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Neuroprotective and Therapeutic Strategies for Manganese–Induced Neurotoxicity

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Abstract

Manganese (Mn) is an essential element required for growth, development and general maintenance of health. However, chronic or high occupational and environmental exposure to excessive levels of Mn has long been known to lead to a progressive neurological disorder similar to Parkinsonism. Manganism patients display a variety of symptoms, including mental, cognitive and behavioural impediments, as well as motor dysfunctions that are associated with basal ganglia dysfunction.

Taking into account the pharmacokinetics and Mn-related toxicity mechanisms, several neuroprotective compounds and therapeutic approaches have been investigated to assess their efficacy in mitigating its neurotoxicity. Here, we will briefly address some of the toxic mechanisms of Mn, followed by neuroprotective strategies and therapeutic approaches aiming to reduce or treat Mn induced neurotoxicity.

Natural and synthetic antioxidants, anti-inflammatory compounds, ATP/ADP ratio protectors and glutamate protectors have been introduced in view of decreasing Mn-induced neurotoxicity. In addition, the efficacy and mechanisms of several therapeutic interventions such as levodopa, ethylene-diamine-tetraacetic acid (EDTA) and para-aminosalicylic acid (PAS), aimed at ameliorating Mn neurotoxic symptoms in humans, will be reviewed.

Keywords

Manganese; Mechanisms of neurotoxicity; Neuroprotective strategies; Therapeutic intervention

Manganese Essentiality and Toxicity

Manganese (Mn) is an essential element required for growth, development and general maintenance of health. In the brain, Mn is an important cofactor for a variety of enzymes, including the antioxidant enzyme superoxide dismutase (SOD), as well as enzymes involved in neurotransmitter synthesis and metabolism [1]. However, with the widespread use of the Mn derivative methylcyclopentadienyl manganese tricarbonyl (MMT), an antiknock

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gasoline agent, a potential health risk may result from increased environmental Mn levels [2]. Indeed, chronic or high occupational or environmental exposures to excess levels of Mn have long been known to lead to a progressive neurological disorder similar to Parkinsonism. This disorder, referred to as manganism, is characterized by excessive Mn accumulation in multiple brain regions, primarily in the basal ganglia [3]. Patients with manganism display a variety of symptoms, including mental, cognitive and behavioural impediments, as well as motor disorders [1, 4]. The motor and behavioural effects of Mn toxicity are considered irreversible, and there is evidence that they can progress even after chronic exposure has ended [3]. Taking into account the pharmacokinetics and Mn-related toxicity mechanisms, several neuroprotective compounds and therapeutic approaches have been investigated to assess their efficacy in mitigating its neurotoxicity. Here we will briefly address some of the toxic mechanisms of Mn, followed by neuroprotective strategies and therapeutic approaches that aim to reduce or treat Mn-induced neurotoxicity.

Manganese Toxicity Mechanisms

Oxidative stress

Oxidative stress (OS), characterized by the generation of reactive oxygen species (ROS), is a convergence point of several other mechanisms of Mn toxicity and also a condition that by itself, leads to neuronal damage [5]. Mitochondria are intracellular target for Mn toxicity [6], where Mn^{2+} can be oxidized to Mn^{3+} becoming a potent pro-oxidizing agent for cellular components [7]. The ensuing oxidation of membrane associated polyunsaturated fatty acid generates lipid peroxides (LPO), affects mitochondrial permeability and triggers cell apoptosis [5, 8].

Neuroinflammation

Another relevant mechanism of Mn neurotoxicity is mediated by inflammatory responses. Though inflammation represents the first line of the cells´ defence against injury, it can also be a source of damage to host cells [9]. Prostaglandins are lipid autacoids involved in the inflammatory response, which are derived from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes [10]. COX is an important source of ROS in the pathologic brain inducing superoxide-mediated cell death and providing a primary source of free radicals with the capacity to modify proteins, lipids and nucleic acids [11, 12]. Enhanced COX activity may deplete the levels of reductive antioxidants, such as glutathione (GSH), increasing the potential for cytotoxic insult and increasing the neurotoxic effects of low doses of ferrous iron (Fe^{2+}) and hydrogen peroxide (H_2O_2). Additionally, inflammation in conjunction with Mn-induced over-activation of glia and release of additional neurotoxic factors may represent crucial components that trigger dopaminergic (DAergic) neurodegeneration [13].

Interference with iron

Mn can also increase cellular uptake of other metals, such as iron (Fe), which in excess may trigger ROS generation [5]. The transfer from Fe^{2+} to the ferric form (Fe^{3+}) in a catalytic reaction with H_2O_2 (or molecular oxygen) known as the Fenton reaction, can yield the highly toxic hydroxyl radical (•OH) via the Haber Weiss reaction [14]. Although most of the

total Fe in healthy brains is stored in ferritin, and levels are typically depleted under inflammatory conditions, ferric ions are readily released after damage to neuronal tissues by yet unknown mechanisms, making those ions available for oxidative catalysis [12].

Disruption of oxidative phosphorylation /ATP decrease

Additionally, Mn toxicity is partially mediated by reduced ATP production since inside mitochondria the metal can inhibit complexes I, II, III and IV of the electron transport chain (ETC), disrupting oxidative phosphorylation [3, 15]. When ATP levels decline, a failure of ion-motive ATPase may occur leading to membrane depolarization, uncontrolled divalent calcium (Ca^{2+}) influx through voltagegated Ca^{2+} channels, and subsequent activation of Ca^{2+}-dependent phospholipases and proteases [16]. Concomitantly there is an increased superoxide anion production via mitochondrial ETC that can catalyse the transition shift of Mn^{2+}–Mn^{3+} leading to an increased oxidant capacity of this metal [17].

Changes in intracellular signalling pathways

Numerous signalling pathways associated with programmed cell death, including c-Jun N-terminal kinases (JNK) and caspases, are known to be activated upon *in vitro* Mn treatments. Indeed, the JNK pathways are activated by cytokines or cytotoxic insults and are often related to stress and cell death [18]; further, caspases play important roles in regulatory networks controlling inflammation and cell death [19]. In addition, Mn-induced neurodegeneration is modulated by the activation of several transcription factors, such as the expression of the human nuclear factor erythroid-derived 2-like 2 (NRF2), which is involved in mitochondrial biogenesis and anti-oxidant responses [20, 21].

Interference with neurotransmission

The ability of Mn to interfere with several neurotransmitter systems has been broadly described. Due to the similarities between Mn neurotoxicity and Parkinson's disease (PD), most research in the area of Mn neurotoxicity has focused on its effect on the biology of DA [22]. In fact, Mn is a potent DA oxidant, leading to the generation of DA quinone products followed by DA depletion. The resulting quinone can initiate superoxide radical formation by the reduction to the semiquinone through NADH or NADPH-dependent flavoproteins, which is then readily oxidized by molecular oxygen to form superoxide radicals [15].

Yet other neurotransmitter systems can be affected by Mn as well, leading to excitotoxicity caused by altered glutamate (Glu) transportation and metabolism. Commonly, Glu is taken up from the synapse by glutamate transporters to astrocytes, where Glu is converted by glutamine synthesis to glutamine (Gln), which can be excreted from astrocytes, be taken up by neurons and converted again to Glu [23]. Mn exposure impairs Glu re-uptake in brain [24], and triggers excitatory neurotoxicity [8]. Excess of extracellular Glu can lead to overexcitation of the glutamate receptor N-methyl-D-aspartate (NMDAR), causing Ca^{2+} overload and cytotoxicity [23]. Furthermore, Mn disinhibition of Glu output to the substantia nigra may result in chronic over-stimulation of DAergic neurons and exacerbation of DA neurotransmission, associated with ROS generation [25].

Mn can also perturb cholinergic mechanisms blocking the release of acetylcholine (Ach) at presynaptic levels. Short- and longterm exposure to Mn can change acetylcholinesterase (AChE) activity: a select number of studies corroborate increased activity of the enzyme [26], while others point to a decrease [27]. Mn-induced AChE targeting may trigger or contribute to ROS generation since Ach plays a neuroprotective role by scavenging superoxide anions [26]. Additionally, AChE inhibition in the rats brain may disrupt the delicate balance between ACh and DA in the cholinergic-DAergic system, where ACh is thought to act directly on DAergic terminals [27, 28].

Gama-Aminobutyric acid (GABA) is another neurotransmitter whose levels are also altered upon exposure to Mn. A 16% increase in striatal GABA concentrations resulting from cumulative low-dose Mn exposure is reported, while other studies showed a significant increase in tissue levels of GABA in rats exposed to a high Mn diet [29]. Actually, the globus pallidus which is a brain region where Mn accumulates contains abundant GABA projections [25, 30]. Notably, being GABA synthesized from Gln by glutamic acid decarboxylase, Mn-induced neurotoxicity was found to be associated with interruption in the Gln/Glu-GABA cycle between astrocytes and neurons [31]. Moreover, cortical Glu afferents project into the striatum where in concert with GABA and DA they regulate motor behaviours [29], which have been shown to be altered upon Mn exposure [32].

Thus, several neuroprotective strategies and therapeutic approaches were investigated aiming experimentally afford protection and as a final objective, contribute to an efficient treatment against Mninduced neurotoxicity.

Neuroprotective Strategies for Mn-Induced Neurotoxicity

Antioxidants

Natural compounds—OS plays a central role in Mn-induced neurotoxicity and therefore the potential neuroprotective effect of several antioxidants has been investigated. Thus far, most of the investigated natural antioxidants are polyphenolic compounds. Polyphenols are a structural class of mainly natural (despite some synthetic or semisynthetic also exist) organic chemicals characterized by the presence of large multiples phenol structural units [33]. Although these compounds can interact with cell signalling pathways, chelate metal ions and have anti-inflammatory effects, the pharmacological actions of polyphenols have been mainly attributed to their antioxidant activity. Recently, it has been shown that polyphenols can exert their antioxidant actions by regulating the transcription of antioxidant enzyme genes [34]. Interestingly it is also achieved that the biological properties of dietary polyphenols are greatly dependent on their bioavailability that, in turn, is largely influenced by their degree of polymerization. The gut microbiota plays a key role in modulating the production, bioavailability and, thus, the biological activities of phenolic metabolites, particularly after the intake of food containing high-molecular-weight polyphenols. In addition, evidence is emerging supportive of the dietary polyphenols on the modulation of the colonic microbial population composition or activity [35]. Additionally, it should be noted that dependent upon the chemical context some polyphenols might also possess prooxidative properties [36].

A study with a polyphenolic extract of a Korean prostrate spurge Euphorbia supina (PPEES) showed that PPEES could effectively inhibit Mn-induced neurotoxicity through antioxidant properties via regulation of endoplasmatic reticulum (ER) stress and ER stressmediated apoptosis. Significantly reduced levels of ROS and malondialdehyde (MDA), which are products of lipid peroxidation, were also observed. Concomitantly, the antioxidants GSH and SOD and catalase (CAT) activities, were increased. *In vivo* work showed also improvement of Mn-induced histopathological alterations in striatum and cerebral cortex by PPEES [37].

Flavonoids are most abundant polyphenols in human diet being quercetin included among the most representative natural polyphenolic antioxidants [38]. Quercetin inhibits excessive free radicals production and is also anti-inflammatory. Its administration has been shown to mitigate the adverse effects of Mn on the histology and functions of the rat´s brain, with inhibition of lipid peroxidation, inflammation and caspase-3 activity, being the latter a crucial mediator of programmed cell death [39, 40]. Resveratrol (3, 5, 4-trihydroxystilbene) is produced in a variety of plant species, particularly in peanuts and grapes [41]. This free radical scavenger also possesses a wide variety of bio-properties, including anti-inflammatory effects [42]. More notably, resveratrol can produce a powerful antiapoptotic effect through its action on several different pathways including a ROS- dependent pathway [41]. The flavonoid can also potentiate cytochrome P450 -mediated neuroprotection upon Maneb exposure, a pesticide containing Mn [42]. Notably, several studies demonstrated beneficial effects of resveratrol at “low” doses, but detrimental outcomes at “high” doses, since the compound can be (auto-) oxidized to generate semiquinones and the relatively stable 4’-phenoxyl radical, which can produce ROS [34].

Silymarin is another flavonoid that can be isolated from the fruits and seeds of the milk thistle (S. marianum). Its positive effects have been ascribed to putative anti-oxidant and anti-inflammatory properties. Silymarin has been shown to protect neuroblastoma cells, characterized by the amount of peroxidised lipids and GSH, also preventing Mn-induced OS in the rats´ brain. Several explanations for the efficacy of silymarin in regulating Mn-induced OS have been proposed: (1) reacting with a damaging free radical and forming a flavonoid radical with a great stability, breaking the free radical chain reaction; (2) chelating metal ions such as Fe; (3) inducing the expression of antioxidative enzymes and GSH biosynthesis [17].

Other important flavonoids are anthocyanins, which are responsible for the blue, purple and red colour of many plant tissues. These compounds possess a high antioxidant potential and have also been shown to inhibit COX enzymes [43], thus exhibiting antiinflammatory properties. The anthocyanins-rich açaí extract can attenuate Mn-induced OS stress in primary cultured astrocytes protecting astrocytic membranes from lipid peroxidation and restoring the GSH/GSSG (reduced over oxidized GSH) ratio and net Glu uptake [44].

Curcumin, another flavonoid, is the principal curcuminoid of the popular Indian spice turmeric; due to its brightly yellow colour, it is commonly used as a food colouring (E100) [45]. Curcumin can scavenge oxygen free radicals, such as superoxide anions and hydroxyl radicals and notably, it can readily cross the blood-brain barrier (BBB). After exposure to

Mn, turmeric maintains the activities of antioxidant enzymes, such as SOD, CAT and glutathione peroxidase (GPx). It has also been suggested that turmeric offers protection against Mn-induced oxidative alterations in brain; this protection is mediated by the inhibition of the formation of end products of lipid oxidation, thus maintaining membrane fluidity and BBB integrity [46].

The carotenoid lycopene is another natural antioxidant, an isoprenoid, a fat-soluble compound [47], normally found in red foods such as tomato, pink guava, watermelon, and papaya. The extensive set of conjugated double bonds makes lycopene a powerful antioxidant with the ability to trap peroxyl radicals and singlet oxygenquenching properties. The singlet quenching ability of lycopene is 2 times higher than that of beta-carotene and 10 times higher than that of alpha-tocopherol. Moreover, lycopene is a strong inhibitor of lipid peroxidation by acting as a good chain-breaking antioxidant and by trapping lipid radicals. Pre-treatment of Mn-challenged rats with lycopene resulted in a significant decrease in lipid peroxide concentrations as well as increased levels of GSH and antioxidant enzymes in brain tissues. It has also been shown that lycopene could contribute to mineral homeostasis binding redox-active metals, such as Fe and copper (Cu). This carotenoid could also efficiently maintain AChE activity within a normal range improving the cholinergic effects of Mn [47].

Melissa officinalis (M officinalis) belongs to the Laminaceae family, being a perennial herb. Recent data from literature have supported a protective role for M. officinalis against Alzheimer disease. Extracts derived from this plant, which include polyphenolic compounds, essential oils, monotherpenoid aldehydes, sesquiterpenes, flavonoids and tannins, have antioxidant and also anti-inflammatory properties. *In vivo* studies showed that antioxidant properties of M. officinalis may be potentially neuroprotective against Mn-induced neurotoxicity, especially in the hippocampus and striatum. Further studies are required to identify the active constituents involved in the antioxidant and neuroprotective activity of this plant [48].

Synthetic compounds—Trolox is a synthetic mimetic of vitamin E that can act as a scavenger of radicals via H-donating groups. In truth, trolox serves as a better antioxidant than vitamin E due to its improved access to the hydrophilic compartments of the cells and due to its ability to trap two membrane lipid peroxyl radicals per molecule [11]. The compound was demonstrated to protect cultured cells from the toxic effects of Mn, while striatal and hippocampal OS and motor impairment in Mn exposed rats were prevented by trolox co-administration [49].

The thiols N-acetylcysteine (NAC) and N-acetylcysteineamide (NACA), represent two other synthetic antioxidants which differ in structure only by a –NH2/–OH functional group [50]. With respect to NAC, this GSH precursor can decrease the toxicity of Mn *in vitro,* thus possibly serving as an indirect antioxidant [49]. NACA has also been shown to protect against Mn-induced toxicity by inhibiting lipid peroxidation, scavenging ROS, and preserving intracellular GSH and mitochondrial membrane potential [51].

Vinpocetine-ethyl apovicaminate, initially synthesized from alkaloid vincamine derived from Vinca minor leaves, has been shown to play a role in scavenging oxidants and free radicals in neurons. Several other studies have suggested that apoptotic pathways were also among the targets for the neuroprotective action exerted by vinpocetine in an *in vitro* Mn toxicity model, with restoration of mitochondrial functions [52].

Additionally, several organochalcogens, i.e. organocompounds containing selenium (Se) or tellurium (Te) atoms bound to on of the carbon, possess antioxidant and anti-inflammatory properties. Indeed, the protective effects of organoselenide and telluride compounds against Mn induced neurotoxicity have been reported [49]. Their ability to scavenge ROS and nitrogen species (RNS) and their GPx mimetic property accounts for their efficacy in attenuating OS both in *in vitro* and *in vivo* rodent models. Furthermore, these compounds also possess anti-inflammatory and neuroprotective properties [20]. It was posited that the efficacy of the organotellurium compound diethyl-2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) in attenuating striatal Mn accumulation might be related to direct competition with Mn for shared transport sites, such as divalent metal transporter (DMT-1). The attenuation of Mn-induced generation of ROS upon treatment with DPTVP has also been reported as well as behavioural recovery and restoration of biochemical parameters that were altered by Mn exposure in striatum. Notably, DPTVP also reversed the Mn-induced inhibition of Glu uptake in striatum [24].

Concerning to seleno- organic compounds, 2-phenyl-1, 2-benzisoselenazol-3[2H]-one (Ebselen) is not a free radical scavenger per se, but mimics the active site of GPx. Thus, Ebselen plays a role in eliminating organic hydroperoxides, in particular of lipid hydroperoxides formed by ROS-induced reactions. Ebselen is also an anti-inflammatory agent [53]. An *in vivo* study revealed that Ebselen significantly rescued locomotor activity in rats treated with Mn. In this study Ebselen reduced Mn body burden with observation of decreased blood and brain levels. Ebselen has also been shown to be a DMT-1 transport inhibitor; thus, it was proposed that Ebselen's attenuation of brain Mn levels was due to DMT-1 inhibition, and reduced uptake of Mn. Concomitantly, OS and neuro-inflammation were reduced as established by observations on increased GSH concentrations and decreased prostaglandins, respectively, in the brain. Decreased serum prolactin levels, which are indirect markers of the DAergic function, support the notion that Ebselen's reversal of the Mn-induced effects is mediated via changes in DA function [32].

Another synthetic antioxidant compound that has attracted increased attention in the last few years is the free radical scavenger 3methyl-1-phenyl-2-pyrazolin-5-one (Edaravone). Their antioxidant effects are exerted via quenching the hydroxyl radical (•OH) and inhibition of •OH-dependent and •OH-independent LPO. Edaravone can also stabilize mitochondrial membranes and inhibit mitochondrialdependent apoptotic pathways by decreasing the release of cytochrome C and the activation of caspase-3. Pre-treatment of astrocytes with Edaravone significantly reduced Mn-induced cell death in a dosedependent manner [8]. This antioxidant can markedly counteract Mninduced OS by reducing the generation of lipid peroxides and protein carbonyls. Edaravone at a dose of 5 mg/kg/day was also demonstrated to significantly prevent the reduction of mitochondrial ETC complex I activity in Mn-exposed rats [5].

Anti-inflammatory compounds

Non-specific cyclooxygenase inhibitors: I is broadly reported that Mn exposure induces neuroinflammation, with COX representing an important mediator given its important contribution to prostaglandins production; two variants exist, COX-1 and COX-2 [32, 54]. In this context, Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that predominantly acts through the non-selective inhibition of COX. This drug protected against increased lipid peroxides generation of prostaglandins induced by Mn *in vivo*. Similarly, another non-specific COX inhibitor, indomethacin, attenuated the neurotoxic effects of Mn, causing a decrease in ROS levels, thus suggesting that COX contributes to neuronal damage given its propensity to ROS generation [11]. However, other authors noted that despite the ability of NSAID to act predominantly through the non-selective inhibition of COX, it is possible that NSAIDs exert neuroprotective effects via alternative pathways, such as the inhibition of nitric oxide synthesis [55].

Selective COX-2 inhibitors: COX-2 inhibitors are selective for COX-2 and help in reducing pain and inflammation; their main mechanism is the inhibition of the conversion of arachidonic acid into active prostaglandins and other compounds. Minocycline is a second-generation tetracycline, with an excellent brain tissue penetration; its anti-inflammatory action is likely mediated by the reduction of COX-2 expression as well as the inhibition o the activation and proliferation of microglia (through the inhibition of inflammatory cytokines) [54]. Systemic treatment with minocycline has been shown to attenuate inflammation in a Mn model of neurotoxicity. The compound minimized the Mn-induced decrease in striatal tyrosine hydroxylase, which is involved in the conversion of tyrosine to DA and this recovery was correlated with a decrease in the number of macrophages in the brain [9, 56].

ATP/ADP ratio protectors: Creatine or methylguanidino-acetic acid is a guanidino compound that can be synthesized endogenously. The brain is supplied by brain creatine synthesis or from the circulating blood, as creatine is capable of crossing the BBB. This ergonomic compound has been used as a supplement for athletes. Recently, approaches for the use of creatine as therapy in neurodegenerative diseases has emerged. The role of creatine in maintaining ATP/ADP ratio at equilibrium in the cell is thought to be the major property behind its neuroprotective ability. Furthermore, creatine is also thought to contribute to the regulation of neurotransmission, protect against OS and mitochondria-induced apoptosis. In agreement, supplementation of astrocytes with creatine prior to exposure to toxic levels of Mn have been demonstrated to prevent or delay the onset of neurotoxicity, by enhancing the survival of astrocytes and providing a substrate for the storage of adequate amounts of ATP [57].

Glutamate protectors

Steroid hormones and selective estrogen receptor modulators: Several studies established that 17β-estradiol (E2), an ovarian steroid hormone, at physiological concentrations exerts protective effects in a number of neurodegenerative disorders. Notably, astrocyttes express several subtypes of estrogen receptors being known that E2 increases the expression of the astroglial Glu transporters, thus reducing extracellular Glu levels [25, 58].

Selective estrogen receptor modulators (SERMs) such as Tamoxifen, have also been studied as neuroprotectants. Several studies have shown that the inhibitory effect of Mn on Glu uptake and Glu transporter (GLAST) expression, were fully reversed by E2 or Tamoxifen. E2/Tamoxifen possibly enhanced astrocytic GLAST expression via increased transforming growth factors (both TGF-alpha and TGF- 1 expression) [25, 58]. It was also reported that E2 and Tamoxifen attenuated Mn-induced ROS generation in astroglial cultures and increased the expression of transforming growth factor β1, a potential modulator of the stimulatory effects of E2/Tamoxifen on Glu transporter function, thus offering a potential therapeutic modality for neurodegenerative disorders characterized by altered Glu homeostasis [58, 59].

Prostaglandins synthetic analogues: The development of small organic molecules that mimic or enhance the action of neurotrophic factors may also provide therapeutic agents for neurodegenerative diseases; cyclopentenone prostaglandins (PGs) are potential candidates. They are formed through the metabolization of prostaglandins, penetrate cell membranes, directly bind to intracellular proteins, and regulate their activities [60, 61]. Previous studies have demonstrated that certain synthetic analogues with a cross-conjugated dienone structure exhibited neuroprotective activities, such as the protection of HT22 neuronal cells against oxidative Glu toxicity. Additional studies established that low concentrations of one of these analogues, designated NEPP11, inhibited Mninduced apoptosis of PC12 cells as well as Glu-mediated cell death [60]. An anti-apoptotic action of NEPP11 was already related to its inhibiting function in JNK pathway, since NEPP11 prevented Mn-induced phosphorylation of JNK [60, 61].

Pinacidil and Nimodipine: Pinacidil is an ATP-sensitive potassium channel opener that evokes K+ efflux through ATP-sensitive channels. It can improve the function of Glu transporters and decline the extracellular Glu in rat primary cultured astrocytes. Nimodipine is a Ca channel blocker that can pass the BBB and inhibit the Ca^{2+} influx induced by over-stimulation of the Glu receptor NMDAR. Indeed both compounds may have neural protective effect on manganism antagonising Glu excitotoxicity [23].

Therapeutic Intervention: Treatment of manganism produces various outcomes in afflicted patients. Symptom severity increases and chance of recovery decreases with prolonged Mn exposure. The foremost therapeutic strategy in treatment of Mn toxicity is to remove the patient from the source of the exposure. If the intoxication is life threatening, the procedures to relieve the critical signs and symptoms should first be employed. As a treatment, chelation therapy can be beneficial in reducing the body burden of Mn, but such treatments may not be able to reverse or improve symptoms [62, 63].

Levodopa: The use of levodopa as DA-replacement therapy is highly effective in ameliorating the symptoms of Parkinsonism and remains the standard drug with which other therapies are compared [64]. Clinically, levodopa has been used to treat extrapyramidal syndromes, but with limited efficacy in improving of clinical symptoms [65, 66, 67]. Mn-induced parkinsonism patients, regardless of the administered levodopa dose, seemed to be unresponsive to levodopa treatment [68, 69]. A more rigorously designed clinical trial leaded

to found that treatment with levodopa among parkinsonian welders did not lead to a significant beneficial effect [68]; levodopa resulted in only a brief improvement that was, however, not sustained [65]. Overall, current evidence suggests that lack of responsiveness to levodopa is a hallmark of Mn-induced Parkinsonism, which distinguishes this disease from idiopathic PD [70, 71].

Chelation therapy: The success of *in vivo* chelation depends on a range of characteristics of the metal, the chelator and the organism. In most studies on the use of chelating agents to treat metal intoxication, focus has been on mobilization (mainly due to renal excretion) of the toxic metal. Thus, a chelating agent forming a stable complex with a toxic metal may shield the metal ion from biological targets, thereby reducing its toxicity, even after administration where mobilization of the metal has yet to take place; it may also expose the metal to the biological environment and prevent it from being scavenged by biological protective mechanisms, thereby increasing its toxicity [72]. A major problem in continued, often life-long chelation treatment is the induction of essential trace element imbalance, which may require correction.

Ethylene-diamine-tetraacetic acid: Ethylene-diamine-tetraacetic acid (EDTA) is a chelator that is poorly absorbed in the gastrointestinal tract (<5%), and is commonly administered by slow intravenous (I. V) infusion of its calcium or zinc (Zn) complexes. In chelation treatment, the monocalcium salts of EDTA are used to avoid hypocalcemic tetani. Acute and subacute exposure to EDTA induces dose- and time-dependent proximal tubular damage and degenerative changes in small intestinal mucosa. The nephrotic lesions are normally completely reversible [72]. Chelation therapy, mainly using CaNa2EDTA has been used with variable success for the treatment in acute and chronic cases of Mn poisoning [73].

The strategy to lower body burdens of Mn in manganism patients relies on increasing Mn excretion in the urine and decreasing Mn concentrations in the blood; however, the clinical symptoms do not appear to be significantly improved [70]. Chelation therapy with EDTA has shown in some cases to produce promising clinical results [73], while in other cases it increased Mn elimination in urine but overall failed to improve the clinical symptoms [62]. The lack of symptomatic improvement after EDTA chelation is not entirely unexpected. Four carboxyl groups in EDTA structure, although essential to its chelating property, render the molecule poorly lipophilic and thus prevent it from effectively crossing the BBB. In fact, radiolabeled EDTA has been used as an extracellular tracer to monitor the leakage of the BBB [70, 74, 75]. Thus, EDTA appears likely to chelate and therefore remove mainly the extracellular Mn ions. A rather poor bioavailability of EDTA to brain parenchyma, where most of Mn ions accumulate, may limit its efficacy in reducing brain burden of Mn. Because EDTA unlikely possesses the ability to repair the damaged neurons, it is essentially of no practical benefit for more advanced neurologic signs and symptoms in the late stage of severe Mn poisoning [70]. Thus, a search for other chelating agents for Mn intoxication is fully warranted [76].

Para-aminosalicylic acid: Para-aminosalicylic acid (PAS) was one of the first antibiotics found to be effective in the treatment of tuberculosis in the 1940s [77, 78]. Fifty years later, PAS continues to be used to treat tuberculosis. PAS is assumed to inhibit dihydropteroate

synthase in Mycobacterium tuberculosis by mimicking the substrate para-aminobenzoate [79]. In addition to the treatment of tuberculosis, it has been found to be efficacious in treatment of severe chronic manganism [70, 80]. Considering its amino, carboxyl and hydroxyl functional groups, PAS is a potential therapeutic Mn-chelating agent. Early studies have shown that PAS could mobilize Mn from the liver and testis of Mn-intoxicated rats [81], and enhance the fecal excretion of Mn in manganeseintoxicated rabbits [82]. In 1992, Ky et al. [80] were the first to report that PAS was clinically successful in the treatment of two cases of chronic Mn poisoning. The patients were treated for three and a half months with PAS-Na; in one patient, the clinical symptoms and signs gradually improved and then disappeared and at follow-up 19 months later no signs of relapse were found. In the other patient, the clinical symptoms and signs were improved and six months later follow-up examination showed that the benefits were long lasting. Another study reported a 17-year follow-up study on one patient with Mn-induced parkinsonism (exposed for 21 years to airborne Mn). PAS-Na treatment for four months significantly alleviated her symptoms, and 17 years later, a clinical normal presentation was observed. Combined with 86 other identified cases in the literature, PAS may be a promising therapy in the treatment of manganism [70].

How does PAS ameliorate Mn intoxication?: Early studies comparing the Mn-chelating abilities of various compounds, found a greater binding capacity of Mn with chelators containing nitrogen (N) and oxygen (O) electron donating centers, than thiol centered chelators [83]. Two putative mechanisms may explain its effectiveness. First, PAS may act as a Mn-chelating agent. In biological matrices, Mn ions can exist in several valent states, with a majority in Mn2+ and Mn3+. Mn3+ as a hard Lewis acid can form a stable complex with hard donor atoms such as oxygen donors in PAS structure. In contrast, the Mn2+ cation has a lower charge density and thus prefers relatively softer donors such as N, which is also present in PAS structure. The exact coordination chemistry of Mn-PAS complex is unknown. It is possible that PAS may form a reasonably stable Mn2 + complex that can be rapidly oxidized to a more stable Mn3+ complex and subsequently remove both Mn2+ and Mn3+ from the intracellular matrix under physiological conditions [70]. Whether PAS chelates physiological Mn or excessive Mn is unknown and whether PAS has a better brain barrier permeability than EDTA is also unknown. These outstanding questions merit further investigation.

The salicylate moiety in PAS structure, which possesses an antiinflammatory effect, may contribute to therapeutic effectiveness of PAS in treatment of neurodegenerative manganism. Recent studies have suggested that nonsteroidal anti-inflammatory drugs, including sodium salicylic acid, may have neuroprotective benefit, because the inflammatory processes have been shown to play a role in the pathogenesis of neurodegenerative diseases. These drugs may facilitate regulation of neurotransmitters, suppress nitric oxide synthesis, and protect against oxidative stress in neurons and neuroglia [70, 76].

PAS mode of action: There are several *in vivo* and *in vitro* studies that address the mechanisms that might afford therapeutic effects in human treated with PAS. In a sub chronic study [76] with rats exposed to Mn a significant accumulation of Mn in targeted

brain areas was analyzed; an altered Cu homeostasis was observed in the cerebrospinal fluid (CSF), choroid plexus, striatum and hippocampus following Mn exposure. PAS appears to be effective in reducing Mn concentrations and restoring Fe and Cu concentrations in body fluids and brain tissues to the normal physiological level. A high-dose and prolonged PAS treatment was necessary for its therapeutic effectiveness. According to Marreilha dos Santos et al (2012) [32] PAS was effective in reducing Mninduced oxidative stress in rats, reflecting its ability to reduce both blood and brain Mn levels. Consistent with attenuated ROS production, F2-isoprostanes (F2-IsoPs) and F4-neuroprostanes (F4-NeuroPs) levels were decreased and GSH levels were increased by PAS. In addition, PAS led to decreased PGE2 levels consistent with a decrease in neuro-inflammation. Moreover, the decrease in PRL levels in Mn + PAS treated rats was significantly different from the Mn group, approaching control levels, indicating a reversal of the effect of Mn on decreased DA function. Finally, the Mn-induced decrease in locomotor activity was significantly rescued by PAS treatment.

A recent study [84] aimed to investigate the effects of PAS-Na on Mn-induced behavioural deficits and the involvement of ultrastructural alterations and GABA metabolism in the basal ganglia of rats. PAS-Na treatment successfully restored these adverse effects to levels indistinguishable from controls. Unexpectedly, PAS-Na failed to recover the Mn-induced decrease in the overall GABA levels, although PAS-Na treatment reversed Mn-induced alterations in the enzyme activities directly responsible for the synthesis and degradation of GABA (glutamate decarboxylase and GABA-transaminase, respectively).

In an *in vitro* study [85] performed in primary cultured basal ganglia neurons, a low level of Mn (50 μM) induced cytotoxicity evidenced by reduced cell viability, impaired membrane integrity, increased oxidative stress and DNA damage, and disrupted amino acid neurotransmitter (Glu/Gln-GABA) balance. However, PAS-Na was able to reverse the aforementioned toxic effects. This study showed that PAS-Na has antagonistic effects on Mn toxicity on amino acid neurotransmitter in basal ganglia neurons *in vitro*. It was also found that PAS-Na increased Gln levels of basal ganglia neurons. These results are consistent with previous *in vivo* study [86] that showed that PAS-Na treatment in rats for 6 weeks or PAS-Na preventive treatment restored Gln, and GABA levels in the Mnexposed rats levels indistinguishable from controls.

In another study [87], the authors explored whether PAS could block Mn-induced neuronal injury in hippocampus *in vitro,* noting that PAS (50–5000 μM) attenuated Mn-induced DNA damage.

Limitations of PAS therapeutic applications: Although PAS or PAS-Na have been shown to possess beneficial therapeutic effects on manganism, their utility is limited first, PAS is associated with gastrointestinal intolerance, causing vomiting, frequent nausea and abdominal discomfort; second, a high dose (5,000 μM) of PAS-Na has a toxic effect on primary basal ganglia neurons [85, 88].

Conclusions

Regardless of the recent advances in drug discovery that have provided multiple strategies to alleviate some symptoms and decrease progression, there is yet no cure for Mn-induced Parkinson-ism [71]. Despite many natural and synthetic compounds that have been tested both in *in vitro* or *in vivo* studies with promising results, the extrapo-extrapolation of these results to humans has yet to be verified. As an exception, in vivo and human cases effectively treated with PAS suggest the latter may be a promising therapy for manganism.

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