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Review Article

Manganese toxicity upon overexposure

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ABSTRACT: Manganese (Mn) is a required element and a metabolic byproduct of the contrast agent mangafodipir trisodium (MnDPDP). The Mn released from MnDPDP is initially sequestered by the liver for first-pass elimination, which allows an enhanced contrast for diagnostic imaging. The administration of intravenous Mn impacts its homeostatic balance in the human body and can lead to toxicity. Human Mn deficiency has been reported in patients on parenteral nutrition and in micronutrient studies. Mn toxicity has been reported through occupational (e.g. welder) and dietary overexposure and is evidenced primarily in the central nervous system, although lung, cardiac, liver, reproductive and fetal toxicity have been noted. Mn neurotoxicity results from an accumulation of the metal in brain tissue and results in a progressive disorder of the extrapyramidal system which is similar to Parkinson's disease. In order for Mn to distribute from blood into brain tissue, it must cross either the blood–brain barrier (BBB) or the blood–cerebrospinal fluid barrier (BCB). Brain import, with no evidence of export, would lead to brain Mn accumulation and neurotoxicity. The mechanism for the neurodegenerative damage specific to select brain regions is not clearly understood. Disturbances in iron homeostasis and the valence state of Mn have been implicated as key factors in contributing to Mn toxicity. Chelation therapy with EDTA and supplementation with levodopa are the current treatment options, which are mildly and transiently efficacious. In conclusion, repeated administration of Mn, or compounds that readily release Mn, may increase the risk of Mn-induced toxicity. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: manganese; MnDPDP; contrast agent; neurotoxicity; EDTA; brain barrier; welder; chelation therapy

INTRODUCTION

The trace element manganese (Mn) is essential for normal development and body function across the life span of all mammals.[1] Mn binds to and/or regulates many enzymes throughout the body. For example, Mn is a required co-factor for arginase, which is responsible for urea production in the liver, superoxide dismutase, which is critical to prevent against cellular oxidative stress, and pyruvate carboxylase, an essential enzyme in gluconeogenesis.[1,2] In brain, about 80% of Mn is associated with the astrocyte-specific enzyme glutamine synthetase,[3] where Mn plays a regulatory role, although it is not a required co-factor.[2]

Interruption of Mn homeostasis has also been associated with a variety of disease states in humans. There are few reports of Mn deficiency in general human populations with self-selected diets, which contain 2–4 mg Mn daily.[4] Skin lesions and bone malformation have been noted in humans on artificial diets with low Mn.[1,5] In rats, long-term dietary Mn deficiency (<1 ppm vs control at 66 ppm) correlates with an increased serum level of calcium and phosphorous and a decreased bone calcium, suggesting an interference of bone metabolism.[6] Low blood Mn in humans has been noted in bone modeling and remodeling diseases, including osteoporosis,[7] Perthe's disease,[8] and also in adults and children with epilepsy (reviewed by Lee[9]). It is suspected that the presence of neurological symptoms in epileptics may correlate with low brain Mn, which may result from a low blood Mn.

Mn is more frequently of toxicological concern because overexposure to the metal can lead to progressive, permanent, neurodegenerative damage, resulting in syndromes similar to idiopathic Parkinson's disease.[10–12] This review will examine the toxicity of Mn upon overexposure, particularly from the perspective of its release into blood from the diagnostic contrast agent MnDPDP

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Abbreviations used: BCM, bile canalicular membranes; DMT1, divalent metal transporter-1; EDTA, ethylene-diamine-tetraacetic acid (edetate calcium disodium); IPD, idiopathic Parkinson's disease; MMT, methylcyclopentadienyl manganese tricarbonyl; MnDPDP, manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5,5'-bis(phosphate), mangafodipir trisodium, TESLASCAN™; MnDPMP, manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5-phosphate; MnPLED, manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate; Tf, transferrin; TPN, total parenteral nutrition.

[also called manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5,5'-bis(phosphate); mangafodipir trisodium; TESLASCAN™]. The general toxicokinetics, routes of exposure, target organs and current clinical intervention will also be discussed.

PHARMACOKINETICS/TOXICOKINETICS

Systemic pharmacokinetics

Serum concentration of Mn in healthy subjects is about 0.05–0.12 μg/dl. After entering, or being injected into, blood, Mn rapidly distributes into other tissues. The terminal elimination half-life in blood was estimated to be 1.8 h after intravenous $MnCl_2$ injection.[13] The whole body terminal Mn half-life was found to be 68–146 days in studies lasting 217–423 days among mice, rats, dogs and monkeys.[14] In humans, the whole-body Mn half-life following intravenous administration varies widely, from a reported shorter half-life of 13–43 days,[11] to a longer half-life of 24–74 days.[15] It should be pointed out that the accurate assessment of terminal elimination half-life requires continuous monitoring of the elimination process by at least three half-lives. Lack of such a practice in some of these studies may account for a wide variability in results.

Based on animal data, Mn distributes, under normal conditions, in brain regions in the following order: substantia nigra > striatum > hippocampus > frontal cortex in a concentration range of 0.3–0.7 μg/g of wet tissue weight.[16] Once Mn enters the brain it persists there for a relatively long time. The cerebral content of ^{54}Mn increased over the first 50 days in the Rhesus monkey.[17] While the half-life of Mn elimination from the brain was not calculated, brain Mn concentration was higher than that in all other sampled tissues after 150 days of dosing, and only slowly decreased in various brain regions over 278 day period in Rhesus monkeys. Thus, the half-life in monkey brain would be expected to exceed 100 days.[17] In the same study, it was found that, on day 278, the relative retention of ^{54}Mn in the cerebrum (Mn concentration in cerebrum/Mn concentration in the whole body) increased, while its relative retentions in most other tissues examined remained fairly constant, suggesting a selective retention of Mn in the brain.[17]

Studies conducted in rats indicate that ^{54}Mn accumulates in the cerebrum during the first 4 days following dose administration; the levels did not decline at 34 or 64 days after dosing.[18,19] The rate of elimination of ^{54}Mn from the brain over a 90-day period of study was slower than that from liver, kidney and skeletal muscle in the same species.[20] The half-life of Mn in 16 rat brain regions was estimated to range from 52 to 74 days.[21] It could be longer had the investigators followed the rigorous study design to monitor brain ^{54}Mn for more than 60 days after intravenous $^{54}MnCl_2$ injection. Nonetheless, these studies show a much slower elimination of Mn from the brain than from many other tissues in rodents as well as primates. Comparable data are not available for humans.

There is currently no established, reliable biological indicator (or biomarker) to evaluate Mn exposure. The suggestion that blood Mn concentration may indicate exposure has been met with much dispute. Some investigators suggest that Mn concentrations in blood seem to be fairly stable over long periods of time in humans exposed to this metal in mining and industrial environments, and thus can be used to reflect the Mn body burden.[22,23] Others, mainly based on animal studies,[13,21,24] point out that Mn is quickly eliminated from the blood circulation and possesses a rather short blood half-life, but a prolonged tissue half-life, following exposure. The discrepancy between blood and tissue half-life, and possibly a large tissue accumulation of Mn, may render the blood Mn level less relevant as an indicator of total body burden of Mn. A recent study among welders conducted by this laboratory shows that career welders have a significantly higher serum Mn compared to control subjects; however the elevated serum Mn concentrations among welders were not associated with welders' length of employment. Thus, the blood Mn may reasonably indicate recent, but not historical, exposure in welders.[25]

Mn distribution into brain

Entry of Mn to brain can occur via three known pathways: through the capillary endothelial cells of the blood–brain barrier, by the choroid plexus of the blood–CSF (cerebrospinal fluid) barrier, or via the olfactory nerve from the nasal cavity directly to brain. The latter is important, as most of the reported toxicities have occurred through the inhalation exposure. This review, however, will focus on the vascular routes, since contrast agents are routinely injected into the bloodstream.

The blood–brain barrier (BBB) lies in and around brain capillary cells and has physical, chemical and metabolic properties that influence movement of selected substrates. The capillary endothelial cells have tight junction proteins, which closely and securely link adjacent cells. The endothelial cells are surrounded by a basement membrane consisting of collagen and other lipophilic matrix proteins, which together slow the diffusion of water-soluble compounds. Astrocytes surround the capillary almost exclusively, covering an estimated 99% of the brain surface of the capillaries,[26] leaving neurons to contact $\leq 1\%$ of brain capillary surface. This connection of endothelial and glial cells with the basement membrane constitutes the physical BBB, which inhibits movement between the blood and brain. Substrates in blood may also be prevented from brain entry by their metabolism at the BBB. The barrier also contains transporter proteins whose distribution and activity influence

movement across the BBB (for details, see the review by Zheng *et al.*[27]).

Substances can also enter the brain through a highly vascularized tissue located in brain ventricles, namely the choroid plexuses. The tissue produces 80–90% of CSF, which surrounds and supports the brain. Substances that enter the CSF can diffuse from there into brain cells since there is no apparent barrier between CSF and the interstitial fluid surrounding neurons and glial cells. The choroidal epithelial cells contain the tight junctions that constitute the blood–CSF barrier, while the capillary endothelial cells within the choroidal plexus tissue lack tight junctions. The total apical surface area of the choroidal epithelium is approximately 75 cm^2, about half that of the blood–brain barrier (155 cm^2).[28] At near-physiologic Mn plasma concentrations (80 nM), brain influx of Mn was reported to occur primarily through the capillary endothelium of the BBB, while Mn influx at high plasma concentrations (78 μM) was primarily via the CSF.[29,30]

Mn ion influx at the BBB has been suggested to occur one or more transporter proteins.[29–33] Mn^{2+} is commonly used as an indicator of calcium flux; thus, these two metals may share common transporters.[34–37] Some have also suggested that Mn influx through cell membranes may be via voltage-gated calcium (Ca) channels, the Na/Ca exchanger, the Na/Mg antiporter or mitochondrial active Ca uniporter.[38] As Mn binds to plasma transferrin (Tf), transport of Mn–Tf complex into the brain has been suggested to rely on a transferrin receptor (TfR)-dependent mechanism, which competes with Fe transport, or vice versa.[39–42] Several studies have suggested that the divalent metal transporter-1 (DMT1) may be involved in Mn influx into brain;[39,41,42] however, recent results have shown that the lack of functional DMT1 in knock-out rats had no apparent effect on brain influx of Mn ion or Mn-Tf.[43] Moreover, some investigators[44] have shown that DMT1 may not exist in brain capillary endothelial cells, which again argues against the involvement of DMT1 in Mn transport into brain.

In contrast to the evidence for brain Mn influx, much less is known about Mn movement out of brain into blood. The brain efflux of Mn across the BBB does not appear to occur through a transporter and is likely to occur slowly by diffusion.[45]

Biotransformation of MnDPDP

MnDPDP is dephosphorylated to an intermediate MnDPMP [manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5-phosphate] and then to MnPLED [manganese (II) *N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate]. This dephosphorylation is thought to occur mainly by alkaline phosphatases rather than acid phosphatases in serum, according to *in vitro* metabolic rates and *in vivo* activities.[46] Zinc (Zn) replaces the Mn ion in all three complexes, with no effect on dephophorylation, resulting in the release of Mn^{2+} from the complexes.[47] The free Mn is thought to bind rapidly to serum proteins, as free Mn ion was not detected in an *in vitro* experiment containing serum proteins.[47] However, it should be noted that the detection limit for this technique was about 2 μM, which exceeds normal serum levels of free Mn by about 100-fold.

The initial plasma half-life for total Mn species following intravenous injection or infusion of MnDPDP is less than 25 min.[48,49] ZnPLED was the only metabolite detected in plasma samples taken 8 h after dosing. In a human study, 5 min after the end of a 20 min infusion of MnDPDP (5 μmol/kg), ZnPLED was also the major metabolite. When MnDPDP was given in an injection lasting less than 1 min, ZnPLED was the major metabolite 15 min after injection. The terminal plasma elimination of all Mn compounds was reported to be 5–11 h.[49]

Chemical species of Mn

Thermodynamic modeling of Mn^{2+} in serum suggests that Mn exists in several forms, including an albumin-bound species (84%), as a hydrated ion (6.4%), and in complexes with bicarbonate (5.8%), citrate (2.0%) and other small molecular weight (MW) ligands (1.8%).[50] These calculations are consistent with the observation of small MW species, slightly larger than the Mn ion, in plasma.[51] Similar modeling of Mn^{3+} in serum predicts that it is almost 100% bound to Tf.[50,52] The metabolism of MnDPDP releases the free Mn^{2+} ion into plasma, where it quickly achieves equilibrium with the serum proteins and ligands.

Mn^{2+} may be oxidized to Mn^{3+}, which is rather reactive and more toxic than $^{2+}$.[53] Mn^{3+} rapidly associates with Tf to form a stable complex.[54] In tissues, Mn may exist primarily in the form of Mn^{2+}. A recent study using X-ray absorbance near edge structure (XANES) spectroscopy failed to identify the presence of Mn^{3+} in mitochondria; yet the authors suggested that Mn^{3+} may exist in a concentration below the detection limit of instrumentation, probably as Mn superoxide dismutase (MnSOD).[55]

ROUTES OF Mn EXPOSURE

Occupational exposure

Occupational exposure to Mn is linked to the majority of the reported cases of Mn intoxication. Neurotoxicity due to inhalation exposure to airborne Mn has been reported in miners in manganese dioxide mines,[56] workers in dry-cell battery factories,[57] smelters[58] and welders.[59,60] While the increased level of public awareness and improved monitoring techniques have reduced the incidence

Table 1. Relationship between aerial Mn and Mn concentrations in blood or urine of chronically poisoned welders

Case no.	Working years	Mn in air (mg/m^3)	BMn[a] (μg/l)	UMn[a] (μg/l)
1	26	0.05–1.08	15.6	4.6
2	23	0.05–5.16	10.0	7.4
3	14	0.07–0.15	13.0	7.8
4	8	0.02–4.15	8.2	2.4
5	25	0.15–21.0	10.5	10.2
6	9	0.30–8.00	36.0	20.0
7	11	0.30–8.00	20.0	20.0

[a] BMn (blood Mn) or UMn (urinary Mn) of samples from chronically Mn poisoned welders were significantly associated with aerial Mn in the breathing zone of work sites. Data were previously presented in Society of Toxicology annual meeting in 1998.

of severe Mn poisoning in occupational settings, the over-exposure to airborne Mn continues to occur. Dr Zheng and his collaborators at the Beijing Institute of Labor Hygiene and Occupational Disease conducted a survey on 3200 welders in 142 factories in the metropolitan area of Beijing, China. Among 421 work sites under annual Mn monitoring (1990–1996), 20% of them showed aerial Mn of 0.42–3.05 mg/m^3, about 2–15 fold higher than that of the Chinese national standard limit (0.2 mg/m^3). The highest level (25.7 mg/m^3) was found to be 128-fold higher than the limit. The dosages of exposure, as calculated by the weight of welding rods, were 5–20 kg (containing 0.3–6% Mn) per working day per person.[61]

Exposure to airborne Mn among these welders had led to cases of Mn intoxication. Among seven patients diagnosed as Mn-poisoned welders, the Mn concentrations in blood ranged was 3–36 μg/l and in urine, 3–20 μg/l. Reconstructing airborne Mn levels at their work site revealed a significant correlation between airborne Mn levels and Mn concentrations in blood and urine (Table 1). Mn intoxication among these workers was probably due to chronic sustained inhalation of airborne Mn.

Environmental exposure

Mn ore is used in the production of steel, aluminum cans, fungicides, fertilizers and electronics. Health risks of exposure to Mn have also been associated with organic Mn-containing pesticides, such as Mn ethylene-bis-dithiocarbamate (MANEB).[62] Mn is also found in the street drug called 'Bazooka', a cocaine-based drug contaminated with manganese-carbonate from free-base preparation methods.[63] Currently, there is a significant concern about airborne Mn exposure from the fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT).[64] Combustion of MMT releases Mn from the tailpipe, primarily as airborne Mn phosphates and sulfate.[65–67] Increased use of Mn-containing products results in greater exposure of large populations to Mn. Mn intoxication has also been reported after ingestion of contaminated water.[64,68]

Medical exposure

The use of Mn as a contrast agent in medical diagnostics provides another potential route of exposure, although there have been no reported cases of Mn intoxication following administration of one or more doses of Mn-containing contrast agent. The low incidence of Mn toxicity following systemic administration of Mn-containing contrast agents is probably owing to acute, less frequent exposure, fast elimination, and low body burden of Mn in clinical diagnosis.

Mn toxicity has also been reported by ingestion in patients receiving long-term parenteral nutrition containing about 1 mg/day of parenteral Mn for adults or more than 40 μg/kg/day for children (as reviewed in Dickerson[4]). Interestingly, Mn deficiency has also been noted in patients on TPN.[1]

Mn OVEREXPOSURE-ASSOCIATED DISORDERS

Mn toxicity is evidenced primarily in the central nervous system and in lung tissue (following inhalation exposure), although cardiovascular and liver, as well as reproductive and developmental, toxicities have been noted. The following discussion will focus on the toxicities which occur upon release of Mn into the blood stream.

Neurotoxicity

In humans, excess brain Mn produces neurotoxicity that may develop into a parkinsonian syndrome (manganism).[64,69] Cumulative evidence suggests that, despite the similarities in extrapyramidal symptoms between Mn neurotoxicity and idiopathic Parkinson's disease (IPD), the sites of Mn-induced neurologic lesions, and therefore the clinical symptoms, are fundamentally different from those observed in IPD. For Mn, the primary targeted brain regions are the globus pallidus and striatum of the basal ganglia, whereas the neurodegeneration in IPD occurs mainly in the substantia nigra.[12,70–73]

Clinical signs and symptoms. Neurotoxicity is evidenced by the physical and psychological symptoms of manganism as well as the neurochemical changes in the brain. Based on our own study on 36 welders diagnosed with the symptoms of typical Mn poisoning in Beijing, the onset of symptoms is between 2 and 34 years (average 16.3 years), the welders having average working duration of 24.4 years (4–40 years). The symptoms

include headache and insomnia (88%), memory loss (75%), emotional instability (35%), exaggerated tendon reflexes (83%), hyper-myotonia (75%), hand tremor (23%), speech disturbances (6%) and festinating gait (3%).

In severe cases, physical signs of Mn neurotoxicity include dystonic movement of the extremities with tremor and a particularly characteristic gait called 'cock-walk' in which patients walk on their toes, leaning forward.[9,11,56,73] Initial psychological symptoms are gradual and mainly psychiatric.[73] It is possible to detect, in the absence of clinical manifestations, early signs of nervous system dysfunction associated with occupational and environmental Mn exposure.[74] Occupational exposure for an average of 7 years was associated with significant decrements in neurological function.[75] The prevalence of abnormal results positively correlated with years of Mn exposure, suggesting cumulative neurotoxicity results from repeated Mn exposure. Other epidemiological studies of industrial workers showed a positive correlation between neurological dysfunction and lifetime integrated or cumulative Mn exposure.[76–78]

Chronic Mn overexposure causes Mn accumulation in brain regions, notably including the basal ganglia structures and, to a lesser extent, the caudate nucleus and putamen.[9,72,73,79] T_1-weighted MRI of patients who have parkinsonism-like symptoms exhibits a high density in the basal ganglia attributed to Mn, especially the globus palladus.[80–84] The Mn accumulation is associated with damage to dopaminergic systems.[85] Mn injection depletes dopamine levels in rat serum,[86] in rat brain,[87] and in globus pallidus and putamen of monkeys.[88]

Mechanism(s) of toxicity. Although the precise mechanism by which Mn induces neurotoxicity is poorly understood, several recent reports have suggested that Mn neurotoxicity may be associated with its interaction with other essential trace elements, including iron,[40,70,85,89–91] zinc,[92] copper[92] and aluminum.[70,85,93] Particularly regarding Mn-induced neurotoxicities, studies have shown that chronic exposure to Mn appears to be associated with altered iron (Fe) concentrations in blood and in CSF, presumably due to Mn–Fe interaction at certain iron–sulfur-containing proteins, which regulate Fe homeostasis.[16,53,89–91] A number of studies have shown that Mn-elicited neurotoxicity may be related to the abnormal iron metabolism. High dietary Mn increased Fe uptake into rat brain, liver and kidneys.[94,95] Furthermore, Mn intoxication in monkey caused an elevated Fe deposition in globus pallidus and substantia nigra pars reticulate.[70] When cultured cells were exposed to Mn compounds, similar increases in cellular Fe uptake were observed.[89,96] The excess accumulation of Fe in neurons may consequently produce the cellular oxidative stress that leads to neuronal damage.

A dysfunction in Fe metabolism has also been seen in IPD patients. High levels of total iron, decreased ferritin, iron-associated oxidative stress, and abnormal mitochondrial complex-I have been repeatedly reported in the postmortem substantia nigra of IPD patients.[97–100] An epidemiologic study has established that serum parameters associated with iron metabolism, such as ferritin, transferrin, total iron-binding capacity, and percentage Fe saturation, are significantly altered in IPD patients compared with normal subjects.[101] MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced Parkinsonism and IPD have also been shown to be associated with the excess oxidative stress, consistent with the accumulation of redox-active metals.[102,103]

The suggestion that transition metals contribute to the neurodegeneration observed in Parkinsonian syndromes is consistent with studies of the toxicity of different Mn valence states. Mn administered as Mn^{3+} more effectively inhibits mitochondrial aconitase activity than that administered as Mn^{2+}.[53] Cells treated with Mn^{3+} accumulate more total Mn and have a higher rate of cell death compared with cells treated with $^{2+}$.[53,54] As Mn can participate in Fenton reactions, it has the potential to increase reactive oxygen species, and subsequent oxidative damage, within cells. A recent human study further confirms the oxidative damage among welders exposed to airborne Mn.[104]

Cardiovascular toxicity

Excess Mn has been reported to be toxic to cardiac muscle cells and tissues from animals, but not necessarily toxic to whole animals or humans. In isolated perfused hearts, infusions of MnDPDP or Mn chloride resulted in increased aortic pressure and coronary vascular resistance. Cardiac tissue Mn concentrations were increased by 60–70-fold ($MnCl_2$) or 8–9-fold (MnDPDP) when given at 1000 μM infusion (0.1 ml/min) into a perfusion of 12.5 ml/min.[105] Mn that entered perfused heart tissue remained in a stable pool and was not removed by a 10 min washout period.[106] The magnesium content decreased after 10 min infusion with MnDPDP and magnesium decreased and calcium increased following $MnCl_2$ infusion.[105,107,108] However, Mn administered as MnDPDP or $MnCl_2$ into conscious dogs resulted only in vasodilation and maintained or increased heart rate or blood pressure.[109] There was no exacerbation of existing cardiac failure. The authors concluded that extensive plasma protein binding and catecholamine release together counteract the effects of Mn toxicity seen in isolated cardiac tissues. They noted that the vasodilation of bovine mesenteric arteries upon MnDPDP administration was consistent with the skin flushing reported to occur upon administration of MnDPDP to humans.[109] Mn appears to affect cardiac function by blocking calcium channels; however, a high concentration of Mn (1 mM) is required to block the calcium channels.[110]

Hepatotoxicity

Biliary excretion is the major elimination pathway for Mn, accounting for $\geq 95\%$ of Mn excretion; consequently any existing liver damage may delay or decrease its elimination and increase the relative amount in plasma.[111] Mn is cholestatic in cattle and rodents.[112–114] Mn and bilirubin, in concert, consistently induce intrahepatic cholestasis in rats and increase the cholesterol content in the bile canalicular membranes (BCM) which line the bile duct. This experimental paradigm is commonly used as a model for intrahepatic cholestasis, because the BCM undergo similar changes to those seen in cases of human cholestasis.[115] A recent study has determined that Mn increases the activity of 3-hydroxy-3-methylglutaryl coenzyme A, the rate limiting enzyme for cholesterol biosynthesis, and that bilirubin decreases cholesterol 7α-hydroxylase, which is important in the conversion of cholesterol into bile acids.[116] Modulation of these enzymes results in increased total cholesterol and decreased bile acid production. Acute liver toxicity was noted in steers after Mn infusion into the duodenum for up to 30 h.[114] The bovine liver's capacity to remove Mn by first pass elimination was only exceeded when mesenteric vein infusions occurred at 84 μmol/min. (In a steer of approximately 200 kg, this rate is about 0.42 μmol/kg/min.)

Reproductive and developmental toxicity

Mn overexposure is evidenced by decreased fertility as well as increased fetal abnormalities. Mn-exposed male workers had significantly fewer children than workers who were not exposed, suggesting decreased fertililty.[117] Reduced fetal body weight, increased number of litters with abnormal limb flexures and increased number of litters and individual fetuses with skeletal malformations were noted when pregnant rat dams were given daily i.v. injections of MnDPDP on gestational days 6–17 (20 μmol/kg).[118] In other studies, dietary Mn or Mn injected s.c. or i.p. did not result in fetal skeletal malformations,[119,120] although the effect on fetal body weight was previously noted.[120] When $MnCl_2$ was administered i.v. at 20 μmol/kg, there were significantly more litters with abnormal limb flexures and skeletal abnormalities compared to control. The effect was even greater at 40 μmol/kg, but was not significantly different at 5 μmol/kg. These studies suggest that repeated, high doses of Mn are deleterious to the developing fetus.

THERAPEUTIC INTERVENTION

Levodopa treatment

Treatment of manganism produces various outcomes among patients. Symptom severity increases, and chance of recovery decreases, with prolonged Mn exposure; therefore it is vital to remove the patient from the source of Mn. Treatment with levodopa has limited benefits in improvement of clinical symptoms. Some reported that a replacement of the lost dopamine could initially improve extrapyramidal symptoms,[9,121,122] but a 5-year follow-up study revealed that the response to levodopa treatment decreased after 2 or 3 years.[123] Furthermore, 10 years after cessation of Mn exposure, the same patients continued to show progression in severity of symptoms.[124]

Chelation therapy

In severe cases of Mn poisoning, chelation therapy has been recommended in order to reduce the body burden of Mn and to alleviate the symptoms. From our own work, the EDTA excretion therapy was conducted on seven Mn poisoned welders. Twenty percent $CaNa_2EDTA$ was administered intravascularly at the dose of 1.0 g daily for 3 days followed by a pause for 4 days as one therapeutic course. The symptoms, as well as blood Mn concentrations and urinary Mn concentrations, were examined before and after each treatment course. The therapy continued for two to four courses, depending upon the outcomes. Results show that EDTA treatment increased Mn excretion in urine and decreased Mn concentrations in blood (Table 2). Although the clinical symptoms did not appear to be significantly improved among these patients because of the short duration of observation, EDTA chelation therapy appears to be useful, at least, in reducing blood Mn levels in acute poisoned patients. The similar conclusion with EDTA therapy was reached by other investigators, who also questioned the efficacy of this treatment to reduce neurological symptoms.[60,73]

CONCLUSION

MnDPDP releases free Mn ion into blood and tissues, where it is quickly bound, distributed and/or retained by

Table 2. Effect of EDTA treatment on blood or urine concentrations of Mn in chronically poisoned welders

Case no.	Before EDTA treatment		After EDTA treatment[a]	
	BMn (μg/l)	UMn (μg/l)	BMn (μg/l)	UMn (μg/l)
1	15.6	4.6	3.40–5.70	12.7–11.6
2	10.0	7.4	15.0–6.0	7.80–14.4
3	13.0	7.8	3.50–5.25	11.4–10.8
4	8.2	2.4	10.2–4.70	5.70–6.50
5	10.5	10.2	9.80–0.30	10.2–8.70
6	36.0	20.0	12.0–5.00	15.0–18.3
7	20.0	20.0	8.20–3.40	12.1–15.2

EDTA, ethylenediaminetetraacetic acid (edetate calcium disodium).
[a] The values for BMn and UMn represent the range of values measured during and after the 2–4 weeks of treatment.

tissues. The brain is the major target organ for Mn toxicity. It retains Mn much longer than other tissues. Following chronic overexposure, Mn can produce a progressive, permanent neurodegenerative disorder, with few options for treatment and no cure. While it remains unclear whether a single dose of MnDPDP would lead to any neurological consequences, care should be taken to avoid repeated exposure to Mn.

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