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Review

Biomarkers of manganese intoxication

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ABSTRACT

Manganese (Mn), upon absorption, is primarily sequestered in tissue and intracellular compartments. For this reason, blood Mn concentration does not always accurately reflect Mn concentration in the targeted tissue, particularly in the brain. The discrepancy between Mn concentrations in tissue or intracellular components means that blood Mn is a poor biomarker of Mn exposure or toxicity under many conditions and that other biomarkers must be established. For group comparisons of active workers, blood Mn has some utility for distinguishing exposed from unexposed subjects, although the large variability in mean values renders it insensitive for discriminating one individual from the rest of the study population. Mn exposure is known to alter iron (Fe) homeostasis. The Mn/Fe ratio (MIR) in plasma or erythrocytes reflects not only steady-state concentrations of Mn or Fe in tested individuals, but also a biological response (altered Fe homeostasis) to Mn exposure. Recent human studies support the potential value for using MIR to distinguish individuals with Mn exposure. Additionally, magnetic resonance imaging (MRI), in combination with noninvasive assessment of γ-aminobutyric acid (GABA) by magnetic resonance spectroscopy (MRS), provides convincing evidence of Mn exposure, even without clinical symptoms of Mn intoxication. For subjects with long-term, low-dose Mn exposure or for those exposed in the past but not the present, neither blood Mn nor MRI provides a confident distinction for Mn exposure or intoxication. While plasma or erythrocyte MIR is more likely a sensitive measure, the cut-off values for MIR among the general population need to be further tested and established. Considering the large accumulation of Mn in bone, developing an X-ray fluorescence spectroscopy or neutron-based spectroscopy method may create yet another novel non-invasive tool for assessing Mn exposure and toxicity.

Contents

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1. Introduction

Manganese (Mn) is a naturally occurring element abundantly present in the environment. While essential to human health, overexposure to Mn is associated with devastating neurologic impairment clinically known as "manganism," a motor syndrome similar to, but partially distinguishable from idiopathic Parkinson's disease (IPD) (Aschner et al., 2007, 2009; Barbeau et al., 1976; Calne et al., 1994; Jiang et al., 2006; Mena et al., 1967; Olanow, 2004). Because the symptoms of Mn intoxication, once established, usually become progressive and irreversible, reflecting permanent damage to neurologic structures, establishing a biomarker for Mn intoxication has become an immensely pressing issue.

According to the National Academy of Sciences (1989), a biological marker or biomarker is defined as an indicator that signifies an event in a biological system or in samples of biological origin. Biomarkers can be divided into three broad interrelated sub-categories: biomarkers of exposure, biomarkers of effect, and biomarkers of host susceptibility (Fowle and Sexton, 1992). The distinction between these three sub-categories is not always clear, as effect and susceptibility usually overlap, and exposure and effect, in many cases, are closely related. However, there are instances in which biological exposure is evident and measurable, and a latency period exists in which biological or physical alterations do not visibly manifest.

No reliable biomarkers have been established to evaluate the effect of Mn exposure or host susceptibility to the metal primarily because a complete scientific understanding of the mechanism of toxicity remains undiscovered. Over the past decade, extensive research using animal models and human populations has led to several potential indicators of Mn exposure and biological effect. The purpose of this review is to identify valid biological indicators of Mn exposure and toxicity, evaluate their feasibility in real-life assessment, and provide a critical comment on the future direction of Mn biomarker investigation.

2. Biomarkers of Mn exposure

A biomarker of exposure is any measurable biological parameter that indicates levels of exposure to a given toxic substance, whether it is an induced protein, enzyme, metabolite, or the toxic substance itself. To be a reasonable biomarker of Mn exposure, ideally the biological measures should display the following: (1) exposure-related changes should be quantitatively and consistently observed in biologic matrices (e.g., blood, urine, feces, breast milk, skin, and hair, etc.); (2) relatively large differences in assessment outcomes between exposure and control groups; and (3) a reasonable threshold or cut-off value above which a Mn-exposed worker can be differentiated from unexposed individuals.

2.1. Mn concentrations in body fluid as a biomarker of exposure

Mn, upon inhalation exposure, readily gains access to the systemic blood circulation. Hence, most Mn biomarker research has initially focused on blood Mn concentration. Rodent studies show that Mn concentrations in the whole blood (MnB) are significantly elevated following Mn administration by injection, oral gavage, or inhalation (Keen et al., 1983; Roels et al., 1997; Salehi et al., 2003; Tapin et al., 2006; Ulrich et al., 1979; Zheng et al., 1998, 2009). Although results from studies of non-human primates suggested similar outcomes, data from human studies on this subject have been controversial (Dorman et al., 2006a,b; Erikson et al., 1992; Sung et al., 2007).

The use of Mn concentration in the blood compartment (i.e., Mn in whole blood, plasma, or serum) as a biomarker of Mn exposure has been investigated among welders (Edmé et al., 1997; Li et al., 2004; Lu et al., 2005; Myers et al., 2003; Park et al., 2006), ferroalloy smelters (Apostoli et al., 2000; Ellingsen et al., 2003; Jiang et al., 2007; Lucchini et al., 1999; Mutti et al., 1996), Mn oxide production workers (Roels et al., 1992), and dry-cell battery workers (Bader et al., 1999). In general, these studies appear to suggest that blood Mn concentration (1) serves as a reasonable indicator of exposure on a group basis; (2) reflects recent, active exposure; and (3) appears to be a modest indicator for distinguishing Mn-exposed workers from control subjects at the individual level. The reason for the lack of sensitivity associated with the use of blood Mn for identifying individual workers appears to be related to the significant discrepancy between blood half-life ($t_{1/2}$) and tissue $t_{1/2}$ of Mn (Apostoli et al., 2000; Lu et al., 2005; Newland et al., 1987; Takeda et al., 1995; Zheng et al., 2000). Toxicokinetic studies, for example, reveal that the plasma $t_{1/2}$ of Mn in rats is less than 2 h (Zheng et al., 2000), whereas $t_{1/2}$ in brain can exceed 50 days (Newland et al., 1987; Takeda et al., 1995). The intracellular distribution of Mn ions may be the underlying basis for the lack of concordance between blood Mn and the actual Mn concentrations in target organs or tissues, particularly in the brain. Presumably, with recent or ongoing exposure, extracellular Mn can be detected prior to its intracellular sequestration. It is tempting to postulate that the mass balance between intra- and extracellular Mn concentrations is such a slow process that the extracellular Mn does not adequately mirror the dynamics of intracellular Mn binding, unbinding, trafficking, and efflux. It is also possible that the ubiquitous nature of Mn in the environment has allowed the body to develop an efficient intracellular sequestration mechanism; hence, at the individual level, the changes of Mn concentrations in the blood compartment become less variable (due to this large tissue buffering capacity). For a group of individuals with prolonged Mn exposure, the differences between Mn-exposed and control subjects may reflect an established mass balance between the two groups.

These characteristics of Mn intracellular distribution have prompted investigations of the feasibility of using the Mn content in erythrocytes (MnE) as the biomarker of Mn exposure. Jiang et al. (2007) conducted an influential study among 18 Mn-exposed smelters. While MnE levels in the smelting workers were not significantly increased compared to controls (possibly due to the small number of study subjects), MnE was significantly correlated with the palladial index (PI) obtained from magnetic resonance imaging (MRI), indicating that increased MnE may reflect Mn accumulation within the CNS. Continuing this line of investigation, this group of researchers recruited 217 subjects from a ferroalloy smelting factory to determine Mn and iron (Fe) concentrations in erythrocytes. The results suggested that MnE was significantly higher in the exposure groups than in controls (Cowan et al., 2009a). More importantly, the study established the concept of the erythrocyte Mn/Fe ratio (eMIR) as an effective biomarker for distinguishing Mn-exposed workers from the control subjects (discussed later in this article).

Mn concentration in saliva (MnV) has also been proposed as a possible non-invasive indicator of Mn exposure. Humans secrete 800–1500 mL of saliva each day, making saliva a readily accessible body fluid for biomarker study. Wang et al. (2008) collected saliva samples from groups of Mn-exposed welders and compared Mn concentrations in saliva with Mn in serum (MnS). A statistically significant increase in both MnV and MnS was observed when Mn-exposed welders were compared to control subjects. The variation in MnV concentrations, however, was only partly associated with the welders' employment years, and the MnV exhibited a much greater variation among tested subjects than MnS. This result led the authors to conclude that the changes in MnV mirror those in MnS, which suggested that this marker does not provide a more sensitive measure or better indication of Mn exposure than MnS.

Other studies have suggested the use of Mn concentration in the urine (MnU) as a potential non-invasive marker of Mn exposure; however since Mn is primarily excreted in the feces via biliary excretion, Mn excreted in urine represents only a small fraction of eliminated Mn. Indeed, little or no significant changes in MnU have been observed in many studies; as a result, some authors have recommended abandoning MnU as a possible biomarker of Mn exposure (Bader et al., 1999; Apostoli et al., 2000; Myers et al., 2003).

2.2. Mn contents in other biological materials

Hair Mn concentration (MnH) has been studied for its feasibility as another non-invasive indicator of Mn exposure. Several studies reported that the average value of MnH tended to increase in workers with increased years of employment, suggesting that MnH might indirectly reflect the Mn body burden (Huang and Cao, 2003; Lin, 2002; Wang and Wu, 2000; Xie et al., 1995; Zhang et al., 1996). Bader et al. (1999) found a significant increase (>300%) in auxiliary MnH when Mn-exposed workers in a dry-cell battery plant were compared to a control population. Cowan et al. (2009a) also observed a large increase of MnH (>2000%) among Mn-exposed smelters. These authors note that the large increase in MnH may be artificial due to external contamination. Eliminating such contamination proves to be highly challenging in real life assessment.

For Mn concentrations in toenail clippings or feces, a high variation among the samples has been observed; for example, the Mn range for feces was between 0.07–15.9 μg/g, and for toenails between 0.02–14.7 μg/g among Mn-exposed welders (Wongwit et al., 2004). Unfortunately, this study did not include a control group for comparison purposes.

2.3. Magnetic resonance imaging (MRI)

The paramagnetic properties of Mn allow identification with non-invasive imaging techniques such as MRI, which can be used for pinpointing Mn accumulation in brain tissues. Mn^{2+} has five unpaired electrons in the 3rd orbital, causing its large magnetic moment, which ultimately results in a shortening of the spin-lattice relaxation time and an increase in signal intensity on T1-weighted MRI. Bilateral symmetrical increases in brain signal intensities due to accumulation of Mn ions can be observed on T1-weighted MRI. Krieger and colleagues (1995) coined the term "pallidus index" (PI) to quantify Mn accumulation in globus pallidus, which is defined as the ratio of the signal intensity in the globus pallidus to that in the subcortical frontal white matter in axial T1-weighted MRI planes multiplied by 100. This term has been used widely as a semi-quantitative indicator of brain Mn status in several human studies (Dietz et al., 2001; Husain et al., 2001; Jiang et al., 2007; Josephs et al., 2005; Kim et al., 1999b,c; Kim, 2004; Kim et al., 2007; Lu et al., 2005; Lucchini et al., 1999, 2000; Nelson et al., 1993; Ono et al., 2002; Sadek et al., 2003; Sato et al., 2000; Shinotoh et al., 1995). While the PI is a valid measure for comparing MRI images of subjects within one study (using the same scanner, MRI sequence parameters, coils, etc.), its numerical value greatly depends on the exact image parameters, as well as on the comparison region of the brain used for the denominator in the PI. Exact numerical values of pallidal indices therefore vary significantly between studies, and are not a proportional measure of the Mn concentration in the brain; they are, however, good indicators for discriminating between Mn-exposed and non-exposed brains. Recently, Chang et al. (2010) showed that the PI calculated from high-resolution 3D T1-weighted MRI correlated better with air Mn concentrations and neurobehavioral performance indicators, when compared to the PI calculated from lower resolution images.

The unique characteristics of MRI make the technique potentially valuable for identifying Mn exposure. First, the increases in Mn signals are generally bilateral and symmetrical in both brain hemispheres. Second, the signals can be detected among Mn-exposed workers who may not exhibit visible signs or identifiable symptoms of manganism (Kim et al., 1999a; Jiang et al., 2007; Lucchini et al., 2000). Third, the signal intensity or the PI values are correlated with MnB or MnE (Kim et al., 1999c; Jiang et al., 2007). Fourth, the high signal intensities can be found not only in the globus pallidus, but also in striatum and nigra in workers with manganism (without terminating exposure and accepting clinical treatment) (Nelson et al., 1993). Finally, the abnormally high intensity may completely disappear after a six month absence from the exposure (Arjona et al., 1997; Kim et al., 1999a). Since Mn may also accumulate in the frontal cortex, which is often used as a reference site, some researchers have reported that the PI value based on the neck muscle is more accurate for reflecting Mn accumulation than the PI value calculated from the white matter in the frontal cortex (Guilarte et al., 2006).

While studying primates exposed to Mn either intravenously or by inhalation, Mn was observed to accumulate in the globus pallidus (GP), caudate, ventricular nuclei, substantia nigra, subthalamic nucleus, ventromedial hypothalamus and the pituitary gland; the characteristic high signal intensities of MRI T1-weighted signals were detected mainly in symmetrical GP (Dorman et al., 2006a,b; Erikson et al., 1992; Misselwitz et al., 1995; Newland et al., 1989; Newland, 1999; Park et al., 2007; Shinotoh et al., 1995; Sung et al., 2007). Consequently, the striatum and the globus pallidus are considered the initial regions of Mn intoxication in non-human primates as seen by MRI scanning (Saleem et al., 2002). Because the target region where Mn preferentially accumulates in the brain is well associated with radiological location of MRI signal hyperintensities, it is reasonable to conclude that MRI scanning has the sensitivity and specificity characteristics necessary to reflect Mn accumulation in the brain. For a more recent review of this subject, please see comments made by Guilarte (2010).

3. Biomarkers of Mn toxicities

Biomarkers of effect are defined as measurable biochemical, physiologic, behavioural, or other alterations within an organism following an exposure that can be recognized as an established or potential health impairment or disease (NAS/NRC, 1989; ATSDR, 1994). Hence, several physiological effects of Mn exposure, based primarily on the hypothesized mechanisms of Mn toxicity, have been suggested as markers for monitoring the degree of Mn neurotoxicity.

3.1. Alterations of Fe and iron-regulatory proteins (IRPs)

Both Mn and Fe are transition elements adjacent to each other in the Periodic Table, and they share similar valence charges and ionic radius. These chemical similarities allow Mn to compete directly with Fe at the molecular level by interacting with proteins and enzymes that require Fe as a cofactor in their active catalytic center, such as mitochondrial Complex I (Chen et al., 2001), aconitase (Zheng et al., 1998), and iron regulatory protein-1 (IRP1) (Li et al., 2005; Zheng and Zhao, 2001). Early studies show that Mn competes with Fe for the same binding site in the active center of iron-regulatory protein IRP1 (Zheng et al., 1998). The altered binding capacity between IRP1 and the stem-loop containing mRNAs that encode various Fe-transport and storage proteins may cause a compartmental shift of Fe from the blood to the cerebrospinal fluid (CSF), resulting in an Fe-deficient status in the blood compartment (Li et al., 2005; Zheng et al., 1998, 1999).

Indeed, human data have shown that systemic Fe levels were reduced in Mn-exposed humans and animals (Ellingsen et al., 2003; Zheng et al., 1999). Moreover, Jiang et al. (2007) further demonstrated that erythrocytes accumulated Mn in a dose-related fashion.

To establish a relationship between Mn exposure and Fe homeostasis, Cowan et al. (2009a,b) recruited 95 Mn-exposed ferroalloy smelter workers (high exposure), 122 power distributing and office workers in the same factory (low exposure), and 106 Mn-unexposed control subjects, and analyzed Mn and Fe concentrations in saliva, plasma, erythrocytes, urine and hair. While Mn concentrations in these biological matrices were elevated in exposed workers, the Fe concentrations in plasma and erythrocytes were significantly lower in Mn-exposed workers than in the control groups. Because changes of Mn and Fe concentrations lean in opposite directions, the authors believed that combining these two measurements into one parameter would further widen the differences between exposed and control individuals so that the sensitivity for assessing Mn toxicity could be increased. Using an integrated biomarker approach, these investigators introduced the concept of the manganese–iron ratio (MIR), which is calculated with Mn concentration in the numerator and Fe concentration as the denominator. Their data showed that, except for saliva, the differences between control and low-exposure groups and between low- and high-exposure groups became considerably greater in the MIR values than Mn concentrations alone in both plasma and erythrocytes (Cowan et al., 2009a). Noticeably, the ratio of Mn and Fe concentrations in body fluids and blood cells does not simply reflect the steady-state body burden of Mn or Fe in tested individuals, but rather signals a biological response (i.e., altered Fe homeostasis) to Mn exposure.

Cowan et al. (2009a) used receiver–operator curves, a technique derived from signal detection theory, to maximize the sensitivity and specificity of biomarkers. They were able to establish a cut-off value (COV) for differentiating erythrocyte MIR (eMIR) or plasma MIR (pMIR) values between control and exposed subjects. Based on the calculated COV, 73 out of 83 (88%) high exposure subjects displayed eMIR above the COV. Similarly, the authors reported that 108 out of 196 total smelters (low and high exposure subjects) studied (55%) displayed an eMIR significantly higher than the COV, whereas 89% of control eMIR values were below this COV. Therefore, it appears that both eMIR and pMIR are suitable markers for distinguishing Mn-exposed workers from controls.

In this same study cohort, Cowan and colleagues did not find any significant health problems or clinically diagnosed neurological dysfunctions between Mn-exposed smelters and control subjects. A Benton test did not reveal any abnormal memory deficits among Mn-exposed smelters, nor did the groove and nine-hole tests detect any significant abnormality in dynamic and static steadiness in tested subjects. Nonetheless, using the Purdue pegboard test, the authors observed that Mn exposure significantly exacerbated the age-related deterioration of fine movement coordination (Cowan et al., 2009b). Furthermore, they reported that both pMIR and eMIR were inversely associated with Purdue pegboard scores. The authors recommended using MIR as a biomarker for assessing not only Mn exposure, but also Mn-associated health risk.

Alteration in proteins related to Fe metabolism may potentially serve as biomarkers for Mn toxicity. For example, the levels of transferrin (Tf), an Fe transport protein, and ferritin (Tf), an Fe storage protein, were significantly increased among Mn-exposed welders (Lu et al., 2005) and smelters (Cowan et al., 2009b), as compared to unexposed controls, while the transferrin receptor (TfR) level, a cell surface Fe transporter, was significantly decreased (Lu et al., 2005). However, the data regarding the use of iron-regulatory proteins as biomarkers of Mn exposure are limited. In addition, variations of these proteins between exposed and unexposed subjects are too large to allow for a clear differentiation of Mn-exposed subjects from controls.

3.2. ^{1}H proton magnetic resonance spectroscopy (^{1}H MRS)

MRS is a noninvasive technique for studying brain metabolites. *N*-Acetyl-aspartate (NAA), a molecule synthesized in neurons from the amino acid aspartate and acetylcoenzyme A, has been used as a neuronal integrity marker. The concentration of NAA in MRS indicates neuronal metabolic function. Total creatine (Cr), including creatine and creatine phosphate, showed relatively stable concentrations in both physiological and pathological statuses, and often served as an internal standard for quantifying changes in a given volume. Choline-containing compounds (Cho) are precursors of acetylcholine and phospholipidoyl choline. Cho has been used to indicate myelin sheath integrity (with phospholipidoyl choline). Myo-Inositol (mI) plays an important role as an astrocytic marker, and functions as a structural basis for a number of second messengers. Glutamate, the main excitatory neurotransmitter, and glutamine, its precursor, are indispensible molecules for a variety of neuronal activities. Since they have difficulty discriminating by MRS *in vivo*, the combined signal of glutamate and glutamine is usually measured and denoted by Glx. Moreover, lipids (Lip) are an essential component of cellular membranes; the phospholipids bind to saturated and unsaturated fatty acids. An injured cell membrane or myelin sheath can release free lipids. A recent study of lead exposed workers revealed a significant decrease in NAA/Cr ratio and a remarkable increase in Lip/Cr ratio in the hippocampus as compared to controls (Jiang et al., 2008).

Increased Mn in the brain has been associated with liver disease, since damaged liver function reduces Mn clearance (Morgan, 1998; Pujol et al., 1993; Kim et al., 2010). Long et al. (2009) used MRS to analyze four major brain metabolites (i.e., Cho, mI, Glx, and NAA) in the basal ganglia among patients suffering from cirrhosis and chronic hepatic encephalopathy (CHE). They observed that the ratios of Cho/Cr and mI/Cr in cirrhosis and CHE patients were significantly decreased compared to controls, whereas the ratio of Glx/Cr was significantly increased. These changes were correlated with the PI values by MRI, which are exclusive to Mn accumulation. Another study using ^{1}H MRS in 20 male welders and 10 age- and gender-matched, non-office control workers displayed no significant differences between the welders and the controls in NAA/Cr, Cho/Cr, and NAA/Cho ratios obtained from basal ganglia (Kim et al., 2007).

Guilarte et al. (2006) performed a longitudinal MRS study on non-human primates with measurements taken at baseline, after a mean of 128 days of Mn administration (cumulative Mn dose = 84 ± 7.8 mg Mn/kg body weight) and a third time approximately 157 days later (cumulative Mn dose = 156.7 ± 9.5 mg Mn/kg body weight). The study found a significant decrease in NAA/Cr in the parietal cortex compared to baseline at the third measurement, while no changes were found for other metabolites assessed (Cho, mI) in any of the other brain regions studied (striatum, thalamus and frontal white matter).

Chang et al. (2009) examined NAA/tCr, Glx/tCr and tCho/tCr ratios among 35 welders and 20 age-matched controls. No significant changes were detected in all metabolites between welders and control subjects. In addition, no correlation between the metabolites and blood Mn or neurobehavioral parameters was found. However, the authors observed a significant decrease of mI/tCr in the anterior cingulated cortex. The authors postulated that the depletion of mI in welders may reflect glial cell swelling and/or detoxification processes resulting from long-term Mn exposure.

More recently, Dydak et al. (in press) used MRS to investigate brain concentrations of active metabolites in the globus pallidus,

putamen, thalamus and frontal cortex of 10 Mn-exposed smelters and 10 age, sex-matched control subjects. Additionally, they used the MEGA-PRESS sequence to determine GABA levels in the thalamus. In addition to a significant decrease of NAA/Cr in the frontal cortex of the exposed subjects, the authors observed a significant increase of GABA (as much as 82%) in the thalamus region due to Mn exposure. Using both GABA levels and the pallidal index, a logistic regression model allows for differentiating the exposed from the non-exposed subjects with 91% accuracy. The authors recommend that the pallidal index and the GABA level combination may be a powerful, non-invasive biomarker for both Mn exposure and pre-symptomatic Mn neurotoxicity.

3.3. Positron emission tomography (PET)

PET scans evaluate the uptake of 6-[^{18}F]fluorodopa or [^{18}F]FDOPA by dopaminergic neurons, which are often significantly compromised in Parkinsonian patients. However, the results for Mn toxicity studies have been inconsistent. Wolters et al. (1989) employed PET to study four Mn-exposed subjects with clinical features of mild Parkinson-type syndromes; results showed decreased cortical glucose metabolism. In patients with Parkinsonian syndromes that developed after chronic Mn exposure, a reduced uptake of [^{18}F]FDOPA in the striatum was detected (Abe et al., 1999; Kim et al., 1999b). Racette et al. (2005) reported cases in which patients with elevated blood Mn concentration showed a progressive, symmetric parkinsonism (characterized by prolonged L-dopa responsiveness) and a reduced [^{18}F]FDOPA uptake by PET, when compared to 10 age-matched patients with idiopathic Parkinson disease (IPD) and 11 normal control subjects. However, other studies found that decreased fluorodopa uptake in the posterior putamen of IPD patients was common, whereas the PET results were normal in manganism patients (Olanow, 2004). The question as to whether the PET scan can be used as a marker for distinguishing manganism from IPD must be further explored. Readers are directed to a recent review article by Guilarte (2010) for a more detailed discussion of nigrostriatal dopaminergic terminal degeneration of following Mn exposure.

3.4. Biomarkers of Mn-induced oxidative stress

Oxidative stress occurs when free radicals are excessively produced through mechanisms of oxygen reduction. One potential source of oxidative stress induced by overexposure to Mn is via oxidation of dopamine and other catecholamines (Sloot et al., 1996). In primates, Mn tends to accumulate in dopamine-rich regions, particularly in the basal ganglia (Newland, 1999). Another possible mechanism by which Mn contributes to excessive production of reactive oxygen species (ROS) is through interaction with intracellular molecules (Anantharam et al., 2002; HaMai et al., 2001; Oubrahim et al., 2001). Several enzymes and products of ROS reactions, such as superoxide dismutase (SOD), malondialdehyde (MDA), glutamine synthetase (GS), metallothionein (MT), or glutathione (GSH), may serve as markers of systemic oxidative stress.

SOD, a cytoplasmic enzyme that catalyzes the decomposition reaction of superoxide free radicals generated by cellular oxidative stress, has been described as a specific superoxide radical scavenger (Oda et al., 1989). The activity of SOD in erythrocytes is directly associated with oxidative status. MDA is a product of lipid peroxidation. An elevated level of MDA reflects, to a certain degree, tissue injury resulting from oxidative damage. Results from animal studies showed an alteration of MDA level in animals exposed to Mn (Chen et al., 2002). Misiewicz and colleagues (1999) also demonstrated an increased serum concentration of MDA in workers engaged in the production of Fe–Mn alloys. Li et al. (2004) observed a marked reduction in antioxidant enzyme activity (SOD) with an increase in lipid peroxidation (MDA) among career welders. These authors suggest that the levels of SOD, MDA, or both, in the systemic circulation may serve as a useful biomarker(s) for oxidative stress status after long-term, low level exposure to welding fumes among career welders.

GS is a Mn-dependant enzyme that plays an essential role in metabolizing nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. In a rat subchronic Mn exposure model, Zheng et al. (1999) reported a 33% increase in GS expression in Mn-exposed rats. They postulated that the increased expression of GS mRNA may result from Mn-potentiated cellular overload of Fe. Increased ROS and the resulting oxidative stress may inhibit enzyme activity, which, in turn, stimulates protein synthesis. Alternatively, the overexpression of GS mRNA may reflect a change in intracellular levels of Mn(II) or the ratio of Mn(II)/Mn(III), since both factors may likely participate in the regulation of cellular GS activity. Erikson et al. (2004) also showed a significant increase of the GS protein level in the hippocampus, but a decreased level in the hypothalamus of rats that inhaled $MnSO_4$. A more recent study from the same laboratory, however, suggests that Mn exposure significantly reduced GS protein levels in the cerebellum and decreases GS mRNA in the striatum (Erikson et al., 2006). The inconsistency between these studies does not support the use of GS as an indicator of Mn-induced toxic effect.

Among other markers of oxidative stress, MT belongs to a family of cysteine-rich, low molecular weight proteins widely present in almost all forms of life (Kägi, 1993; Vasak and Kägi, 1994). Beyond its well-recognized role as a metal binding protein, MT has been shown to act as a free radical scavenger (Bauman et al., 1991; Suzuki et al., 1993). Results from animal studies suggest a significant decrease in MT mRNA in brain tissues after Mn exposure (Dorman et al., 2004; Erikson et al., 2004, 2006). However, other researchers report an increased MT mRNA in young rats following Mn exposure (Dobson et al., 2003a), or no effect in brain MT levels (Dobson et al., 2003b) compared to controls. Whether the alteration of MT protein level corresponds to the decrease in MT mRNA is still uncertain.

GSH functions as a non-enzymatic reducing agent, and also as an antioxidant by trapping free radicals (Meister and Anderson, 1984). Several studies discovered a marked decrease in GSH levels and GSH peroxidase activity in the striatum of aged rat after subchronic exposure to Mn (Donaldson, 1987; Erikson et al., 2004; Liccione and Maines, 1988; Spina and Cohen, 1989). In patients with IPD and other neurodegerative diseases, GSH levels are significantly decreased (Sian et al., 1994). Therefore, the possibility of using GSH as a biomarker for assessing oxidative stress status induced by Mn cannot be ignored.

3.5. Other biomarkers of Mn effects

Dopamine (DA) is a hormone and neurotransmitter present in various animals, including both vertebrates and invertebrates. Prolactin (PRL) is an indirect indicator of dopaminergic function. Homovanillic acid (HVA) is a downstream metabolite of DA. All three molecules have been tested as possible biomarkers for Mn exposure (Alessio et al., 1989; Aschner and Aschner, 1991; Mutti et al., 1996; Takeda, 2003). Increased PRL levels have been observed in Mn-exposed male workers with previously manifested neurotoxicities (Mutti and Smargiassi, 1998; Smargiassi and Mutti, 1999). The information on these molecules is limited, however, and inconclusive. More recently, the possibility of using signal transduction molecules as potential molecular biomarkers of manganese neurotoxicity has been raised. For example, Mn exposure has been shown to proteolytically cleave an oxidative stress sensitive kinase, namely PKCdelta (Kitazawa et al., 2005;

Latchoumycandane et al., 2005). Additionally, a recent study indicated that Mn exposure up-regulates Prion proteins (Choi et al., 2010). Additional research is necessary to determine the efficacy of molecules associated with DA as biomarkers of Mn toxicity.

4. Comments and recommendations

The nature of Mn intracellular distribution and tissue accumulation underlies the discrepancy between Mn concentrations in blood and in targeted tissues, particularly in the brain. Recognizing this fundamental biological property can help identify situations in which an appropriate biomarker can be applied to define Mn exposure or its toxic effect under an appropriate assessment scenario. As suggested by the literature, blood Mn is useful for distinguishing Mn-exposed subjects from unexposed cohorts on a group comparison basis, which is a useful tool for epidemiological studies. The mean value obtained from a group study, however, cannot be readily used when attempting to distinguish one individual from the rest of the study population. The Mn/Fe ratio (MIR) in plasma or red blood cells appears to yield a sensitive measure of Mn exposure; hence at individual level, we recommend further investigating the feasibility of using plasma MIR to distinguish Mn-exposed workers from control subjects. In addition, for active workers, MRI scan with a detectable PI, combined with a noninvasive assessment of GABA by MRS, provides convincing evidence of Mn exposure even when clinical signs and symptoms of Mn intoxication are not observed.

For subjects with long-term, low-dose Mn exposure, particularly those with residential exposure, or those who were previously, but not currently exposed to Mn, neither blood Mn nor MRI can provide a valid measure of historical exposures. The plasma or erythrocyte MIR may be a sensitive measure; however, the cut-off values for pMIR or eMIR among the general population need to be further explored, verified, and established.

It should be pointed out that nearly 40% of Mn accumulates in bone (Schroeder et al., 1966). Recently, a rapid development in X-ray fluorescence spectroscopy and neutron-based spectroscopy has made it possible to detect low levels of a variety of metals, including Pb, in bone (Nie et al., 2006). It seems likely, and even probable, that a detection of Mn mass stored in bone may create yet another novel non-invasive method for assessing Mn exposure and toxicity.

Conflict of interest

All authors declare no conflict of interest.

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