the published literature indicating a broad, 200-fold range in the recommended daily Mn dose for adults on TPN ranging from a low dose of 0.18–0.91 μmol (0.01-0.05 mg) to a high dose of 40 μmol (2.2 mg). It should be acknowledged that infants, especially premature infants, receiving TPN, may not have intact or mature homeostatic control of the metal. In addition to bypassing the homeostatic barrier of the gastrointestinal tract where Mn absorption is normally tightly regulated (Davidsson et al., 1989), many infants and children on TPN solutions suffer from hepatic dysfunction and cholestasis, compromising their biliary excretion of Mn. Finally, further risk of Mn-induced toxicity is associated with the overall neurophysiological immaturity of the developing brain, perhaps allowing entrance for more Mn than would occur in the adult brain.

Mn intoxication associated with TPN solutions providing ≥ 0.1 mg Mn/day is well established (Bertinet et al., 2000; Nagatomo et al., 1999; Ono et al., 1995). These patients developed elevated serum Mn levels (Takagi et al., 2002), and they exhibit symmetrical high intensity MRI lesions in the globus pallidus consistent with the preferential accumulation of Mn at this site, in association with characteristic psychiatric symptoms and clinical signs of Mn-induced parkinsonism-like syndrome. Withdrawal from the TPN solutions significantly decreases Mn levels in both the blood and CNS (Bertinet et al., 2000). In children chronically receiving TPN high Mn blood levels and abnormal neurological signs have been reported, along with MRI findings indicative of Mn deposition in the brain (Kafritsa et al., 1998). Once removed from Mn supplemented TPN, Mn brain levels tend to decline over time, blood Mn levels normalize and some case reports suggest a good prognosis without long-lasting neurodevelopmental sequelae (Kafritsa et al., 1998).

Mechanisms of Mn Neurotoxicity

Oxidative Stress

Oxidative stress has been implicated as a contributing mechanism by which Mn may be cytotoxic (Aschner, 1997). The oxidation of dopamine by Mn is a potential mechanism by which Mn-induced oxidative stress may occur, especially since Mn can accumulate in dopamine-rich brain regions of rodents and primates (e.g., basal ganglia) following prolonged exposure (Sloot et al., 1996). Another possible mechanism is that Mn, through its sequestration

in mitochondria (Galvani et al., 1995), interferes with proper respiration, thereby leading to excessive production of reactive oxygen species. One laboratory reported inhibition of complex I of the electron transport chain after treatment of PC12 cell cultures with Mn chloride (Brouillet et al., 1993). Another laboratory showed evidence suggesting that the ATPase complex is inhibited at very low levels of mitochondrial Mn, and that complex I is inhibited only at higher concentrations (Gavin et al., 1999). Although, trivalent Mn is more effective at inhibiting complex I (Archibald and Tyree, 1987; Ali et al., 1995; Chen et al., 2001), the divalent form is by far the predominant species within cells and is largely bound to ATP (Ali et al., 1995; Gunter et al., 2002). Nevertheless, in biological media, Mn of any valence will spontaneously give rise to small amounts of trivalent Mn. Interestingly, HaMai et al. (2001) demonstrated that even trace amounts of trivalent Mn can cause formation of reactive oxygen species (ROS) (HaMai et al., 2001).

Glutamate excitotoxicity

It has been shown that ROS will interfere with glutamate removal by inhibiting the high affinity glutamate transporters (Trotti et al., 1998). The ensuing increase in extracellular glutamate levels is potentially excitotoxic to neurons, representing a cause of Mn neurotoxicity. Glutamate uptake is attenuated in astrocytes that are exposed to Mn (Hazell and Norenberg, 1997; Erikson and Aschner, 2002b; Erikson et al., 2002c) and that GLAST gene expression is significantly decreased due to that exposure (Erikson and Aschner, 2002b; Erikson et al., 2002c). However, little data exist on the in vivo effects of Mn exposure on glutamate transporter gene expression and protein levels. Information that is critical for fully evaluating the neurotoxicity of Mn.

Neurotoxicity Studies using a neonatal model

Research projects that examine these putative mechanisms of Mn neurotoxicity in the neonatal brain are scant. Recently, our laboratory has shown that rats exposed to airborne Mn during gestation and during post-natal days 1-19 had significantly decreased glutathione (a prevalent endogenous antioxidant) levels as well as decreased gene expression of metallothionein and glutamine synthetase (two markers of oxidative stress) in the striatum (Erikson et al., 2006). It should be noted that compared to older rats, the neonatal rat striatum was impacted by Mn exposure to a greater degree (Erikson et al., 2004b; 2006). The striatum is also a brain region where marked changes in dopamine (Tran et al., 2002) and GABA metabolism have been observed in Mn exposed rat pups. Overall, it appears that the striatum is a vulnerable brain region in terms of Mn neurotoxicity in the neonate. This is in contrast to the adult brain in which other regions such as hippocampus, midbrain and olfactory bulb in addition to the striatum, have increased Mn concentrations (Dorman et al., 2003; 2004), altered neurotransmitter levels (Erikson et al., 2002a) and oxidative stress (Dobson et al., 2004; Erikson et al., 2005; 2006) due to Mn-exposure.

Direction for Future Research

Understanding the mechanisms in which Mn is transported into the neonatal brain is important when trying to dissect normal transport mechanisms from abnormal ones (i.e., during toxicity). To date it appears that the neonatal brain handles excessive Mn (via DMT-1 upregulation and to a lesser extent Tf/TfR) similarly to the adult brain (Garcia et al., 2006). In terms of neurochemical alterations associated with Mn-exposure in early life, both dopamine (Tran et al., 2002) and GABA (Garcia et al., 2006) have been implicated. One thing that remains unknown is the reversibility of these changes. In our recent study described in the previous paragraph, some of these developing rats were allowed to recover from Mn exposure for three and half weeks and upon termination (PND 45) many of the biomarkers did not normalize despite Mn levels returning to normal (Erikson et al., 2005). No data are available in terms of

assessing the neurological effects of Mn-exposure during early life and long term consequences of this exposure. Specifically, does Mn-exposure during early development increase the risk for developing neurodegenerative diseases in later life? It is known that changes in Mn-containing proteins have been observed in many neurodegenerative diseases, including Alzheimer's disease (Markesbery, 1997), amyotrophic lateral sclerosis, and Parkinsonian-like syndrome (Pal et al., 1999; Malecki, 2001), as well neurobehavioral deficits (May, 2000; Normandin et al., 2002) are associated with Mn-exposure. Therefore longitudinal studies that examine both markers of oxidative stress and neurotransmitter biology in subjects who have a history of Mn exposure during critical neurodevelopmental periods are necessary. These studies could include recovery periods ranging from several weeks to months after exposure to high manganese levels and ultimately will expedite the development of therapies for affected individuals.

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Table 1

Manganese concentration in human milk and select infant formulas

Investigator	Country	Analysis Method	Study Description (# Subjects)	Human Milk µg Mn/L	Infant Formula µg Mn/L
Krachler et al., 2000	Austria	ICP-MS	Human Milk (27) Infant Formula (4)*	Observed Range: 1.8-22.3	Observed Range: 32.8-55.0*
Casey et al., 1985	U.S.	GFAAS	Human Milk (22)	Observed Range: 1.97-3.94	
Stastny et al., 1984	U.S.	GFAAS	Human Milk (116) Infant Formula (7)**	Observed Range: 1.9-27.5	Observed Range: 70-1289**
Al-Awadi and Srikumar, 2000	Kuwait	GFAAS	Human Milk (17)	Observed Range: 3.8-6.0	

Data are Ranges of Means for each parameter.

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GFAAS=Graphite Furnace Atomic Absorption Spectrophotometry

ICP-MS=Inductively Coupled Plasma Mass Spectrophotometry

* Represents four different formulas tested, with at least six samples of each.

** Represents seven different formulas tested, Similac (Ross Laboratories) had the lowest Mn, whereas Enfamil (Mead Johnson and Company) had the highest. Note: As of March 1983, Enfamil is formulated to contain 105 µg/L.