Manganese Neurotoxicity

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ABSTRACT: Manganese is an essential trace element and it is required for many ubiquitous enzymatic reactions. While manganese deficiency rarely occurs in humans, manganese toxicity is known to occur in certain occupational settings through inhalation of manganese-containing dust. The brain is particularly susceptible to this excess manganese, and accumulation there can cause a neurodegenerative disorder known as manganism. Characteristics of this disease are described as Parkinson-like symptoms. The similarities between the two disorders can be partially explained by the fact that the basal ganglia accumulate most of the excess manganese compared with other brain regions in manganism, and dysfunction in the basal ganglia is also the etiology of Parkinson's disease. It has been proposed that populations already at heightened risk for neurodegeneration may also be more susceptible to manganese neurotoxicity, which highlights the importance of investigating the human health effects of using the controversial compound, methylcyclopentadienyl manganese tricarbonyl (MMT), in gasoline to increase octane. The mechanisms by which increased manganese levels can cause neuronal dysfunction and death are yet to be elucidated. However, oxidative stress generated through mitochondrial perturbation may be a key event in the demise of the affected central nervous system cells. Our studies with primary astrocyte cultures have revealed that they are a critical component in the battery of defenses against manganese-induced neurotoxicity. Additionally, evidence for the role of oxidative stress in the progression of manganism is reviewed here.

KEYWORDS: manganese; neurotoxicity; manganism; oxidative stress; reactive oxygen species

INTRODUCTION

Manganese is a trace element that is essential in the diet of all animals. While this metal is inhaled from the atmosphere, diet is normally a far greater source of human exposure to manganese. Because there are homeostatic systems of regulation for absorption and excretion of manganese in the body, the levels found in tissues are usually very stable, regardless of intake levels. However, manganese can accumulate in certain brain regions following elevated exposures, and manganese-induced neuro-

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Ann. N.Y. Acad. Sci. 1012: 115–128 (2004).
doi: 10.1196/annals.1306.009

toxicity can ensue. The symptomatic cases of this neurotoxicity are known as manganism, and clinically this presents with a Parkinson-like motor dysfunction. Little is known about the detailed mechanisms of manganese neurotoxicity. Studies on manganese transport and other biochemical end points are reviewed here. In particular, evidence for the involvement of oxidative stress in the progression of manganism is discussed.

ESSENTIALITY

Manganese is found in all body tissues as it is essential for many ubiquitous enzymatic reactions, including synthesis of amino acids, lipids, proteins, and carbohydrates.[1] Also particularly noteworthy for neurotoxicity studies is the requirement for manganese in the reactions catalyzed by arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese-dependent superoxide dismutase, to name a few.[2] The National Academies' Institute of Medicine has set dietary reference intakes in their 2002 report.[3] The report sets an adequate intake (AI) level for manganese at 2.3 mg per day for men and 1.8 mg per day for women. The tolerable upper intake level (UL) is set at 11 mg for adults. Studies have demonstrated that the female gastrointestinal tract is more efficient at absorbing manganese than in men.[4] Iron availability may be related to this difference in absorbance. Furthermore, adjustments in the manganese requirement were made in consideration of pregnancy (2.0 mg/day), lactation (2.6 mg/day), and the developmental stages of childhood (range of 0.003–2.2 mg/day, depending on age and sex).[3]

Manganese deficiency can cause a wide range of problems, including impaired growth, skeletal defects, reduced fertility, birth defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism.[5,6] However, these effects were observed in lab animals and this deficiency is not clinically recognized in humans.

SOURCES OF MANGANESE

Most adults have a daily intake of manganese below 5 mg manganese/kg, with a reported range of 0.9 to 10 mg manganese per day.[1,7] Grains, tea, and green leafy vegetables contain the highest amounts of manganese in the normal adult male diet as reported in the Total Diet Study.[8] The manganese content of human milk has been found to vary with stages of lactation.[9–11] There are also reports from both rat and human studies of much higher manganese absorption in the neonatal period.[12–15] This evidence is consistent with the higher manganese levels believed to be required for brain development at early stages. Infant formulas tend to have more manganese than human milk, and this has been a cause for some concern.[16,17]

Individuals receiving total parenteral nutrition (TPN) make up a subpopulation that is at even higher risk for manganese toxicity. The TPN solution can be formulated to contain manganese, but sometimes the manganese is found as an unintended contaminant.[18,19] Because the normal regulating mechanisms for manganese metabolism are bypassed (i.e., the gut), 100% of the manganese in the TPN solution enters the body as compared to approximately 5% of that taken orally. There have been reported intoxications from TPN solutions containing 0.1 mg Mn/day. The symptoms

and manganese measurements were consistent with other forms of manganese toxicity; withdrawal from the TPN alleviates symptoms.[20,21]

Airborne manganese can exist as fumes, aerosols, or suspended particulate matter.[7] This manganese "dust" can be inhaled and deposited in parts of the upper or lower respiratory tract, where the manganese can then be absorbed into the bloodstream. The levels of manganese in the air vary, depending on the industries nearby, wind erosion, and other factors. Ferroalloy production, iron and steel foundries, and combustion emissions from power plants and coke ovens make significant contributions to the concentration of manganese in air.[22] The average levels reported by ATSDR[7] for urban and nonurban air are 33 and 5 ng Mn/m^3, respectively. Nevertheless, the average daily Mn intake from ambient air is estimated to be <2 μg Mn/day.[23,24]

The gasoline additive, methylcyclopentadienyl manganese tricarbonyl (MMT), is a somewhat controversial source of additional airborne manganese. This compound may be used as a replacement for lead as an antiknock agent, and the debate surrounding its use in the United States was recently reviewed in *Science*.[25] Upon combustion in automobile engines, MMT yields a complex mixture of phosphate, sulfate, and oxide forms of manganese. It has been used in Canada for over 10 years, and studies of the Canadian cities with the most traffic have shown the air manganese content to be near or below the current inhalation (RfC) reference concentration for inhalable manganese, which is 0.05 μg Mn/m^3 as set by the United States Environmental Protection Agency.[24,26–28]

ABSORPTION AND TRANSPORT

Only about 1–5% of the manganese ingested by humans is absorbed into the body by the gastrointestinal tract under normal conditions.[4,29,30] This value is reportedly higher when measurements are taken less than 24 h postingestion, but similar studies in animals indicate that much of the manganese that is retained for shorter time periods is localized to the liver and intestinal tract and eliminated through biliary excretion.[31] As such, it would not reach the brain or other systemic tissues in significant amounts.

The molecular details of oral manganese absorption are not well understood. There is one line of evidence suggesting an active transport process,[32] and another group has demonstrated absorption through a simple passive diffusion-like process.[33] Furthermore, there are many other factors that have been found to affect manganese absorption, including dietary manganese levels,[1,29,34–36] dietary levels of various minerals,[37–39] age and developmental state of the individual,[13–15,40] and especially iron status. There seems to be an inverse relationship between body iron stores and manganese absorption, perhaps due to competition for transport machinery such as DMT-1 (divalent metal transporter, also known as DCT-1 or nramp-2).[41,42] Several studies have demonstrated that iron deficiency increases transport of orally administered manganese into the body as well as delivery to the brain.[1,43–45]

Absorption of manganese via the lungs has only recently been investigated and it seems to depend largely on particle solubility. Whereas $MnCl_2$, which is a soluble salt, is quickly taken into the bloodstream, insoluble MnO_2 given at similar doses was very slowly absorbed and at much lower overall levels.[46] This report also showed that the soluble salt was more readily delivered to the brain. More recently,

Dorman *et al*.[47] also showed that inhaled $MnSO_4$ was cleared from the lung faster than the less soluble phosphate or tetroxide manganese compounds, and transport into the brain and other tissues reflected this pattern based on particle solubility as well.

Blood manganese is largely bound to β-globulin and albumin (~80%), and a small percentage of trivalent (3+) manganese is found complexed to transferrin.[48–50] Nevertheless, because of the large number of unoccupied binding sites, transferrin has been implicated as a potential transport system for manganese to traverse the blood-brain barrier and other membranes.[51] No other specific transporters are known for blood manganese. Typical serum concentrations of manganese are in the range of 0.8–2.1 μg Mn/L. Neonates generally have the highest levels, a decreasing trend is observed through the first year, and adults have the lowest serum manganese content (reviewed in refs. 52 and 53).

Distribution of manganese to the body tissues is fairly homogeneous. Increased concentration of manganese is found in tissues rich in mitochondria and pigmentation. Bone, liver, pancreas, and kidney tend to have higher manganese levels than other tissues.[54,55] Liver especially accumulates manganese after high exposures, and most absorbed manganese is excreted in bile. Liver disease, therefore, is a risk factor for increased accumulation of manganese in the brain.[56–58]

Transport of manganese into the central nervous system (CNS) has been directly investigated in a limited number of studies. Together, these reports implicate three sites of manganese entry into the brain. The cerebral capillaries, the cerebrospinal fluid (CSF; via choroid plexus transport), and the olfactory nerve are all potential locations of manganese import.[59–61] Acute bolus intravenous injections of large amounts of manganese leads to a saturable transferrin-independent transport across the blood-brain barrier via either active or passive processes.[59,62] The choroid plexus, the site of CSF production, is where ^{54}Mn first appears in rodent brain after bolus injection into the circulation.[63,64] However, at relevant manganese exposure levels, the capillary endothelium seems to represent the route that is physiologically most germane to manganese entry into the CNS. Furthermore, the likeliest modes of transport are by transferrin/transferrin receptor and DMT-1.

Iron, manganese, and other metals are able to be complexed and carried by some of the same transporters. Transferrin/transferrin receptor and DMT-1, especially, are thought to transport both of these metals, with iron being far more prevalent under normal circumstances. Evidence from Suarez and Eriksson[65] and Aschner and Gannon[66] strongly suggests transport of trivalent manganese complexed to transferrin into the brain capillary endothelium. As such, the high concentration of transferrin receptors in the nucleus accumbens and caudate putamen, which provide efferent fibers to areas rich in manganese (ventral pallidum, globus pallidus, and substantia nigra), is consistent with transferrin-mediated manganese transport.

The role of DMT-1 in brain manganese transport is currently an area of intense investigation. It has been suggested that much of the manganese that gains access to the CNS does so via DMT-1 in brain endothelium. Absorption of manganese in the gut is thought to be mediated by DMT-1.[52] Studies of the Belgrade rat, which carries a mutation in the DMT-1 gene, show that (in addition to frank deficiency in uptake of iron) the homozygote demonstrates lower uptake of radiolabeled manganese than the heterozygote.[67] The Belgrade rat may also be a good model for dissecting the mechanisms of manganese transport into the brain. Additional experiments to elucidate the role of DMT-1 in manganese transport into rat brain endothelial cells are under way.

It has been well documented that xenobiotics can travel directly to the brain via the olfactory system.[68] Axonal transport of manganese has also been conclusively demonstrated.[61,63,69–72] Delivery of inhaled manganese is likely through direct intra-axonal transport,[73] and it has been reported in rat, mouse, and freshwater pike after intranasal instillation.[69,73,74] Additionally, Dorman *et al.*[75] have studied inhalation of various manganese-containing particulates and also found delivery along the olfactory route. However, the significance of the contribution of this pathway toward manganese toxicity is not yet clear. The striatum and other nonolfactory brain structures do not seem to accumulate much manganese through this route.[61,75,76] Further, there are substantial physiological differences known between human and rodent nasal and brain anatomy that complicate interpretation of comparative studies.[77]

Manganese toxicity studies have revealed that distribution of the metal to the various brain regions is not homogeneous and may even differ across species. Magnetic resonance imaging (MRI) techniques show that, in exposed humans and macaque monkeys, manganese concentrations are highest in striatum, globus pallidus, and substantia nigra.[21,78–80] Mixed results have been observed in rodent studies, however. Brenneman and coworkers[81] have reported that rat striatum and globus pallidus do not preferentially accumulate manganese after excess exposure. However, a very recent study in our laboratory showed that, after dietary iron deprivation, manganese accumulated in globus pallidus, hippocampus, and substantia nigra of rat brain.[45] This suggests that iron deficiency in humans might also lead to a higher tendency toward manganese accumulation in brain regions normally rich in iron. Previous work has demonstrated higher absorbance and accumulation in the brain in iron-deficient animals.[45]

TOXICITY

Inhalation of particulate manganese is the most recognized occupational risk for human toxicity. This manganese dust in various forms irritates lungs of humans and animals, causing an inflammatory response,[82] as do many other particulates. Studies thus far have not clarified whether this response is specific to manganese or is representative of the general reaction to inhaled particulates. A recent report shows that oxidative stress in lungs and heart is observed after 5-h inhalation exposure to concentrated ambient particles containing a mixture of metals including Mn.[83] This suggests that the lung inflammation may be a general response to inhaled metal particulates. Nevertheless, there are significant neurological effects specific to manganese particulate inhalation. Impotence and loss of libido have been reported in manganese-exposed workers,[7] but the later-stage neurological effects are the most compelling cause for concern about manganese exposure.

Chronic exposure to high levels of inhalable manganese (>1–5 mg Mn/m^3) is the most frequently observed cause of manganese-induced neurotoxicity.[7,84,85] Ingestion of very large amounts of manganese in water from contaminated wells has also been reported to cause neurotoxicity.[86,87] However, confounding variables in both of these studies make the interpretation questionable. Additionally, Vieregge and coworkers[88] studied human populations chronically consuming high levels of manganese and they were unable to confirm adverse health effects even after 10 years of exposure.

The disorder known as manganism is strongly associated with elevated levels of manganese in the brain. Specifically, structures of the basal ganglia—caudate putamen, globus pallidus, substantia nigra, and subthalamic nuclei, all of which contain substantial levels of nonheme iron—represent regions of highest manganese concentration.[85] The earliest symptoms associated with abnormal manganese accumulation are psychiatric. Compulsive or violent behavior, emotional instability, and hallucinations are characteristic, and patients may also suffer from fatigue, headache, muscle cramps, loss of appetite, apathy, insomnia, and diminished libido. The most severe forms of manganism present with prolonged muscle contractions (known as dystonia), decreased muscle movement (known as hypokinesia), rigidity, and muscle tremors. The physical traits of this disorder thus resemble Parkinson's disease, but there are distinguishing features.[78,85,89] While generalized bradykinesia and rigidity are

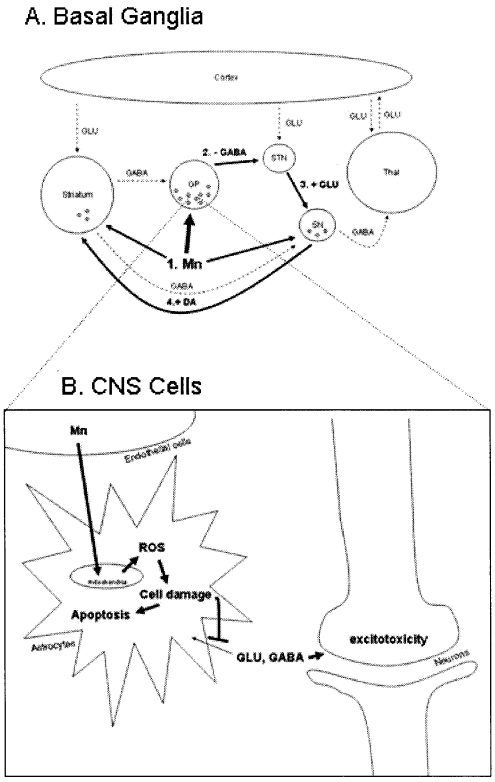

FIGURE 1. Manganese-induced neurotoxicity. **(A)** Regions of the basal ganglia are affected by manganese accumulation: (1) Manganese preferentially accumulates in globus pallidus (GP), leading to (2), decreased GABA innervation into subthalamic nuclei (STN), which leads to (3), unregulated glutamate (GLU) input into the substantia nigra (SN) and (4), increased dopamine input into the striatum. **(B)** Manganese induces oxidative stress through mitochondrial perturbation, leading to astrocytic dysfunction and imbalanced extracellular neurochemistry. (Adapted from ref. 107.)

found in both syndromes, the dystonia of manganism is a neurological sign attributed to damage to the globus pallidus[78] and is only minimally observed in Parkinson's patients. Other features of manganism that differ from parkinsonism were noted in a comprehensive survey of patients with these disorders, and they include less frequent resting tremor, a propensity to fall backward, little or no sustained response to levodopa therapy, and normal fluorodopa uptake, as observed by positron emission tomography (PET).[78,85]

Glutamate from cortical neurons along with γ-amino-butyric acid (GABA) and dopamine from other basal ganglia structures all influence striatal control of motor activity.[90] In Parkinson's disease, the nigrostriatal pathway is affected due to demise of dopaminergic neurons in the substantia nigra. Based on these observations, a model has been proposed in which the etiological damage in manganism is likely to occur to the output pathways downstream of the nigrostriatal dopaminergic pathway (FIG. 1A).[78,85,91]

Animal studies are providing insight into the hypothesis that the developing brain and nervous system may be substantially more sensitive to accumulation of manganese than adult brain. A recent study by Dorman *et al*.[92] found that rat neonates chronically exposed to oral manganese chloride ($MnCl_2$) accumulate more brain manganese than adult rats given equal doses (0, 25, or 50 mg/kg body wt/day). This evidence may suggest increased susceptibility for the developing nervous system, but an alternative explanation is that it could reflect the increased requirement for manganese in the developing CNS.[52] Additionally, there are species differences to consider. The literature available on manganese toxicity in nonhuman primates reports data similar to those obtained from humans with manganism.[52,78] However, rodent studies have yielded mixed findings concerning regional brain manganese distribution and neurochemical and neuropathological responses to manganese exposure.[81,92–94] Further, the behavioral changes observed in manganese-poisoned humans are not replicable in rodents, which further confounds interpretation of results from those studies in assessing the consequences of human exposure.

MANGANESE-INDUCED OXIDATIVE STRESS

Oxidative stress and its effects on mitochondrial energy metabolism have lately been implicated in a wide range of pathological processes, and especially in neurodegenerative conditions such as Parkinson's or Alzheimer's disease.[95] Furthermore, the intense investigation surrounding the free radical theory of aging is leading many scientists to believe that aging mitochondria are the primary culprits.[96] They are more susceptible to oxidative damage and less efficient at repairing this damage than young mitochondria. Indeed, there is good evidence that in multicellular organisms, such as *C. elegans*, oxidative stress is an important factor in limiting life span.[97] Witholt *et al*.[98] recently investigated increased risk to manganese-induced damage using a preparkinsonian rat model treated with low cumulative doses of manganese. They report exacerbation of both neurochemical and motor function changes in the senescent group. A previous report showed that exposure of neurons to MMT resulted in rapid increases in reactive oxygen species followed by mitochondrially induced apoptosis.[99,100] However, it is noteworthy that combustion of MMT in cars yields various manganese salts, the most abundant being phosphate and sulfate.[23,24]

Oxygen radicals can damage components of the electron transport and oxidative phosphorylation machinery, and this leads to generation of more reactive oxygen species (namely, superoxide). The new radicals exacerbate the damage and a "downward spiral" ensues.[101] In this scenario, cells are ultimately subjected to energy failure as ATP production declines. The membrane potential is lost as the mitochondria undergo permeability transition, which then leads to cell death.[102] This mitochondrial dysfunction coincides with decreased cerebral metabolic rates in Alzheimer's disease, Parkinson's disease, Huntington's disease, and other neurodegenerative disorders.[102,103] Whether the mitochondrial demise has a causal role or appears as a secondary effect in these disorders is still a subject of intense debate. Albin *et al*.[104] reviewed a variety of basal ganglia toxicants and concluded that the probable mechanism of action for almost all known basal ganglia neurotoxins is inhibition of mitochondrial function. Studies of this interrelationship are clouded by the fact that mitochondrial function declines as a normal part of the aging process, and age itself is a risk factor for these neurodegenerative diseases. Altogether, the literature seems to point to a strong association between aging, mitochondrial impairment, and oxidative stress.

Optimal brain function is dependent upon cross talk between multiple cell types. In particular, astrocytes produce trophic factors, regulate neurotransmitter and ion concentrations, and remove toxins and debris from the extracellular space around the neurons. Therefore, oxidative impairment of astrocytic functions has the potential to indirectly induce and/or exacerbate neuronal dysfunction.[105] Specifically, removal of the neurotransmitters, GABA, glutamate, and dopamine, from the extracellular fluid can be altered by manganese treatment.[45,106–108] Neurons vicinal to the affected astrocytes are then potentially made susceptible to excitotoxicity or other downstream dysfunction because of the imbalanced extracellular neurochemistry (see FIG. 1B).

On the subcellular level, manganese is most concentrated in mitochondria.[109] However, the overall percentage of manganese found in the mitochondria of specific brain regions did not increase after manganese exposure in neonatal rats,[81] which indicates that there is not additional selective uptake into this organelle at higher manganese levels. Nevertheless, decreased complex I activity, increased oxidative damage, and altered activities of antioxidant defense enzymes have been demonstrated in Parkinson's disease.[95] This supports a growing body of literature on oxidative stress in neurodegeneration.

Gavin *et al*.[110] showed evidence suggesting that the ATPase complex is inhibited at very low levels of mitochondrial manganese and that complex I is inhibited only at higher manganese concentrations. In another study, treatment of striatal neurons with manganese showed dose-dependent losses of mitochondrial membrane potential and complex II activity.[111] Collectively, these results indicate that manganese may trigger apoptotic-like neuronal death secondary to mitochondrial dysfunction. However, it is possible that necrosis may be involved to some extent as Roth *et al.*[112] found that caspases were not involved in manganese-induced neuronal death.

Zwingmann and colleagues recently reported that neurons treated for 5 days with $MnCl_2$ are extremely susceptible to oxidative stress and energy failure through the resulting mitochondrial dysfunction,[113] whereas astrocytes fare slightly better after the same treatment. When the cells were cocultured, comparative NMR data showed "disturbed astrocytic function and a failure of astrocytes to provide neurons with substrates for energy and neurotransmitter metabolism, leading to deterioration of

neuronal antioxidant capacity (decreased glutathione levels) and energy metabolism". These results are consistent with previous reports from our lab and others demonstrating the important role of astrocytes in effectively buffering the extracellular environment to protect the more sensitive neurons. It has also been reported in many cases that astrocytes have higher levels of glutathione and some other antioxidant defenses than neurons.[114,115]

A final factor in manganese toxicity is the oxidation state of the metal. It has been shown that trivalent manganese is more effective at inhibiting complex I,[116–118] but the divalent form is by far the predominant species within cells and is largely bound to ATP.[118] Nevertheless, manganese in any state will spontaneously give rise to infinitesimal amounts of trivalent manganese, and HaMai *et al*.[119] demonstrated that trivalent manganese, even at trace amounts, can cause formation of reactive oxygen species. Interestingly, the mitochondria also paradoxically rely heavily on manganese for antioxidant protection as it is the critical cofactor for the important superoxide dismutase enzyme specific to this organelle. In fact, mice lacking the mitochondrial isoform of SOD have a mean life span of 8 days, whereas mice deficient in cytosolic or extracellular SODs have a very benign phenotype.[97]

The mechanisms of manganese toxicity in the brain are slowly being elucidated. At present, the preponderance of evidence indicates that oxidative stress and mitochondria play major roles in the manganese-induced degenerative processes that lead to dysfunction in the basal ganglia. It is well established that astrocytes are critical in defending the more sensitive neurons, and we will continue to study their role in preventing degenerative mechanisms. Furthermore, there is insufficient data to determine whether any subpopulations are in fact more susceptible to manganese-induced neurodegeneration. These issues clearly warrant future investigations.

ACKNOWLEDGMENTS

This work was supported by NIH Grants NIEHS 10563 (to M. Aschner) and NIH T32-ES-07331 Multidisciplinary Training in Molecular Toxicology (to A. W. Dobson) and by the RJR–Leon Golberg Fellowship in Pharmacology and Toxicology (to K. M. Erikson).

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