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Review Article

IS MANGANESE INDUCED NEUROTOXICITY A POTENTIAL MODEL FOR PARKINSON'S DISEASE? AN OVERVIEW ON ITS COMPLICATIONS

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ABSTRACT

Parkinson's disease (PD) is one of the most common movement disorders, affecting over 6 million people annually across the globe. During normal aging, approximately 0.1-0.2% of the dopaminergic neurons in substancia nigra pars compacta (SNpc) is lost annually. But this rate is accelerated in patients with PD and symptoms manifests when 70–80% of these neurons have been lost. Manganese (Mn) is a vital trace mineral necessary for normal development and biological functions whereas it is also labelled as environmental toxic factor by World Health Organization (WHO). It is an essential co-factor for many of the enzymes which include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Excessive exposure to Mn is referred to as manganism, which is a well-recognized occupational and environmental hazard which can lead to an extrapyramidal syndrome manifested by impairment in iron homeostasis, cellular excitotoxicity, mitochondrial dysfunction, oxidative stress and induction of protein aggregation. Mn induced neurotoxicity is one of the models for screening anti-parkinsonian agents. Complications, cellular disturbances, advantages and the major drawbacks of the model are the limelight of the present study.

Key words: Parkinsons disease; Manganese madness; Substantia nigra pars compacta; Neurotoxicity; Oxidative stress; Anaemia;

INTRODUCTION

PD is the most common movement disorder, affecting over 6 million people annually across the globe. The disease is characterized by the formation of intra-cytoplasmic protein aggregates known as lewy bodies, in the adjacent neurons of the SNpc [1]. During normal aging, approximately 0.1-0.2% of the dopaminergic neurons in SNpc is lost annually. But this rate is accelerated in patients with PD and symptoms manifests when 70–80% of these neurons have been lost [2]. The majority of PD cases are idiopathic, but 10% of cases report with a family history of PD, and the growing number of mutations are also been associated with familial and sporadic forms of the disease [3]. PD is mainly age related [4]. In addition to genetic defects, a range of environmental and occupational factors, such as pesticides like paraquat, to more ubiquitous metals such as Mn, is been implicated as one of the risk factors in PD [5]. The mechanism by which metal ions are pathogenic may be related to their pro-oxidant, free radical-generating action and/or to their association with metalloenzymes such as super oxide dismutases [6]. Amongst all the PD cases developed from various factors, specifically Mn induced PD has gained lot of importance in the past two decades because of the escalating cases of Mn poisoning seen in the workers of different occupations like welding, mine working, dry-alkaline battery production and ferromanganese alloy production. Based on experimental animal studies, Donaldson (1987) suggested that Mn might accelerate the central nervous system (CNS) aging [7]. Mn induced neurotoxicity is influenced by brain cell types, their origins and developmental stages, as well as by the chemical speciation of Mn [8]. Hence it is mandatory to understand the molecular mechanisms by which Mn causes neurotoxicity and also to understand the factors that aggravate Mn induced neurotoxicity. The present review may help the different Mn induced neurotoxicity studies which are in pipeline and also for the future investigations.

Chemical nature of mn

Mn is the 12th most abundant element, 5th most abundant metal on earth, and 4th most mined metal. It was named from the magnetic stone in which it was first found. Mn exists in a number of physical and chemical forms in the earth's crust, in the atmosphere's particulate matter, and in water. Mn does not occur as the free metal and is found in more than 100 minerals of which the most common ones are pyrolusite (MnO_2), psilomelane ($Ba(Mn^{2+})(Mn^{4+})_8O_{16}(OH)_4$), rhodochrosite ($MnCO_3$), rhodonite ($MnSiO_3$), braunite ($3Mn_2O_3.MnSiO_3$) [9]. Mn outer electron shell can donate up to seven electrons, Mn can assume 11 different oxidation states. The environmental important states of Mn are $Mn^{2+,}$ Mn^{4+} and Mn^{7+}. In living tissue, Mn has been found as Mn^{2+}, Mn^{3+} and possibly as Mn^{4+}[10]. Mn^{5+}, Mn^{6+}, Mn^{7+} and other complexes of Mn at higher oxidation states are generally unrecognized in biological materials. Typically Mn is found in compounds with a coordination number of 6 and lacking octahedral coordination complexes. Mn tends to form very tight complexes with other substances [11]. As a result, its free plasma and tissue concentrations tend to be extremely low [12].

Importance of mn in biological homeostasis

One hundred fifty years back the biological importance was recognized [13, 14]. Mn is a vital trace mineral necessary for normal development and biological functions. It is an essential co-factor for many enzymes (Table I) which includes oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases[15]. Mn is a fundamental micronutrient, a transition metal, and a trace element that is required for normal cell growth and development and is also potentially toxic [16-18]. In humans diet consisting of Fruits, vegetables, grains, nuts, tea, and several spices are the major source of Mn [19]. Normal levels of Mn in humans range from 4.2-16.5 µg/L in blood, 0.40-0.85 µg/L in serum, and 1.0-2.0µg/g dry weight in the brain [20]. Mn concentrations are at zenith in putamen and globus pallidus. Similarly some research studies proved hippocampus having highest concentration of Mn in rats [21].

Mn acts as a cofactor for a myriad of metalloenzymes, which are involved in signal transduction as well as in DNA and neurotransmitter biosynthesis such as the mitochondrial protein superoxide-dismutase, a critical enzyme in attenuating oxidative stress[22], arginase, which is a key enzyme for biosynthesis of urea in liver, pyruvate carboxylase, an essential enzyme in gluconeogenesis[23], as well as glutamine synthetase, an astrocyte-specific enzyme that converts glutamate into glutamine [24, 25]. Another biological significance of Mn that has received relatively miniature consideration is its interaction with the cell surface integrin receptor namely, vitronectin receptor and extracellular matrix (ECM) proteins in an Arginylglycylaspartic acid (RGD; Arg/Gly/Asp)-dependent process. Integrin–ECM interactions are critical for appropriate neuronal development. Mn is important in bone formation, fat and carbohydrate metabolism, blood sugar regulation, calcium absorption, immune system functioning,

regulation of cellular energy, connective tissue growth and blood clotting . Protein kinases require the formation of an adenosine triphosphate (ATP)-divalent metal cation complex for the phosphoryl transfer of the γ-phosphate of ATP to a protein substrate [27]. Typically, Magnesium (Mg^{2+}) serves as the essential metal ion for catalysis, however Mn^{2+} and other divalent cations may support nucleotide binding and subsequent phosphoryl transfer [28]. The mounting evidence proving the nexus between Mn and PD suggests that there exist a possibility that a dysregulation of Mn homeostasis over a lifetime can play an important role in the etiology of PD [29].

Table 1: Role of Mn as a cofactor for the activity of respective metalloenzymes

Metalloenzymes	Significance of metalloenzymes mediated by Mn as a cofactor
Superoxide dismutase	Attenuating oxidative stress
Arginase	Biosynthesis of urea
Pyruvate carboxylase	Gluconeogenesis
Glutamine synthetase	Conversion of glutamate to glutamine
Endonucleases	Synthesis of deoxyribose nucleic acid (DNA)
Nucleotidases	Synthesis of DNA
Ribonucleases	Synthesis of DNA

Table 2: Summary of manifestations observed in imbalanced cellular Mn levels

Decreased Mn plasma levels
• Irreversible ataxia • Skeletal muscle abnormalities • Increased brain convulsibility • Defective mucopolysaccharide synthesis • Membrane deficits • Teratogenicity • Abnormal carbohydrate and lipid metabolism • Birth defects • Impaired fertility • Bone malformation • Weakness and susceptibility to seizures
Increased Mn plasma levels
Schizophrenic symptoms- • Compulsive and violent behaviour • Emotional instability • Hallucinations Motor impairments- • Prolonged muscle contractions(dystonia) • Decreased mucle movement (hypokinesia) • Rigidity • Muscle tremors Cognitive deficits- • Memory impairment • Reduced learning capacity, • Decreased mental flexibility • Cognitive slowing • Difficulty in visuomotor and visuospatial information processing Chronic Mn exposure include symptoms- • Tiredness • Sleep disturbances • Aggressiveness • Behavioural disturbances Nonspecific complaints- • Disorientation • Impairment of memory and judgment • Acute anxiety • Emotional lability • Compulsive behaviour • Flight of ideas • Visual hallucinations • Illusions and delusions

Cellular imbalances of mn

Decreased cellular Mn level

The decrease of Mn levels in plasma interferes with the catalytic activity of some enzymes such as galactosyltransferase an enzyme defective mucopolysaccharide synthesis and membrane deficits along with abnormal carbohydrate and lipid metabolism [31,32]. Mn deficiency can contribute to birth defects, impaired fertility, bone malformation, weakness, and enhanced susceptibility to seizures [33] (Table II).

Increased cellular Mn level

The homeostatic level of free Mn in tissue and cells is maintained by various transporters and by binding to various proteins. Despite its necessity for proper metabolic function, excessive exposure to Mn is a well-recognized occupational and environmental hazard which can

lead to an extrapyramidal syndrome, referred to as manganism [16,34]. Manganism is associated with elevated brain levels of Mn, primarily in those areas known to contain high concentrations of non-heme iron, especially the caudate, putamen, globus pallidus, SNpc, and subthalamic nuclei [34]. Exposure to Mn results in its increased cellular concentration which results in systemic toxicity. Even the cases of Mn acute exposure results in hyperactivity accompanied by elevated brain levels of catecholamines and their metabolites, which are also one of the the characteristic features of schizophrenia a psychiatric disorder [35]. Symptoms include compulsive and violent behaviour, emotional instability and hallucinations. Upon chronic exposure, the disease progresses and the patients may develop prolonged muscle contractions (dystonia), decreased muscle movement (hypokinesia), rigidity, and muscle tremors [36]. Neurotoxicity of Mn is the limelight of the present study.

Clinically, manganism is a CNS disease which is manifested following exposure to high concentrations of Mn oxides is first described in the 19th century [37]. Despite being essential, Mn is known to be a neurotoxicant for about 150 years [38]. Mn neurotoxicity was first identified as an extra-pyramidal syndrome in miners exposed to high concentrations of Mn ore [39,40,41,42]. Mn toxicity is also characterized by cognitive deficits such as memory impairment, reduced learning capacity, decreased mental flexibility, cognitive slowing [43] and difficulty with visuomotor and visuospatial information processing [44].

Nonspecific complaints are followed by signs and symptoms of organic psychosis including disorientation, impairment of memory and judgment, acute anxiety, emotional lability, compulsive behavior, flight of ideas, visual hallucinations, illusions and delusions. Psychomotor slowing and cognitive decline evolve later. This is usually followed by disturbances of gait and excessive salivation, which are the first manifestations of a movement disorder, an extrapyramidal syndrome clinically resembling PD [45,46], with peculiar neurological features [47].

Typical symptoms of chronic Mn exposure include tiredness, sleep disturbances, aggressiveness, and behavioral disturbances (manganese madness). The medical term, locura manganica or manganese madness, was coined to characterize this initial neuropsychiatric syndrome in miners of Mn ores in Chile, Australia and Taiwan [48]. Delayed neurological disturbances encompass the extrapyramidal system, characterized by walking difficulties, dystonia, and kinesia resembling many clinical features of PD [42]. Mining, steel manufacturing, and welding represent occupational exposures that are associated with Mn exposures to high Mn levels [49,50,51]. An organic Mn compound, methylcyclopentadienyl manganese tricarbonyl (MMT), used as an octane booster or anti-knock agent in gasoline, has also been proven to cause adverse health effects in humans [52,53].

Exposure to Mn from mining, working in Mn metal and alloy smelters, dry-cell battery production, working with fertilizers and fungicides containing Mn, and welding are examples of industrial work which may cause damage to the CNS and peripheral nervous system (PNS) which can be irreversible and progressive [54]. Despite the similarities in extrapyramidal symptoms between Mn neurotoxicity and IPD (idiopathic PD), numerous reports suggest that the sites of Mn-induced neurological lesions are fundamentally different from those observed in IPD. For instance, the primary targeted brain regions in Mn intoxication are the globus pallidus and striatum of the basal ganglia, whereas the neuro-degeneration in IPD takes place mainly in the substantia nigra [55,56,57,58,59]. Chronic Mn intoxication has been known as an important cause of secondary Parkinsonism since the report of Couper in 1837 [41]. Bilateral neuronal degeneration in the globus pallidus along with a marked decrease of myelinated fibres and mild-to-moderate proliferation of astrocytes are common features observed post-mortem [59,60].

Manganism- a historical background

The Mn was recognized as an element in 1774 by the Swedish chemist, Carl Wilhelm Scheele and isolated by Johan Gottlieb Gahn coevally [61]. Until the 1960s, most manganism cases were occupational and were diagnosed in miners. Later on, cases of manganism were found in workers engaged in the ferromanganese-alloy industry and the manufacturing of dry-cell batteries [62]. During the last three decades, welding has come gradually into focus as a high-risk occupational factor for the development of manganism [43,63,64,65]. Recent studies suggest that high levels of Mn in drinking water (>300 mg/L) is associated with reduced intellectual function in children [66,67].

Similarities and differences between manganism and pd

It should be noted that there are both similarities and differences between manganism and PD. Among the similarities, there are generalized bradykinesia and widespread rigidity. On the other hand, manganism differs in notably less frequent resting tremor, more frequent dystonia, a particular propensity to fall backwards and a failure to achieve a prolonged therapeutic response to levodopa. Moreover, PD is characterized by degeneration of the nigrostriatal pathway. Neuronal loss in PD is most significant in the SNpc, while the histopathological changes in human manganism are located in the globus pallidus, where neuronal loss and gliosis occur; the damage extends less prominently to the caudate nucleus and the putamen. The most important among these differences is the lack of clinical response to levodopa in manganism [55,57].

disturbances in the cellular mechanisms by mn leading to pd

Elevated Mn levels in the brain have been associated with impairment in iron homeostasis, cellular excitotoxicity, mitochondrial dysfunction, oxidative stress, induction of protein aggregation, and alteration in the homeostatic conditions of other divalent metals that share similar transporter systems with Mn. Mn-induced neuronal damage is caused by elevated intracellular calcium ion and altering expression of N-Methyl-D-aspartic acid (NMDA) receptors (NRs) subunit messenger ribo-nucliec acids (mRNAs) and proteins. These results give insight into the neurochemical alterations that take place in Mn-treated neurons [68]. Mn exposure causes up regulation of α- synuclein and down regulation of Tyrosine hydroxylase (TH) and Parkin (PARK2) in dopaminergic neurons, resulting in increased oxidative stress and eventual cell death [69].

Oxidative stress

Free radicals generation during cellular metabolism seems to be the main cause of molecular damage underlying aging. Induced stress enhances this process, because the deleterious effects of free radicals are progressive and accumulative [70,71]. Induced stress also increases lipid peroxidation that is usually considered as an estimator of oxidative stress. Oxidative stress has been hinted as a critical feature in Mn neurotoxicity; indeed, the mechanism seems to be related to the ability of the metal to enhance the formation of reactive oxygen species (ROS's) and oxidation by-products of catecholamines [72,73]. Mitochondria are an important cellular source of ROS and are highly susceptible to oxidative damage. Under resting conditions, between 2.0% and 5.0% of molecular oxygen consumed by mitochondria is partially reduced by the electron transport chain (ETC) to form superoxide (O_2^{-}) and subsequently hydrogen peroxide (H_2O_2). Elevated mitochondrial-ROS generation can inhibit one or more of the components of the respiratory chain, further accelerating the rate of O_2^{-} Formation [74]. ultimately leading to cell death [75]. Interestingly, Mn preferentially localizes within these organelles [76,77], reflecting its high affinity for the calcium uniporter [78,79]. Adenosine triphosphatase (ATPase) complex is inhibited at very low levels of mitochondrial Mn, and that complex I is inhibited only at higher concentrations [79]. Although, trivalent Mn is more effective at inhibiting complex I[10,80,81] the divalent form is by far the predominant species within cells and is largely bound to ATP [80]. Moreover, O_2^{-} produced in the mitochondrial ETC catalyzes the transition shift of Mn^{2+} to Mn^{3+} through a set of reactions analogous to those mediated by SOD, resulting in increased oxidant capacity of Mn [82,83].

Oxidative stress has been implicated as a contributing mechanism by which Mn may be cytotoxic [83]. The oxidation of dopamine DA by Mn is a potential mechanism by which Mn-induced oxidative stress may occur, especially since Mn can accumulate in DA-rich brain

regions of rodents and primates (for example, basal ganglia) following prolonged exposure [84]. Another possible mechanism is that, through its sequestration in mitochondria [85], interferes with proper respiration, thereby leading to excessive production of ROS's. Adams et al in 1991 investigated that Mn increases uric acid levels in the striatum and in the brainstem of the rat [86]. The products of xanthine oxidase activity on hypoxanthine and xanthine include uric acid and ROS. It is postulated that DA auto-oxidation following Mn exposure activates intracellular signaling cascades, particularly nitric oxide synthase (NOS), through oxidative stress-mediated Necrotic factor-κB (NF- κB) activation and thus potentiates dopaminergic toxicity [87].

On the other hand, in recent years several research studies have revealed that stress can interact with certain chemicals increasing their potential toxicity, especially when they are administered at high doses [88,89,90,91,92,93,94]. The effects of stress are mainly mediated by glucocorticoids, which have paradoxical functions in the brain. While basal levels of glucocorticoids are essential for neuronal development, plasticity and survival, stress levels can increase the vulnerability of neurons to metabolic insults, potentially by altering the neuronal defense capacity against oxidative damage [95,96].

Mn on dopaminergic action

Mn toxicity triggers enhanced auto-oxidation of DA by Mn^{3+} which efficiently oxidizes as compared to Mn^{2+}. It has also been suggested that the oxidation state of Mn specifically Mn^{3+} is an important factor that contributes to its cytotoxicity [10,72,98,99]. The deficiency of striatal DA is generally regarded as expression of Mn toxicity in experimental animals [100]. Mn is apparently taken up by axonic transport to the γ-amino butyric acid (GABA) *i.e.,* GABAnergic and dopaminergic synaptic terminals in the circuit between the substantia nigra and striatum, which suggests a relationship between Mn and the release of these neurotransmitters [101]. Repeated Mn exposure has long-term effects on the regulation of exocytotic DA release in the striatum, which may be involved in the mechanism underlying Mn toxicity [102], Stanwood et al (2009) observed 20% reduction in TH positive neurons in the SNpc following Mn treatment and concluded that acute Mn exposure induces cytoskeleton dysfunction prior to degeneration and that chronic Mn exposure results in neurochemical dysfunction with overlapping features to PD [103]. The mechanism that Mn stimulates DA autoxidation in the dopaminergic neurons, a process accompanied by an increase of quinones [104,105] and that of protein-bound cysteinyl DA, and cysteinyl dihydroxyphenylacetic acid (DOPAC) is similar to that observed in PD. Interestingly, in the treatments of PD, the therapeutic manipulation of DA concentration is often used by replacing DA primarily with the DA precursor levodopa [106].

Activation of apoptosis implications by Mn in the neurodegenerative diseases

Apoptosis is a phenomenon that has a crucial role in various physiological and pathological processes. Activation of apoptosis may be implicated in neurodegenerative disorders including PD. Studies have proven that both Mn and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogue 1-methyl-4-(2-ethylphenyl)-1,2,3, 6-tetra-hydropyridine (2Et-MPTP), which is metabolized by Monoamine oxidase-A (MAO-A) to 1-methyl-4-(2-ethylphenyl)-pyridinium ion induce apoptosis and decreases cell viability in *in-vitro* studies with pheochromocytoma-12 (PC12) cells [107]. Only Mn induced apoptosis and decrease in cell viability was inhibited by the antioxidant ascorbic acid (AA). A study revealed that the antioxidant AA and N-acetylcystein (NAC) strongly inhibited both apoptosis and decrease in cell viability induced by Mn [108]. The mechanism by which Mn aggravates cell death is slightly controversial; although it is likely that both apoptosis and necrosis contribute to the cytotoxic process [109,110].

Inhibitory effect of Mn on acetylcholine-esterase (AChE) activity

The inhibition of AChE prevents the hydrolysis of acetylcholine (ACh), leading to accumulation of ACh in the synaptic cleft and overstimulation of muscarinic and nicotinic ACh receptors. Decreased activity of AChE, the enzyme responsible for ACh hydrolysis, was shown to be associated with increased oxidative and nitrosative stress [111]. Significant inhibition of AChE activity was observed following prolonged exposure to Mn in adult [112,113] and neonatal rat brain [114,115]. The excessive Mn exposure is associated with behavioural changes and altered cognitive function. Mn induced cortical cholinergic dysfunction is compatible with these cognitive deficits, as well as with dementia observed later on in the clinical course of manganism [116,117,118].

Mn in Mitochondrial dysfunction

As discussed earlier, since Mn preferentially accumulates in the mitochondria of basal ganglia, it has been suggested that the mitochondria are target organelles for Mn toxicity [119,120] .Intracellular Mn^{2+} is sequestered in the mitochondria of the brain and liver via the Ca^{2+} uni-porter [76,121]. Mitochondria are the primary pool of Mn in the cell; however, nuclei have also been implied to preferentially accumulate this metal [79,122,123]. The efflux of mitochondrial Mn^{2+} is mediated preferentially through an active but slow Na^{+}-independent mechanism; a Na^{+}-dependent mechanism also contributes minimally. This slow Mn efflux has been suggested to account for the net accumulation of Mn in mitochondria. Mitochondrial dysfunction is the foremost perpetrator of the nigrostriatal dopaminergic neuro-degeneration leading to PD [4,124].

Mn alone disturbs mitochondrial respiration and inhibits the antioxidant system, thus consequently straining cell ability to combat oxidative stress [125]. ROS production in cells exposed to Mn is attributed to indirect consequence of the toxic actions of Mn on mitochondrial function since a number of mitochondria-disrupting agents have been shown to promote formation of ROS [126,127,128]. Mn inhibits mitochondrial aconitase activity and disrupts mitochondrial energy production in brain [129]. By inhibiting both the sodium-dependent and sodium-independent exporter, Mn promotes calcium accumulation within the mitochondria, thus activating the permeability transition pore, which ultimately leads to disruption of mitochondrial function. Brain tissues/cells are dependent on glucose-derived oxidative metabolism not only for energy needs, but also, for the synthesis of tricarboxylic acid (TCA) cycle-related neurotransmitters, such as glutamate, aspartate, GABA, and Ach [130].

Inhibition of ATP synthesis is one of the proposed mechanisms of the Mn neurotoxicity. This Mn-induced impairment in energy metabolism could be attributed to decrease in the activities of enzymes of glucose oxidative metabolism. Enzymes located in the mitochondrial matrix are known to be vulnerable to Mn toxicity and inhibition of the TCA cycle enzyme, aconitase, has been suggested as one of the mechanisms underlying Mn neurotoxicity [129].

It was also demonstrated that Mn can directly inhibit α-ketoglutarate dehydrogenase complex activities in both liver and brain mitochondria and induce a dose-related lowering of the activities of two other TCA cycle enzymes, namely, citrate synthase and malate dehydrogenase. Thus, these studies show that, Mn exerts inhibitory effects on TCA cycle [131] and on oxidative phosphorylation [132]. Neuro-degeneration in the striatum may be linked to a cascade of oxidative damage related to the ease with which Mn can be readily oxidized from the Mn^{2+} to the Mn^{3+} oxidation state [10,82]. Some studies also proven that Mn disrupts calcium homeostasis and other mitochondrial functions [78,119,133].

Anaemia- a risk factor for Mn neurotoxicity

Iron is must for the normal oxidative metabolism within the cell, with this maintenance of homeostatic concentrations of iron is under control at both the systemic and cellular levels. The globus pallidus along with numerous other areas of the brain are normally enriched with Mn and iron. The co-accumulation of iron along with Mn in the globus pallidus raises the concern that iron may be a contributing factor facilitating neuronal cell loss during Mn intoxication. Iron is capable of generating ROS via the Fenton reaction, leading to oxidative stress, lipid peroxidation, and finally cell death. The divalent metals are transported by the same transporter as that utilized by iron. As a consequence, cellular levels of proteins involved in divalent metal

uptake, storage, and release are dependent mainly upon iron status. The importance of iron in regulating overall oxidative events and energy production within the cell, it is not surprising that iron homeostasis is mediated by a number of processes that also affect divalent metal disposition. In the condition of iron deficiency, increased expression of transferrin (Tf), transferrin receptors (TfR), are expected, whereas ferritin (Ft) and metal transport protein1 (MTP-1), levels will decrease [30]. MTP1, is the duodenal enterocyte basolateral iron exporter, is also expressed in the cells of the reticuloendothelial system (RES) and is likely to be involved in iron recycling of these cells [134]. The intestinal absorption and cellular uptake of Mn occurs via the same transport systems as that of the iron [102,135]. Iron deficiency and iron chelating agents can potentially increase or decrease expression of these proteins, depending on their function [110,136,137]. Earlier studies by Aschner's group [138,139] suggest that iron homeostasis may play an important role in the regulation of Mn transport across the BBB.

Mn exposure by oral administration increased the binding of iron regulated protein-1 (IRP-1) to iron responsive element (IRE) containing RNAs encoding Ft in the brain-cerebrospinal barrier (BCB) and selected regional BBB, particularly in striatum, as well as in brain parenchyma. The ensuing increase in the expression of TfR and decrease in Ft in those brain regions may facilitate the influx of iron into the brain compartment. Interference of iron transport by Mn may contribute to Mn-induced neurotoxicity [140]. It has been suggested that Mn and iron transport across membranes is mediated by the same carrier [141]. The research study investigated that, the divalent metal transporter-1 (DMT-1) defective rat reticulocytes display decreased Tf-bound iron uptake [142]. These observations strongly suggest an intimate role of DMT-1 in iron uptake via TfR - mediated endocytosis. DMT-1 and the TfR co-localize in the vascular endothelial cells that make up the BBB [143]. DMT-1 is also expressed by the glial end feet that surround subcortical blood vessels, suggesting it plays a role in iron uptake from endothelial cells into brain. The absence of DMT-1 activity at the BBB would be expected to reduce brain uptake of Tf-bound iron and presumably other divalent cations [144].

Accordingly, there is an opposite relationship between the status of iron and uptake of Mn in that iron deficiency will lead to increased Mn levels in the body. Several research studies revealed that iron status has a direct effect on the levels of Mn within the body [145,146]. Both Mn and iron are transition elements adjacent to each other in the Periodic Table, and they share similar valence charges and ionic radius. These chemical similarities make Mn to compete directly with iron at the molecular level by interacting with proteins and enzymes that require iron as a cofactor in their active catalytic center, such as mitochondrial Complex I [81], aconitase [129], and IRP-1. Early studies show that Mn competes with iron for the same binding site in the active center of IRP-1.The altered binding capacity between IRP-1 and the stem-loop containing

mRNAs that encode various iron-transport and storage proteins may cause a compartmental shift of iron from the blood to the CSF, resulting in an iron deficient status in the blood compartment [129,147].

The study by Wei, et al (1999), concluded that the chronic Mn exposure alters iron homeostasis in systemic circulation by depressing plasma iron and in cerebral compartment by elevating CSF iron. Overexpression of TfRs in the choroid plexus by Mn treatment suggests that Mn, by acting on IRP1, may promote the expression of TfR, which partly facilitates the influx of iron from blood to CSF. This infers that Mn-induced Parkinsonism may result from a compartmental shift of iron into the brain, leading to oxidative stress in sensitive brain regions [148].

Toxicokinetics of mn

The routes of Mn exposure are mainly through dietary intake, dermal absorption, and inhalation [33]. The main route of Mn absorption is the gastrointestinal tract, but absorption also occurs via the lung [98]. A study has shown that after an intratracheal instillation, which is a surrogate of inhalation exposure, the Mn concentrations were higher in brain following the administration of the soluble salt $MnCl_2$, than following the administration of the insoluble oxide (MnO_2) [149].

Mn is essential for neuronal development and function. It penetrates cerebral extracellular liquid in two forms: Mn^{2+} and Mn^{3+}. There, especially as Mn^{3+}, it may combine with Tf secreted by oligodendrocytes, and can be found in bound or nonbound form. Nonbound Mn can be taken up by the choroid plexus cells and the cerebral parenchyma. It has been proposed that neurons can obtain Tf-bound Mn by an endocytotic mechanism mediated by receptors; it is subsequently sequestered in synaptic vesicles [101,150].

Mn is primarily absorbed as Mn^{2+} and upon absorption some Mn^{2+} is oxidized to more reactive Mn^{3+}, which can bind to Tf in a stable complex. Several forms of Mn^{2+} are found in serum, with 84.0% as an albumin-bound species, 6.4% as a hydrated ion, 5.8% as bicarbonate complexes, 2.0% as citrate, and 1.8% as small molecular weight ligands [151,152,153]. The Mn^{3+} instead is almost totally bound to Tf in serum. From blood plasma Mn can enter the brain by crossing the cells of the BBB or through the choroid plexuses [154,155]. Mn is actively transported across cell membranes by several types of cells, such as biliaric cells and astrocytes.

Repeated excessive Mn exposure may result in brain Mn accumulation, due to different mechanisms regulating Mn kinetics at the BBB that could possibly favour a more rapid influx and a slower efflux from the brain [156]. The highest quantity of Mn, after crossing BBB, gets accumulates in pallidum, thalamic nuclei and substantia nigra [157] Generally, Mn is regulated by its uptake rate by the enterocytes within the intestinal lining and by its overall hepatic elimination in bile. At the cellular level, maintenance of normal Mn balance is regulated by a cascade of events involving its cellular uptake, retention, and release [30,158]. The pulmonary route is the main gate way of Mn into the body. Although the brain has the capability of retaining it for longer periods of time than in other organs, probably due to its difficulty in eliminating the excess of this metal [159]. After intestinal absorption, Mn is transported to the liver via the hepatic portal vein, where oxidation to Mn^{2+} and Mn^{3+} takes place; these forms pass across the BBB into the brain. It is anticipated that Mn transport at the BBB maintains a healthy brain Mn concentration [101].

Mn induced neurotoxicity

An experimental model

Since the report of Couper in 1837, chronic Mn intoxication has been known as an important cause of secondary Parkinsonism [41].

An adequate PD animal model should have some typical features, they are, a normal set of nigrostriatal dopaminergic neurons at birth followed by a selective gradual loss of these cells beginning in adulthood, easily detectable and quantifiable motor deficits, generation of lewy bodies, and the model should have a relatively short time course to mimic the pathogenesis of PD (about 3–6 months) which would allow a rapid screening of therapeutic substances and strategies [160]. When choosing an animal model for IPD, it must be consider the degree of similarity or difference between the anatomy, physiology and behaviour between humans and animals. The existing models have been useful for understanding the aetiology of the disease and offer resources for proving novel treatments [161]. However, the loss of the nigrostriatal dopaminergic pathway that has been replicated in animals, either unilaterally or bilaterally, using a variety of selective toxins or by genetic manipulations, is rapid and not progressive, and for those derived through genetic manipulations relevant to human PD, the loss, although more progressive, may be limited in extent or may not even occur at all [162,163]. The effects of Mn as a PD model have been investigated due to the fact that its toxicity (referred to as manganism) shares neurological symptoms with several clinical disorders commonly described as ''extrapyramidal motor system dysfunction'', and in particular, IPD [50,55]. The mode of exposure, duration, severity and route of administration of Mn into the body, and intermittency of exposure remains to be defined. The mode of exposure is probably an important determinant of symptom development. High dose acute intoxication may produce different

symptoms than chronic low dose exposure. Cumulative exposure may be an important aspect of disease development. It is also unclear what minimal duration of chronic exposure is necessary to cause symptoms [164].

Experimental animal models

The effect of Mn varies with experimental conditions, form of Mn, route of administration, length of exposure [165] and age [100,166]. Inhalation of $MnCl_2$ and manganese acetate ($Mn(OAc)_3$) mixture produces similar morphological, neurochemical and behavioural alterations to those observed in PD, suggesting a useful experimental model for the study of this neurodegenerative disease [167]. Rats were placed in an acrylic chamber inhaling 0.04M $MnCl_2$ and 0.02M $Mn(OAc)_3$ one hour three times a week for six months [168]. $MnCl_2$ (100 mg/kg) was given twice daily by gastric gavage for 7 consecutive days. The last half daily dose was given on the 8^{th} day, one hour before sacrifice. All the $MnCl_2$ doses were given in 5 ml/kg of distilled water [100]. *In-vivo* Mn exposure via intraperitoneal injection expedites unidirectional influx of iron from the systemic circulation to cerebral compartment [147]. The route of administration can influence the distribution, metabolism, and potential for neurotoxicity of Mn-containing compounds [169]. Pharmacokinetic factors that may contribute to the increased efficiency of brain Mn delivery following inhalation include greater Mn absorption from the lungs and slower clearance of absorbed Mn from the circulation [170]. Mn concentrations were higher in brain following the administration of the soluble salt $MnCl_2$, than following the administration of the insoluble MnO_2 [169].

Advantages of the model

Mn induced PD is progressive and bilateral, which makes it more reliable. The Mn induced models have been useful for understanding the etiology of the disease and offer resources for proving new treatments [161,168].

Drawbacks of the model

Despite the similarities in extrapyramidal symptoms between Mn neurotoxicity and IPD, numerous reports suggest that the sites of Mn-induced neurological lesions are fundamentally different from those observed in IPD. Human manganism at the morphological level is characterized by neuronal loss and reactive gliosis in the globus pallidus and SNpc without lewy bodies, the intra-neuronal protein aggregates that distinguish PD. Though rarely reported, damage to the striatum (caudate nucleus and putamen) and subthalamic nucleus may occur, while the SNpc is less likely to be affected [55,56,57,58,59]. In contrast, IPD is predominantly characterized by neuronal loss in the SNpc [33]. As in early stages of PD, the presence of an intact, functioning sub portion of the nigrostriatal system could allow l-DOPA treatment to be efficient.

The study suggest that the motor alterations induced by the inhalation of the combination of $MnCl_2$/ $Mn(OAc)_3$ are related to nigrostriatal dopaminergic function, providing new insight in understanding Mn induced neurotoxicity as a suitable PD experimental model. Furthermore, these findings may have particular significance to the role of chronic overexposure to the mixture of Mn compounds in the pathogenesis of PD [171]. The most important among these differences is the lack of clinical response to levodopa in manganism [55,57].

The influence of factors such as exposure duration and severity, route of administration of Mn into the body, and intermittency of exposure remain to be defined. The mode of exposure is probably an important determinant of type of symptoms. High dose acute intoxication may produce different symptoms than chronic low dose exposure. It is also unclear what minimal duration of chronic exposure is necessary to cause symptoms [164].

Need to address the neurotoxic setback of mn

Mn neurotoxicity is becoming of great concern because of several factors. Its industrial use will further increase in view of new technological applications both in the metallurgic as well as chemical sectors. The Mn-based pesticides are extensively used, both in industrialized and in developing countries. In addition, the adoption of an organometallic compound such as methyl-cyclopentadienyl manganese tricarbonyl (MMT), Cl^{-2} or antiknock 33X (Ak-33X) as a lead substitute in gasoline could become an additional source of environmental exposure [172]. Mn is labeled by WHO as an environmental toxic factor that induces brain dysfunction more frequently by industrial accidents. The globus pallidus may be more vulnerable than other brain regions to chronic Mn treatment, due to its constitutive low levels of Copper/Zinc-superoxide dismutase (Cu/Zn-SOD) [173].

CONCLUSION

Despite the intensive research work carried out on Mn, the efficiency and reliability of Mn induced PD model is still under scientific debate. Because, the region involved in the neurodegeneration is basal ganglia where as in case of IPD it is SNpc. On the brighter side, the model offers an advantage over other traditional methods like MPTP and 6-OHDA induced models as Mn induced PD is progressive which happens in IPD and not rapid.

But the different cellular mechanisms involved in the Mn induced neurotoxicity are similar to that of MPTP and 6-OHDA induced neurotoxicity.

Apart from regulating motor functions, basal ganglia also influences many of the cortical functions like sensory, limbic, and cognitive. As Mn neurotoxicity is resulting in many of the cognitive and behavioral deficits, there must be parallel involvement of both basal ganglia and some parts of cerebral cortex.

Therefore longitudinal studies that examine the underlying molecular disturbances caused by Mn neurotoxicity in both basal ganglia and cerebral cortex is required to establish Mn induced PD Model as a standard model for PD's.

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