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Manganese metabolism in humans

Pan Chen[1], Julia Bornhorst[2], Michael Aschner[1]

[1]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461, [2]Department of Food Chemistry, Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany D-14558

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1. ABSTRACT

Manganese (Mn) is an essential nutrient for intracellular activities; it functions as a cofactor for a variety of enzymes, including arginase, glutamine synthetase (GS), pyruvate carboxylase and Mn superoxide dismutase (Mn-SOD). Through these metalloproteins, Mn plays critically important roles in development, digestion, reproduction, antioxidant defense, energy production, immune response and regulation of neuronal activities. Mn deficiency is rare. In contrast Mn poisoning may be encountered upon overexposure to this metal. Excessive Mn tends to accumulate in the liver, pancreas, bone, kidney and brain, with the latter being the major target of Mn intoxication. Hepatic cirrhosis, polycythemia,

hypermanganesemia, dystonia and Parkinsonism-like symptoms have been reported in patients with Mn poisoning. In recent years, Mn has come to the forefront of environmental concerns due to its neurotoxicity. Molecular mechanisms of Mn toxicity include oxidative stress, mitochondrial dysfunction, protein misfolding, endoplasmic reticulum (ER) stress, autophagy dysregulation, apoptosis, and disruption of other metal homeostasis. The mechanisms of Mn homeostasis are not fully understood. Here, we will address recent progress in Mn absorption, distribution and elimination across different tissues, as well as the intracellular regulation of Mn homeostasis in cells. We will conclude with recommendations for future research areas on Mn metabolism.

2. INTRODUCTION

Mn is the 12th most abundant element and 5th most abundant metal on the earth. This metal has a silver-grey color and is very easy to oxidize. Thus, Mn is not found as a free element, but usually exists as oxides, carbonates and silicates. The naturally occurring and most stable isotope is ^{55}Mn, and 18 radioisotopes have been discovered, with a half-life from seconds to million years. Although found in a negative oxidation state (-3), Mn commonly exists in positive oxidation states (+2, +3, +4, +6, and +7). In living organisms, the most commonly oxidized states are Mn^{2+} and Mn^{3+}. Mn^{2+} is the most stable form, while Mn^{3+} is a powerful oxidant, which is usually disproportionated to Mn^{2+} and Mn^{4+}, or forms complexes with proteins, such as transferrin (Tf) (1). Natural earth erosion releases tons of Mn into the air, soil and waterways on an annual basis, which is subsequently available for absorption by microorganisms, plants and animals. Given its physical and chemical properties, Mn is widely used in various industrial settings. In manufacture, Mn is incorporated in production of batteries, ceramics, steel, cosmetics, leather, fireworks and glass. In energy consumption, the combustion of gas releases Mn phosphate into the atmosphere secondary to the usage of an antiknock gasoline additive - methylcyclopentadienyl Mn tricarbonyl (MMT). In agriculture, Mn is present in various pesticides and fungicides, such as Maneb and Mancozeb, which may result in adverse health effect in farmers and others. In medicine, given its paramangnetic property, Mn serves as a contrast agent in medical magnetic resonance imaging (MRI) (2, 3).In the infant food industry, Mn is commonly added to total parenteral nutrition (TPN) at significant concentrations as an essential nutrient (4).

Dietary consumption is the primary route of Mn intake for majority of people. Drinking water contains Mn levels ranging from 1μg/L up to 2 mg/L depending on the locations and contamination (5). In human daily diets, rice, nuts (hazelnuts, almonds, and pecans), whole grains (wheat germ, oats, and bran) and legumes contain the highest levels of Mn, leafy green vegetables, tea, chocolate and seafood (clams and mussels) are also abundant in Mn. Multivitamins and other daily supplements also contain Mn, although the levels vary.

Although Mn is required for various physiological activities, accumulation of excessive Mn in human body can result in severe toxicity. The primary target tissue of Mn toxicity is the brain, and “manganism” refers to a variety of psychiatric and motor disturbances caused by excessive Mn accumulation. Reduced response speed, irritability, mood changes and compulsive behaviors are first noticed in the patients (6); later on, the symptoms get more prominent with four-limb dystonia, an upright stance, tremors at rest and a signature high-stepping gait (7, 8). These symptoms resemble, however, are not identical to symptoms of idiopathic Parkinson’s disease (PD) (9). Mn preferentially accumulates in the globus pallidus. Although dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) are affected by excess Mn, the effect is not as prominent as in PD patients, and loss of DAergic neurons is not as common as in PD. In addition, patients with manganism do not respond to levodopa therapy as well as PD patients (10). Other than neurological symptoms, liver impairments are found in most patients, with micronodular cirrhosis, elevated transaminases and unconjugated hyperbilirubinemia (8). Mn overexposure can also impair cardiovascular function, causing abnormal electrocardiogram, increased heartbeat, shorter P-R interval and lower diastolic blood pressure (11).

3. MN ABSORPTION AND DISTRIBUTION IN HUMANS

Manganese is absorbed by ingestion, inhalation and dermal permeation, and also administered in intravenous injection. It is rapidly absorbed in the gastrointestinal (GI) tract and in the lung, then distributed into different tissues through blood circulation. Liver, pancreas, bone, kidney and brain are the organs containing the highest Mn levels in human body. It has to be clarified that Mn level in the brain is not the highest among these organs, however, the brain is the major target of Mn-induced toxicity as most of the patients with Mn intoxication show symptoms of neurological dysfunction. Thus how Mn crosses the blood-brain barrier (BBB) and accumulates in the brain is of special interest. We will review the processes of Mn uptake, distribution and elimination, as well as the factors that regulate these processes.

3.1. Exposure routes and absorption

3.1.1. Ingestion

Oral exposure is the most common route for Mn absorption. Drinking water, Mn-rich vegetables,

nuts, vitamins, supplements and infant formula are the major Mn food source. In adults, approximately 3-5% of ingested Mn is absorbed through the gastrointestinal (GI) tract, and females tend to have a higher absorption rate (3.5.5 ± 2.1.1%, ~2.3. mg/day) than males (1.3.5 ± 0.5.1%, ~1.8. mg/day), which is possibly affected by iron status (4, 12, 13). The average intake of Mn is 2.3. to 8.8. mg per day in western diets (14). Currently, the formal recommended dietary allowance for Mn has not been established yet, but the estimated safe and adequate daily dietary intake of Mn for adults is 2–5 mg per day, and the lowest Mn level in water with observable adverse effect is 4.2. mg per day for a 70-kg individual (15). However, the number can be much higher in infants or children, due to a higher demand for Mn and a less developed regulatory system at early developmental stages. A daily consumption of 3 μg Mn is sufficiently for an infant up to 6 months of age, and a daily intake of 1.2. and 1.5. mg is adequate for children of 1-3 years old and 4-8 years old, respectively (13). The World Health Organization (WHO) recommends Mn concentration in drinking water < 400 μg/L (16). In the US the human health benchmark is set at 300 μg/L (17). In contrast, in Bangladesh the level may be as high as 2 μg/L in the water supply (5), a level which is associated with altered classroom behavior in school-aged (8 to 11 years old) children (18). Infant formula especially soy-based formula is another Mn food source that may result in Mn accumulation in infants (19, 20). However, it can be as 10-fold higher than the recommended level due to lack of maximum requirement of Mn levels in formula production (14).

Ingested Mn is rapidly absorbed in the intestine, where it enters cells through passive diffusion or active transport. In human intestinal cells, Mn is transported in a biphasic pattern with a saturable process similar to other divalent cations, such as iron and calcium (21). It takes about one hour to activate the cellular components (mainly transporters), followed by a progressive acceleration of Mn uptake after a steady-state condition (21). In rat intestinal cells, a high affinity, low capacity, active transport mechanism is reported to regulate Mn absorption (22). The divalent metal transporter 1 (DMT1) is considered to be mainly responsible for active Mn influx, although it also transports other divalent cations. Several factors regulate the absorption of Mn. Mn importers are not necessarily Mn-specific transporters, as they also regulate influx of other metals, such as iron (Fe), copper (Cu), zinc (Zn), calcium (Ca), etc. Therefore, the presence of other metals in the biological media (blood, extracellular fluid, etc) will compete with Mn absorption.

Individuals with Fe deficiency are at higher risk of Mn poisoning as Mn absorption in the GI tract can increase under low Fe conditions (23). Similarly, the expression of Fe/Mn transporters is altered and Mn levels in the brain is upregulated in iron-deficient rats (24) and pigs (25). Approximately 75% of human milk Mn is bound to lactoferrin (26), and the absorption of this complexed Mn could be inhibited by excess ferric lactoferrin in the brush-border membrane vesicles from the small intestine of infant monkey (27). Furthermore, addition of Ca to human milk significantly decreased Mn absorption in both male and female adults; in contrast, addition of phytate, phosphate and ascorbic acid to infant formula, as well as iron and magnesium to wheat bread had no significant effect on Mn absorption (28). In rats, when complexed with albumin or albumin-like proteins, Mn tends to discharge from the intestine, but the transferrin complexed or carrier-free Mn does not (29). Age is another factor known to influence Mn absorption. Infants and children tend to absorb higher amount of Mn from diet due to a large demand of Mn in body development compared with adults. In neonatal rats fed with human milk, bovine milk or infant formula, the absorption of Mn from these milk diets decreased significantly with age (30). Moreover, the Mn retention rate (80%) in rat pups (<15 days) was much higher than in older pups (40%) or adults (31). Although most of Mn uptake is through ingestion, it is considered relatively safe due to efficient liver elimination.

3.1.2. *In utero* exposure

In utero Mn exposure is often neglected as the direct link between Mn exposure and health effects is obscure. However, there has been an increasing number of studies correlating *in utero* Mn exposure and infant health. Average Mn concentration (78.7.5 mg/L) in umbilical cord blood is higher than in the mother's whole blood (54.9.8 mg/L) and an inverted U-shaped curve has been noted between mothers' whole blood Mn levels and birth weights, as well as between umbilical blood Mn levels and birth weights (32). Similar results were observed in other studies (33-35), indicating both low and high maternal blood Mn levels were associated with impaired infant health.

3.1.3. Inhalation

Most of clinically reported cases of Mn intoxication are due to occupational exposure. Inhalation of airborne Mn is the major exposure route in occupational Mn intoxication. Industrial workers, especially miners (36), smelters (37) and welders (38), breathe in a significant amount of Mn-containing fume and dust, thus are the adult population with the highest risk for Mn-induced toxicity. Inhaled Mn is absorbed in the lung and enters the circulation. It can be rapidly transported to the olfactory bulb and enter the brain by two Zinc transporters ZIP8 and ZIP14, bypassing the liver and BBB (9). In rats exposed to 0.0.92 mg $MnSO_4/m^3$, the level of Mn in the lung was elevated; at 0.9.2 mg $MnSO_4/m^3$, Mn concentrations in the lung, striatum and bile were significantly increased (39).

3.1.4. Intravenous administration and dermal exposure

Intravenous administration of agents containing high levels of Mn is another Mn exposure route, which bypasses the regulation at the GI tract resulting in 100% absorption of the metal (4). For example, premature infants fail to absorb sufficient amounts of nutrients due to an undeveloped GI tract or certain diseases. Thus, they are commonly supplemented with total parenteral nutrition (TPN) by intravenous injection, which contains many trace elements required for life support. Infants taking TPN are of special concern of Mn poisoning. In addition, intravenous abuse of methcathinone, containing manganese dioxide as a byproduct of synthesis, has been reported to result in manganism (40-43). The absorbed amount can reach from 60 to 180 mg per day, far beyond the average intake from diet (41-43). Furthermore, Mn exposure through skin is also a risk factor for individuals with contact to organic forms of Mn, such as the gasoline supplement methylcyclopentadienyl manganese tricarbonyl (MMT) (2).

3.2. Distribution and regulation

In the human body, liver (1.2-1.3 mg/kg), pancreas (1.04 mg/kg), bone (1 mg/kg), kidney (0.98 mg/kg) and brain (0.15-0.46 mg/kg) are the organs containing highest Mn levels (44). After absorbed in the GI tract or the lung, Mn enters the blood stream and then quickly distributes to different tissues.

3.2.1. Blood

The normal Mn concentrations in human blood ranges from 4 to 15 μg/L (2), and females tend to have a ~30% higher Mn level than males (45), probably due to a higher absorption rate in women. Currently, the mechanism behind Mn absorption in the intestine and delivery to the plasma remains unclear. Most of the blood Mn (~60%) is distributed in soft tissues, the rest is rapidly delivered to the liver (30%), kidney (5%), pancreas (5%), colon (1%), bone (0.5.%), urinary system (0.2.%), brain (0.1.%) and erythrocytes (0.0.2%) (46).

Erythrocytes are responsible for Mn distribution due to its ability to carry the Mn ion with the presence of various Mn transporters, including DMT1 and transferrin receptor (TfR) on the cell surface (47, 48). The divalent Mn^{2+} and trivalent Mn^{3+} are the two major Mn species in the blood, although the exact ratio of these two species remains unknown. Mn^{2+} is the predominant form in the blood and exists in complexed with different molecules, including albumin (84% of total Mn^{2+}), hexahydrated ion (6%), bicarbonate (6%), citrate (2%), and transferrin (Tf) (1%); almost all Mn^{3+} is bound to transferrin to form a more stable complex (49-51). Mn^{3+} is a highly reactive oxidant and usually gets reduced to Mn^{2+}. Interestingly, Mn^{2+} can be oxidized to Mn^{3+} by ceruloplasmin, which is an abundant plasma protein synthesized in the liver and able to oxidize iron and copper (52). In mice, ceruloplasmin has been shown to regulate Mn levels in the blood and kidney, as well as the brain and lung to a lesser extent; meanwhile, this protein also elevates brain oxidative stress probably via an extracellular ceruloplasmin—manganese redox mechanism upon chronic Mn exposure (52). Transferrin is also produced in the liver and secreted in the plasma. This circulating protein binds to both Mn^{2+} and Mn^{3+}; together with transferrin receptor (TfR), Tf regulates Mn^{3+} transport to the brain in a way similar to Tf/Fe^{3+} transport, but this process is less efficient compared with other transporter mediated Mn transport processes (53).

3.2.2. Liver

Liver is the primary organ to regulate body Mn levels through endogenous gut losses of Mn (29). The liver cells express various Mn transporter on the cell membrane, including DMT1 (54), transferrin/transferrin receptor (Tf/TfR) (55, 56), ZIP14 (54, 57) and citrate transporters (58), which regulate Mn influx. Meanwhile, Mn exporters including SLC30A10 (59), ferroportin (60) and SPCA1 (61) are also expressed in the liver, regulating efflux of excess Mn. Thus, liver plays an important role in Mn storage, redistribution and elimination. As the blood passes through the liver, a small but adequate amount of Mn required for physiological functions remains in the plasma, while excess Mn is sequestered by liver cells and conjugated to bile, which then passed to the intestine and excreted in the feces (29, 62).

3.2.3. Bone

Bone is another tissue with extensive Mn accumulation with a normal concentration of 1 mg/kg (14, 63) and approximately 40% total body Mn (62). Thus, bone Mn can be used as a biomarker for Mn exposure. Using *in vivo* neutron activation analysis (IVNAA) bone Mn measurements, Pejović□Milić and colleagues found that the mean Mn level (2.9.±0.4. μg Mn/g Ca) in the hand bone of welders exposed to Mn rich environment, was significantly higher than the non-occupationally exposed subjects (0.1.±0.7. μg Mn/g Ca) (64). In adult rats, atomic absorption spectrometry (AAS) revealed that Mn concentrations in bone reached a steady state after 6-week of Mn exposure, with approximately 2-3 fold increase of bone Mn levels before exposure (65). The half-life of Mn in femur, tibia and humerus bones was 77, 263 and 429 days, respectively; the average half-life of rat skeleton bone was 143 days, which was about 8.5. years in human (65). In addition, Mn concentrations in striatum, hippocampus and cerebrospinal fluid (CSF)

was found to be relevant to bone Mn levels (65), indicating a possible redistribution of bone Mn to the central nervous system and a risk factor for developing manganism.

3.2.4. Pancreas

The distribution of Mn in the pancreas and kidney is less studied. Kodama and colleagues studied Mn distribution in Wistar rats by subcutaneous delivery of 15 mg of Mn/kg daily for 10 days. They found that Mn concentration in the pancreas was significantly increased from 1.4. to 13.3. μg/g wet tissue, and Mn was found in the high-molecular-mass protein fraction (66). Interestingly, they identified that Mn bound to the Zn binding site of protein zymogen of carboxypeptidase B (pro-CBP), which was the primary Mn-binding protein in the pancreas (66). In pancreatic islets isolated from ob/ob mice incubated with 0.2.5 mM Mn for 1 hour, the intracellular Mn concentration was about 25-fold higher than that of the extracellular medium which was stimulated by 20 mM D-glucose due to inhibition of Mn efflux in the islets (67). Interestingly, in Korean diabetes patients, blood Mn concentrations were significantly lower than the control group, suggesting blood Mn may regulate glucose homeostasis (68).

3.2.5. Kidney

Kidney also contains high levels of Mn. In Korean population, people with renal dysfunction have significantly lower blood Mn concentrations than the healthy group (68), indicating kidney may plays a role in mediating blood Mn levels or *vice versa*. In rats exposed to Mn by oral gavage, the kidney and prostate glands of male rats showed the most obvious lesions. Animals had viscous, gritty urine, and even urinary bladder stones, and tubulointerstitial nephritis with tubular proteineous and glomerulosclerosis was also reported. However, female rats were affected, indicating a sex-preference of Mn intoxication in these rats (69). The absorption of Mn in the kidney is regulated by several Mn transporters, including ZIP8, ZIP14 and DMT1 in the epithelial cells of proximal tubules in the kidney, as knockdown of these three transporters significantly reduced Mn uptake (70).

Mn excretion happened primarily in the apical side of the proximal tubule cells (70) although the mechanism has not been revealed yet.

3.2.6. Brain

The human brain is the most susceptible organ to Mn intoxication, as neurological disorders are the most obvious and severe symptoms seen in people with Mn poisoning, although brain Mn levels are lower than those in liver, pancreas, bone and kidney. Neurons are more susceptible to Mn intoxication possibly due to their long lifespan and high energy demand. Brain Mn can be detected by T1-weighted magnetic resonance imaging (MRI) given the paramagnetic property of Mn ion. In both industrial workers exposed to high levels of Mn and individuals carrying genomic mutations without high environmental Mn exposure, MRI studies showed that in the brain, Mn preferentially accumulates in the globus pallidus, followed by putamen, caudate, midbrain, cerebellum, subthalamic and dentate nucleus and sparing of the thalamus and ventral pons (8, 48, 59, 71). In rats, the highest Mn intensity was found in the globus pallidus, the thalamus and the substantia nigra pars compacta, followed by caudate putamen, axon bundles, and cortex (72). Mn enters the brain via three routes: the blood-brain barrier (BBB), the blood-cerebrospinal fluid (CSF) barrier and the olfactory tract.

Following oral uptake the BBB and the blood-CSF barrier are the two main interfaces regulating the brain Mn homeostasis. While the BBB separates blood from brain interstitial fluid and consists basically of capillary endothelial cells (73), the blood-CSF barrier is composed of epithelial cells of the choroid plexus, which separate the blood from the CSF (74). Mn has been shown to cross both barriers (75, 76). However based on *in vitro* studies using porcine models of the brain barriers, the blood-CSF barrier is considered as a major route for Mn into the brain. (76). In direct comparison with the *in vitro* BBB model, the blood-CSF barrier model was much more sensitive toward Mn illustrated by a disturbance of barrier properties. Additionally Mn crosses the blood-CSF barrier model site-directed, most probably by an active Mn transport toward the brain facing compartment. The protection afforded by the BBB and blood-CSF barrier is essential for regulating Mn homeostasis and is essential for neuronal survival and proper central nervous system functioning. In this context concerns are rising about the risk of an elevated dietary Mn exposure of infants due to their immature BBB (77). This might contribute to increased rates of Mn uptake and deposition in infant brain and tissue (78). The olfactory route provides a pathway for inhaled Mn, which comes into contact with the olfactory epithelium, to pass directly to the brain, thereby circumventing the two brain barriers (79).

3.3. Elimination

As mentioned above, an average of 2.3. to 8.8. mg Mn is absorbed daily (14). However, only 2.3. mg/day required for men and 1.8. mg/day for women (4). The extra Mn needs to be eliminated. The turnover of ingested Mn is relatively fast, with an average retention of 10 days (80). Most of excess Mn is conjugated to bile by the liver and get eliminated via fecal excretion (29, 62). Liver plays a critical role in this process as it is reported that the liver is the major source of endogenous gut losses of Mn (29).

Rats fed with Mn diet absorbed about 8% of ingested Mn and then 37% of the absorbed Mn was excreted through endogenous hepatobiliary elimination of Mn (29). Therefore, individuals with hepatic problems are at higher risk of Mn intoxication. In addition to the primary fecal hepatobiliary elimination, Mn excretion through urine (81), milk (82) and sweat (83) has also been reported but with very limited amount. However, the ratio of elimination via different routes may change under certain circumstances. For example, when using hexameric Mn dendrimer as a MRI contrast agent, Mn clearance through renal routes was increased dramatically equal to or over the amount eliminated through hepatobiliary route (84).

4. MN METABOLISM REGULATED BY MN TRANSPORTERS AT CELLULAR LEVEL

Mn can cross the neural barriers by means of transporters and in different oxidation states (85). Although Mn transporters have been vigorously investigated, a conclusive result is presently not available since information from different papers and research groups are contradictory (85-87). Also the process itself as well as the transported Mn-species are strongly debated.

4.1. Transported Mn-species

Focusing on the transported species first, the three most relevant species entering the brain are Mn^{2+}, $Mn^{2+/3+}$ citrate and Mn^{3+} transferrin (Mn-Tf). Evidence from animal models, as well as human CSF suggests that Mn-citrate is the major Mn-related species entering the brain (88-90). Brain influx rates have been compared in several animal studies and pointed out to a higher rate for Mn-citrate than unbound or protein-bound Mn (88, 90). Speciation studies performed in human by Michalke and coworkers found Mn from CSF correlated with Mn-Tf, the physiological Mn carrier in serum, as long as total Mn concentration was below 1.5. µg/l. Above 1.9. µg/L, Mn in serum and CSF were positively correlated with Mn-citrate in serum (89, 91, 92). Whether elevated concentrations of Mn-citrate in serum or plasma could be a valuable effect biomarker for increased total Mn concentration in CSF (and brain) need to be proven in further studies.

4.2.1. Mn influx: TfR, DMT1, ZIP8/ZIP14, calcium channels, citrate transporter, DAT, choline transporter, ceruloplasmin

The valence status might account for the transport properties of Mn species at the respective barrier. Mn^{3+} influx from the blood into the CNS is facilitated via Tf receptor-mediated endocytosis. Synthesized in the liver and released to the blood, Tf is binding Mn as a plasma-carrier (93, 94). The TfR expressed in neurons, microglia, astrocytes and cells of the neuronal barriers, recognizes, binds and transports Mn into the CNS (95, 96). Intracellularly, it is suggested that specific organelles have different concentrations of TfR with high concentrations in the Golgi cisternae, the plasmalemmal pits and the vesicle membrane (97). As an early event in response of Mn exposure an upregulation of TfR trafficking has been observed (98, 99).

However, the majority of Mn in the body is in the divalent oxidation state. Thereby the best studied importer is the divalent metal transporter 1 (DMT1), also known as divalent cation transporter 1 (DCT1), natural resistance-associated macrophage protein 2 (NRAMP 2) or solute carrier family 11 member 2 (SLC11A2). DMT1 is reported to have a wide range of substrates as Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} with the following transport affinity (reflecting transport efficacy): Mn>Cd>Fe>Pb~Co~ Ni>Zn (100). Recently, a study defined the conformational changes underlying transition-metal ion transport in the SLC11 family, providing molecular insight to its coupling to protons (101). DMT1 expression in the brain is prominent in neurons, whereas studies report on lower as well as varying protein expression in DMT1 in non-neuronal cells such as astrocytes, microglia and oligodendrocytes, and the two principal cell types that form the brain barriers (102). DMT1 in the brain is highly expressed in the SN, globus pallidus (GP), hypothalamic nucleus and striatum making them more susceptible to Mn accumulation and toxicity (103). DMT1 is located at cellular membranes, endosomal membranes as well as the outer mitochondrial membrane (104). Since the expression of DMT1 in brain capillary endothelial cells remains debatable, it is suggested that Mn might access the brain without the involvement of DMT1. While some studies imply a physiological role for the transport of Mn by DMT1 (105, 106), others suggest no direct evidence supporting its role (76, 107). Since Mn and Fe share and compete for Tf as well as DMT1, an altered amount of either Fe or Mn in the brain may result in a dysregulation of the other, causing altered homeostasis (summarized in (108)).

Next to Fe, Mn has also been reported to compete for transporters of other divalent metals. Mn has also been reported to be transported by a family of Zn transporters. Zn-interacting protein 8 (ZIP8) and 14 (ZIP14) are transmembrane proteins that belong to the SLC39 family of genes which are expressed on the apical surface of brain capillaries. These divalent metal/bicarbonate ion symporters are known to transport Mn, Zn, and Cd under normal conditions (109). ZIP8 appears to be more important for Mn than Zn homeostasis (110). Both ZIP8 and ZIP14 have high affinity for Mn and ZIP8 overexpression has been shown to stimulate intracellular accumulation of Cd and Mn (111, 112). Since the expression of these two transporters is lower

in the brain than in other tissues, they may be more important in regulating body Mn levels by controlling the absorption through the liver and lung (113). Specifically, ZIP8 is proposed to regulate Mn metabolism in the liver, which in turn regulates Mn content in other organs and tissues, including kidney, brain, heart and whole blood. ZIP8, localized to the hepatocyte canalicular membrane, functions to reclaim Mn from biliary excretion which is supposed to be the mechanism underlying the association of the ZIP8 locus with whole-blood Mn and the severe Mn deficiency in patients with ZIP8 mutations (114). A study performing whole-exome sequencing in children demonstrate that variants in ZIP8 impair the function of Mn-dependent enzymes, most notably ß-1,4-galactosyltransferase, a Golgi enzyme essential for biosynthesis of the carbohydrate part of glycoproteins. SLC39A8 deficiency linked for the first time Mn deficiency with inherited glycosylation disorders. As therapeutic step dietary galactose supplementation is suggested to be effective (110). However, Tuschl *et al.* (2016) demonstrated that ZIP14 functions as a pivotal manganese transporter and they identified a novel autosomal recessive disorder of Mn homeostasis caused by homozygous mutations in ZIP14 that lead to early-onset rapidly-progressive parkinsonism–dystonia with distinctive brain magnetic resonance imaging (MRI) appearances and neurodegenerative features on post-mortem examination. Additionally the patients show no excessive Mn in the liver, possibly due to the bypassing of hepatic uptake by Mn and subsequent biliary excretion in the absence of ZIP14. Next to the findings in patients they show that mutations in ZIP14 impair manganese transport *in vitro* and lead to manganese dyshomeostasis and altered locomotor activity in zebrafish with ZIP14 null mutations (115).

Furthermore ZIP14 KO mice exhibited excessive Mn accumulation in the brain associated with impaired motor function (116). Being expressed in the nasal respiratory epithelium and olfactory receptor neuron dendrites ZIP8 and ZIP14 might further play a role in uptake of inhaled Mn through the olfactory pathway, bypassing the neuronal barriers (113).

In addition, the Mg transporter HIP14 and Ca channels located in the plasma membrane are reported to be involved in Mn uptake, and therefore, may play a role in Mn accumulation in the brain. Mn has been shown to enter cell membranes trough store-operated Ca channels which are expressed in brain endothelial cells (117). Besides store-operated calcium channels also voltage-gated Ca channels as well as ionotropic glutamate receptor channels have reported permeability to Mn (118). The expression of voltage-gated Ca channels is higher in dopaminergic neurons of the midbrain, which could contribute to their selective vulnerability induced by Mn (119). Use of a Ca channel blocker resulted in an inhibited Mn uptake in human erythrocytes (120). Mn^{2+} can acutely inhibit ATP-dependent Ca^{2+} signaling in astrocytes by blocking Ca^{2+} entry through the receptor-operated cation channel, TRPC3. Consequently critical homeostatic functions necessary for metabolic and trophic support of neurons might be comprised (121).

As already noted above, Mn-citrate is thought to be the major Mn-related species entering the brain, indicating citrate transporters might represent another putative Mn transporter system. It has been suggested that a Mn-citrate tridentate complex with a non-coordinated central carboxylate recognition moiety could be a substrate for the organic anion transporter or a monocarboxylate transporter (MCT). Evidence for an H^+-dependent mechanism suggests the possibility that MCT-1 mediates Mn-citrate uptake (75). However, the role of citrate as an efficient *in vivo* Mn transport needs to be further investigated.

In addition, the dopamine transporter (DAT) has been posited to be involved in Mn uptake in the brain. Being highly expressed in in the axons, dendrites and cell bodies of neurons in the SNpc, globus pallidus and striatum it normally functions to induce reuptake of dopamine into presynaptic vesicles (122). Studies point out that DAT and Mn overload impact each other. The usage of DAT inhibitors as well as DAT knockout animals resulted in a reduced Mn accumulation in certain brain areas as the striatum, while affecting Mn accumulation in brain regions not expressing DAT (123, 124). Selectivity to the DAT was verified, since inhibition of the serotonin transporter or norepinephrine transporter did not show this effect (125). Acute Mn administration in animals as well as patients chronically exposed to Mn show decreased DAT levels (123, 126). It has also been observed that the presence of Mn induces the internalization of DAT in transfected HEK cells (127).

Chronic Mn exposure is associated with decreased levels of choline in the hypothalamus and thalamus (128). However, whether choline transporters play a direct role in Mn import, need to be investigated in further studies (129).

At the neuronal level, α-synuclein (α-Syn) is believed to contribute to Mn homeostasis in neurons (130). Overexpression of increased intracellular Mn levels, whereas levels of Ca, Zn, K, P, and S were significantly decreased with not altering the expression patterns of DMT1, voltage-gated Ca-channels and ferroportin. Thus, α-Syn may act as an intracellular Mn store and neurotoxicity associated with PD might be mediated via regulation of transition metal levels and the metal-binding capacity of α-Syn (130).

In addition, the Cu-dependent ferroxidase ceruloplasmin (Cp) is actually discussed to contribute to Mn uptake. While previously it was proposed that

Cp oxidized Mn to its trivalent state and loaded onto Tf (93, 131), a more recent study did not find any difference between control and a ceruloplasminemic mouse in trivalent Mn bound to Tf. The data pointed out further that Cp affects the tissue distribution of Mn and increases oxidative stress in the brain (132).

4.2.2. Mn efflux: Ferroportin, SLC30A10, NCX

In addition to import, Mn efflux plays a central role in regulating intracellular concentrations of this metal in the CNS. Compared with Mn import, less is known about Mn transporters/channel proteins that participate in Mn efflux. Currently, plasma membrane localized exporters include ferroportin (Fpn), SLC30A10 and sodium-calcium exchanger (NCX).

Fpn is the only known mammalian Fe exporter, and is expressed in brain cells including neurons, astrocytes, the endothelial cells of the BBB, oligodendrocytes, the choroid plexus and ependymal cells (133). However, whether or not Fpn exports Mn and plays a role in Mn homeostasis remain controversial. While some studies report the induction of Fpn protein by Mn exposure (134, 135), other reports indicate treatment with Mn has no effect on gene expression (136). Xenopus laevis oocytes expressing human Fpn showed lower intracellular Mn and higher extracellular Mn (135). In an animal model by using flatiron (ffe/+) mice, a genetic model of Fpn deficiency, evidence suggest that Fpn deficiency impairs Mn metabolism. The authors suggest further that flatiron mice provide an excellent genetic model to explore the role of this exporter in Mn homeostasis (137). In addition, in *C. elegans,* a genetic contribution on the Mn export has been shown. *pdr-1* (PD related-1) imparts a risk for autosomal recessive, early-onset PD, and encodes for the E3 ubiquitin ligase parkin. Within the ubiquitin proteasome system that targets substrates for degradation, parkin functions in multiple processes, among them is the stabilization of cytoskeletal components associated with actin filaments in neuronal and non-neuronal cells. Recently, studies in *C. elegans* have shown that a genetic predisposition of PD by means of a loss of *pdr-1*, can modulate Mn export through altered transporter expression of Fpn. Overexpression of *fpn-1.1.* in worms lacking *pdr-1*, showed evidence for attenuation of several endpoints of Mn-induced toxicity (138).

SLC30A10 (or ZnT-10) is one of the 10 solute carrier family 30 (SLC30) transporters (ZnTs, Zn transporters). Interestingly, although SLC30A10 mediated Mn efflux, SLC30A1-8 transport Zn. Molecular characterizations as well as crystal structures revealed fundamental differences between a crucial Zn binding site in the transmembrane domain and the corresponding putative metal binding site of SLC30A10 (139) (140, 141). Residues in the transmembrane and C-terminal domains together confer optimal manganese transport capability to SLC30A10 (141). SLC30A10 is localized to the plasma membrane and is functional in manganese metabolism by effluxing cytosolic Mn (142, 143). Recent findings identified the SLC30A10 gene as the disease-causing gene in an inherited Mn overload syndrome. Mutations of the SLC30A10 gene result in parkinsonism with hypermanganesemia along with dystonia, polycythemia, characteristic MRI brain findings in the basal ganglia, and chronic liver disease (8, 144). Currently, it is the only known protein associated with the first hereditary or familial form of Mn-induced parkinsonism underlining its critical role in regulating CNS Mn homeostasis. The new form of familial parkinsonism has been reported first in 2008 by Tuschl *et al* in a 12-year old girl with hypermanganesaemia, liver cirrhosis, an extrapyramidal motor disorder and polycythaemia (145). The brother had the same symptoms but did not survive. While not being exposed through elevated Mn from environmental or occupational sources, the patient had ~ 10-fold increase in blood Mn levels and the MRI studies point out Mn deposition in the basal ganglia (145). Almost 10 years later, about 13 families have been reported on, for a total of at least 25 affected individuals with the same clinical picture as just described (146, 147). All affected patients carried homozygous mutations in the gene coding for SLC30A10 and thirteen causative mutations have been reported so far (147). The patients have never been exposed to high Mn-containing environment while all patients exhibited 10–20 fold increase in blood Mn levels concomitant with Mn deposition in the basal ganglia. This indicates that homeostatic control of Mn was compromised in the cases (8, 146, 147). Recent findings provided insights in the molecular mechanisms. *In vitro* as well as *in vivo* studies discovered that SLC30A10 functioned as a cell-surface-localized manganese efflux transporter that protected against Mn toxicity. A mutation of SLC30A10 resulted in a heightened sensitivity to Mn toxicity (139, 143). Experiments using a full-body, constitutive Slc30a10 knockout mice unexpectedly exhibited extensive alterations in their thyroid while brain and liver were largely unaffected (139). Thus far, the relationship between thyroid function and Mn toxicity has received little attention, and whether human patients of Mn toxicity develop hypothyroidism is unknown and needs to be determined in future studies.

Recent cardiac studies suggested the role of sodium-calcium exchanger (NCX) in Mn accumulation and retention. Studies examined the temporal features of cardiac Mn^{2+} efflux by implementing MRI and inhibiting the NCX with SEA0400. This inhibition was shown to result in an increase of Mn^{2+} blocks manganese efflux in mouse and rat tissues (148-150). More recently, the

inhibition of the NCX channel (inhibitor KB-R7943) was shown to increase cellular Mn levels in immortalized mouse striatal neuroprogenitors (151). However, additional studies are needed in order to determine the role of NCX under normal Mn neuronal homeostatic conditions.

4.3. Intracellular Mn transporters

Despite being expressed in the plasma membrane, transporters can be further expressed in the organelles and thus ensure subcellular Mn transport. Regarding their intracellular distribution, only a limited number of studies is available. Data suggested that the lysosomes, the Golgi apparatus, the endosome, mitochondria as well as the nucleus may be significant pools for intracellular Mn (152-156).

4.3.1. Endosome: TfR and DMT1

In addition to the membrane transporters, Mn can be transported into the cytosol via the ligand-receptor endocytosis mechanism, which is mediated by TfR and DMT1. In mouse hippocampal and striatal neuronal cells, Mn^{3+}/Tf/TfR complex has been found in a region close to the mitochondrial network, presumably endosomes, via the endocytic transport (53). Later, Mn^{3+} dissociates from Tf/TfR complex after endocytosis. As a unstable and reactive oxidant, Mn^{3+} has to be reduced to Mn^{2+}, a process which is mediated by ferrireductase to avoid oxidative stress (9, 53, 157). Analogous to TfR, DMT1 is also expressed in the endosome and transports Mn^{2+} from endosome lumen to cytoplasm (9, 157).

4.3.2. Lysosome: PARK9/ATP13A2

The ATP13A2 gene (PARK9) encodes a lysosomal type 5 P-type ATPase. Its substrate specificity and physiological function are unknown, however studies suggest it is involved in lysosomal degradation of proteins, Mn homeostasis, and most recently Zn transport (158, 159). Mutations in ATP13A2 have been associated with an autosomal recessive levodopa-responsive early-onset parkinsonism, known as Kufor–Rakeb syndrome (160, 161). In *in vitro* studies ATP13A2 protected cells as neuronal cultures from Mn-induced cell death in response to Mn treatment (160, 162, 163). In primary rat neurons, ATP13A2 levels were increased in the presence of excess Mn, while expression of wild-type ATP13A2 lowered intracellular Mn levels and prevented Mn-induced neuronal death (163). These data show the involvement of ATP13A2 in Mn homeostasis which is further underlined by polymorphisms of ATP13A2 to modify the effects of Mn on motor function in an elderly population (164). To modify the activity of ATP13A2 is another approach for a therapeutic strategy. The catalytically active ATP13A2 offers cellular protection against rotenone-induced mitochondrial stress, which relies on the availability of the lipids phosphatidic acid (PA) and phosphatidylinositol(3,5)bisphosphate (PI(3,5)P2). Thus, the N-terminal binding of PA and PI(3,5)P2 emerges as a key to unlock the activity of ATP13A2, which may offer a therapeutic strategy to activate ATP13A2 (165). Besides regulating Mn toxicity, ATP13A2 is also known to regulate α-Syn toxicity. ATP13A2 expression can suppress α-Syn toxicity in yeast and rescue α-Syn-induced dopaminergic degeneration in primary neuronal culture (160). However, a recent study did not find neuroprotection when ATP13A2 and α-Syn were co-expressed using viral vector technology in the SN in rats (161). Another study supports a relationship between ATP13A2, Mn, and α-Syn accumulation *in vivo*. Older ATP13A2-deficient mice showed Mn enhanced sensorimotor function, increased autofluorescence in the SN, and increased insoluble α-Syn in the ventral midbrain. The authors also found increased Mn concentration in the brain with higher levels in Mn-treated ATP13A2 mutants as compared to the control animals (158). However, another study in a different ATP13A2 knockout mouse showed age-dependent motor impairments, gliosis, accumulated ubiquitin protein aggregates, and endolysosomal abnormalities but no aberrant α-Syn up to 18 months of age (166). Besides the existing *in vitro* and *in vivo* studies, no direct Mn efflux activity for ATP13A2 has been demonstrated up to now. Therefore, additional studies are necessary especially also whether patients who harbor mutations in this gene also exhibit Mn deposition in specific brain regions.

4.3.3. Golgi: SPCA1, HIP14, SLC30A10, calcium channels

Synchrotron X-ray fluorescence nanoimaging has established the Golgi apparatus is the cellular site of preferential accumulation of Mn (153, 167). A Mn transporter localized in the Golgi apparatus is the secretory pathway Ca^{2+}/ Mn^{2+} ATPase isoform 1 (SPCA1) encoded by ATP2C1. It is a known Ca^{2+}/ Mn^{2+} transporter pump and is the only known P-type ATPase having a high affinity to transport Mn^{2+} (168, 169). Silencing of SPCA1 as well as deficiency resulted in an extreme sensitivity to high Mn concentrations (140, 169). SPCA1 carrying specific mutations that could potentially increase its Mn^{2+} pumping activity as the point mutation Q747A has been shown therapeutically useful in the management of manganism (170). Mn has further been shown to induce degradation of GPP130, a membrane protein that cycles between the Golgi and endosomes (171). Therefore, it is suggested that G PP130 may be involved in Mn homeostatic regulation. GPP130 is viewed as a Mn sensor, potentially useful for monitoring Mn levels. SPCA1 is required for Mn to reach the Golgi lumen where it binds to GPP130 and induced GPP130 oligomerization in the Golgi. This

results in sorting to the oligomer and secretion of Mn from the cell (172).

Additional Mn transporters expressed in the Golgi apparatus are HIP14, SLC30A10 as well as calcium channels which have already been described in this review. Additionally, variants in ZIP8 impair the function of a Golgi enzyme essential for biosynthesis of the carbohydrate part of glycoproteins (110, 170). At higher concentrations Mn may not be stored properly in the Golgi apparatus and other organelles may be impacted by Mn. An drug-induced collapse of the Golgi apparatus, results in the striking intracellular redistribution of Mn with Mn accumulation in the cytoplasm and the nucleus (153). This lead to the assumption that the Golgi apparatus might be a storage site and critical target for Mn toxicity and altered functions might be involved in Manganism.

4.3.4. Mitochondria: DMT1, TfR and Ca uniporter

Excess Mn has been reported to disrupt energy production and induce oxidative stress in the mitochondria (173-175). In addition, the highest Mn accumulation rate was observed in the mitochondria of astrocytes and neurons, compared with other organelles after chronic Mn exposure (176). However, the regulation of Mn homeostasis in this organelle remains largely unknown. Mn transporters expressed in the mitochondria include DMT1 in the outer mitochondrial membrane (104), the TfR, Ca transporter as well as citrate transporter (177, 178). Cytosolic Mn^{2+} is imported in the mitochondrial lumen by Ca uniporter, while excessive Mn is supposed to be exported through Na-independent mechanisms (179-181). Gavin *et al.* (1999) further indicated that a slow efflux of Mn by mitochondria accounts for the excess accumulation of Mn ions in this subcellular organelle (177). Other studies did not observe Mn accumulation in the mitochondria (152, 154).

4.3.5. Nucleus: unknown

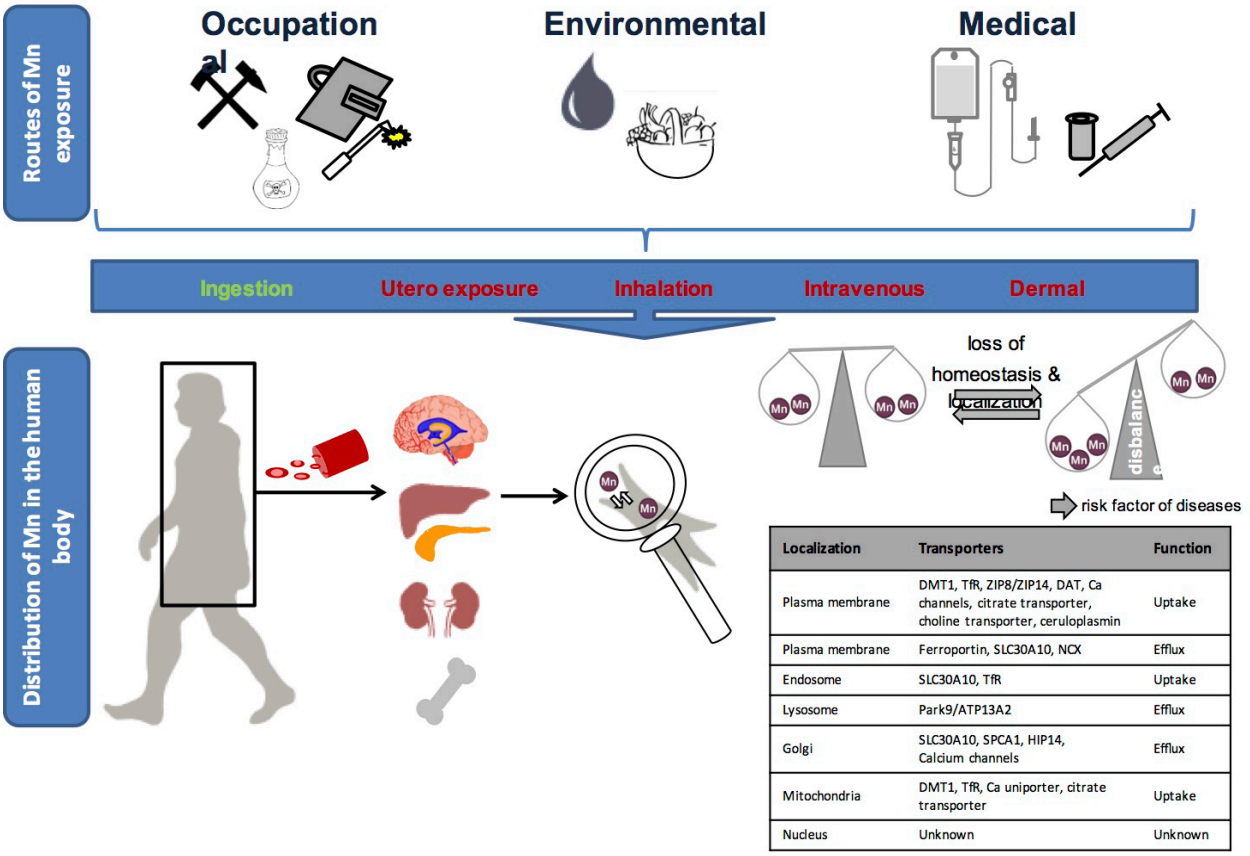
From experimental evidences the nucleus is supposed to be the largest intracellular Mn pool. For example, in rat striatum and globus pallidus, the highest Mn level was observed in the heterochromatin and nucleolus, after moderate Mn exposure (176). But to our knowledge there is no mechanistic study to investigate the reason for its high capacity to accumulate Mn. Discussed are hypothesis that Pirin, a highly conserved nuclear protein that is exclusively localized within the nucleoplasm and predominantly concentrated within dot-like sub-nuclear structures, may play a role in Mn transport (154). The highly conserved metal binding site in the N-terminal β-barrel of Pirin may allow Mn to replace Fe and therefore offer a depot for Mn ions (182).

5. SUMMARY AND FUTURE DIRECTIONS

In the last decade, research on Mn-related toxicity has advanced the understanding on mechanisms associated with Mn intoxication, providing scientific evidence for Mn regulation and potential therapeutics to treat people with Mn poisoning.

Given that Mn is a required nutrition but also an abundant metal on the earth, every day people have contact with Mn through environmental, occupational and medical exposure routes (Fig. 1). Currently, dietary consumption is the primary Mn exposure route, about 3-5% of ingested Mn is absorbed in blood stream via the GI tract through passive diffusion or active transport, regulated by Mn transporters and Mn binding proteins. Mn absorption via ingestion accounts for the highest Mn amount and is also the safest way. Abnormal Mn uptake though upon *in utero* exposure, inhalation, intravenous administration and dermal exposure (Fig. 1) can bypass the absorptive regulation afforded by GI tract, exceed the capability of liver elimination or directly deposit in the brain, with ensuing Mn intoxication. Once in the blood stream, Mn is rapidly distributed to different tissues. The liver, bone, pancreas, kidney and brain are the five major organs (from high to low) containing highest Mn level in human body (Fig. 1). Importantly, the brain is the primary target of Mn poisoning although it is not the tissue with the highest Mn concentration. Mn gets delivered in the brain through the blood-brain barrier (BBB), the blood-cerebrospinal fluid (CSF) barrier and the olfactory tract, which is regulated by various Mn transporters. The daily absorption amount usually exceeds the actual need, thus extra Mn has to be eliminated. Fecal hepatobiliary excretion is the primary elimination route, through which excess Mn is conjugated in the bile and excreted in feces. Besides, Mn can be excreted though urine, milk and sweat as well, although the amount is very limited.

As a requisite nutrition at low levels but also a toxicant at high concentrations, Mn in the human body has to be tightly regulated. At the cellular level, this homeostasis is achieved by various types of Mn transporters and regulators. On cell surface, DMT1, TfR, ZIP8/ZIP14, DAT, calcium channels, citrate transporter, choline transporter and ceruloplasmin regulate Mn influx from the cell matrix, while SLC30A10, Fpn and NCX mediate cytosolic Mn efflux. Intracellularly, the Mn/Tf/TfR complex can be packed in endosomes for endocytosis and finally released into cytosol by endosomal DMT1. On the other hand, PARK9/ATP13A2 transports cytosolic Mn into lysosomes, meanwhile, SPCA1, HIP14, SLC30A10 and calcium channels are able to pack Mn in the Golgi apparatus. Once Mn is sorted out in lysosomes and the Golgi, it is secreted from cells. In addition, the mitochondrion and nucleus are the two organelles



Localization	Transporters	Function
Plasma membrane	DMT1, TfR, ZIP8/ZIP14, DAT, Ca channels, citrate transporter, choline transporter, ceruloplasmin	Uptake
Plasma membrane	Ferroportin, SLC30A10, NCX	Efflux
Endosome	SLC30A10, TfR	Uptake
Lysosome	Park9/ATP13A2	Efflux
Golgi	SLC30A10, SPCA1, HIP14, Calcium channels	Efflux
Mitochondria	DMT1, TfR, Ca uniporter, citrate transporter	Uptake
Nucleus	Unknown	Unknown

Figure 1. Manganese metabolism and regulation in humans. Human exposure to Mn arises from both natural and anthropogenic sources, including environmental, occupational and medical exposure. Mn enters human body via ingestion, utero exposure, inhalation, intravenous administration and dermal exposure. Once absorbed, Mn enters the blood stream and gets distributed via blood circulation. This process is regulated in various organs including blood, liver, pancreas, kidney, bone as well as the brain. On the cellular level, Mn homeostasis is maintained by both membrane transporters and subcellular transporters. The membrane transporters include importers and exporters. Mn is further subcellular distributed and regulated in endosome, lysosome, Golgi, mitochondria and nucleus. A loss of Mn homeostasis is documented to lead to devastating neurological impairment. This disease, termed as “manganism” shares a similar neuropathology as Parkinson’s disease (PD).

with the highest Mn concentrations, and they play a role as Mn pools for its storage. The uptake, efflux and subcellular transport of Mn are facilitated by various transporters to obtain a homeostasis (Fig. 1). However, environmental or genetic risk factors may disrupt this balance and result in Mn intoxication (Fig. 1).

Given the exposure routes, absorption, distribution and elimination of Mn described above, several groups of people are at high risk of Mn poisoning. The first group are neonates. Infants tend to absorb various metals including Mn for their development; however, the elimination system is still developing, thus less capable to extrude excessive Mn. The most important infant foods-formula and milk, both contain significant amounts of Mn, which might be a potential risk and require a new industrial standard. Importantly, premature infants receiving total parental nutrition (TPN) are at highest risk, as they absorb 100% of Mn from TPN. Second, children exposed to high levels of Mn through drinking water or living close to high Mn environment have a higher risk of Mn poisoning as developing children tend to absorb higher levels of Mn. Third, industrial workers performing welding, smelting, mining, manufacture of steel, batteries, glass, ceramics, cosmetics, leather, fireworks and other textiles should be aware of potential risk. Fourth, patients with liver problems, such as hepatic encephalopathy or liver failure, are less capable to eliminate extra Mn. They tend to accumulate Mn in the body even without high environmental exposure. Fifth, people carrying certain genetic mutations, such as SLC30A10, ZIP14, PARK9 and SPCA1, are also at risk of Mn intoxication. Sixth, people under iron deficiency condition tends to have Mn accumulation in their body as iron deficiency will increase the activity of Fe/Mn transporters and upregulate the absorption rate of Mn. Last but not least, methcathinone abusers has become another susceptible group with the spreading of this drug.

Although the toxic effect of Mn in neonates and children has been well-studied in TPN, administration by government to limit Mn contents in infant foods and nutrition supplements remains a contemporary health

issue. Establishing standards is of great urgency especially in TPN and potentially in infant formula and milk as well.

While great strides have been made in the past years, the complete picture of Mn metabolism and homeostasis remains to be mapped out. Advanced biomarkers or measuring techniques are needed to monitor body Mn levels, especially for chronic Mn accumulation. As blood Mn is very dynamic and only represents current body Mn levels in a relatively short term, patients with low blood Mn after chelation therapy might still suffer from Mn-induced neurotoxicity due to slow release of Mn from other tissues accumulated in the past. Bone Mn is a good biomarker for this purpose, given that bone stores the largest amount of Mn in human body and bone Mn has a long half-life (skeleton bone 8.5. years). Recent techniques such as *in vivo* neutron activation analysis (IVNAA) allow non-invasive measurement of bone Mn levels, thus are of great help to access chronic Mn accumulation and monitor Mn levels across developmental stages in children. Brain is the primary target of Mn poisoning. As for regulation of Mn homeostasis, although we have identified a few transporters capable to transport Mn, a large numbers of regulator proteins remain to be identify. Besides, among these known transporters, actually none of them has been proved as a Mn specific transporter. Most of them facilitate couple metal ions influx or efflux with highest affinity to other metals rather than Mn. SLC30A10 could be a potential candidate, but more research is needed to confirm that. In addition, within a cell, the nucleus is the largest Mn storage site with the highest Mn concentration. However, regulation of Mn homeostasis in this organelle remain blank due to very few intracellular transporters identified there. A forward or reverse genetic screen to find these transporters or regulators is of great interest and priority to understand Mn homeostasis in cells and in human body.

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Send correspondence to: Pan Chen, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Forchheimer 209, 1300 Morris Park Avenue, Bronx, NY 10461, Tel: 718-430-4047, Fax: 718-430-8922, E-mail: pan.chen@einstein.yu.edu