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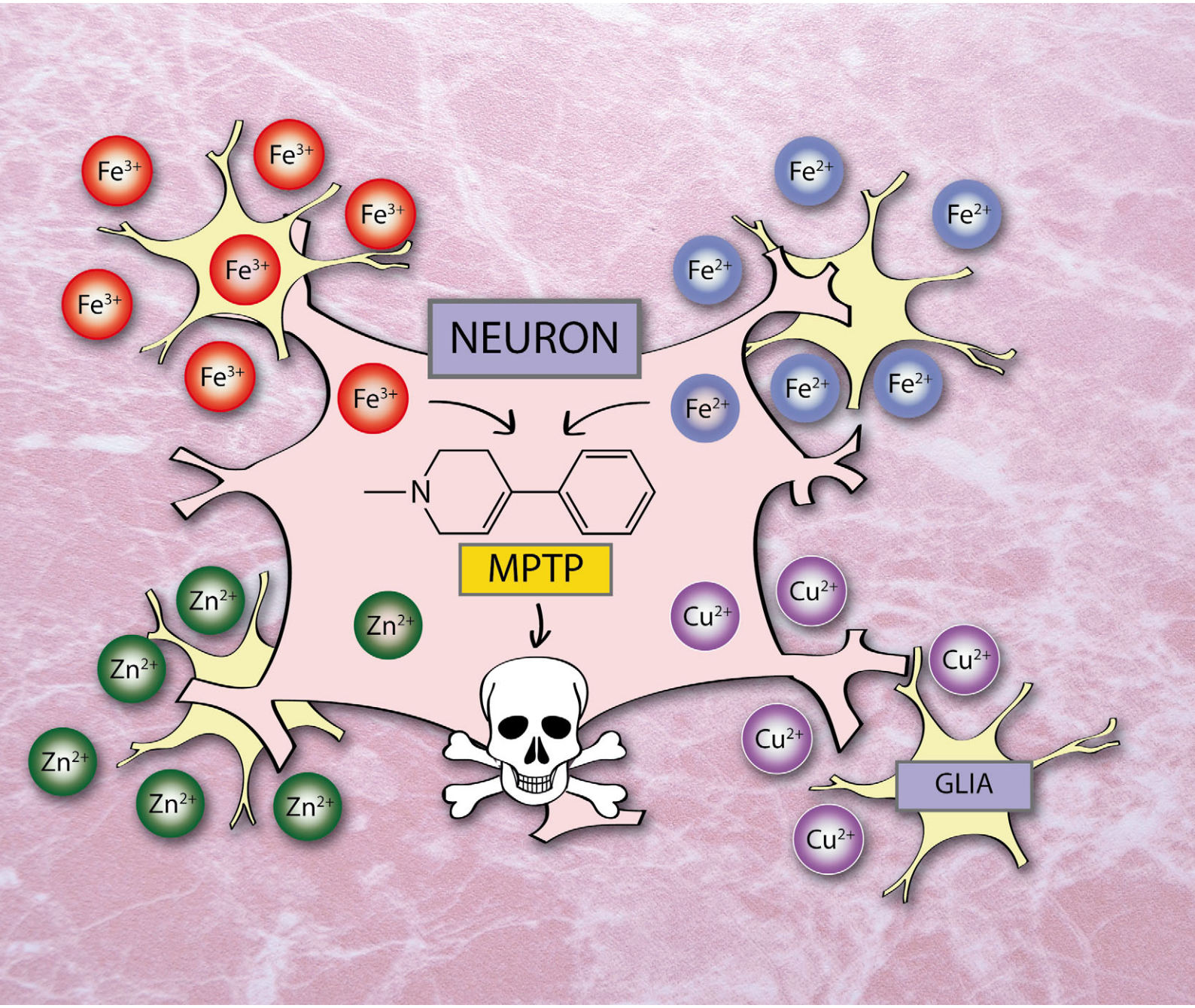

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CRITICAL REVIEW

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Metallobiology of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent toxin used to selectively destroy dopaminergic neurons in the substantia nigra and induce parkinsonism. MPTP is metabolised to the 1-methyl-4-phenylpyridinium ion (MPP^+) in glia, after which it enters the neuron via the dopamine transporter and results in elevated levels of oxidative stress. The mechanism through which MPP^+ causes cell death is thought to involve redox-active metals, particularly iron (Fe). This review will examine how cellular metal metabolism is altered following MPTP insult, and how this relates to metal dyshomeostasis in idiopathic Parkinson's disease. This includes both cell damage arising from increased metal concentration, and how metal-binding proteins respond to MPTP-induced neurotoxicity. Implications for using MPTP as a model for human Parkinson's disease will be discussed in terms of cell metallobiology.

Introduction

Globally, Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting around 1% of people aged 60 years and older.[1] PD was first described by James Parkinson in 1817, who reported on symptoms including tremor, poor posture and muscle loss in six individuals.[2] Traditionally the motor symptoms of PD, including rigidity, tremors and bradykinesia, were thought of as the cardinal features of the disease. PD is more commonly considered a syndrome that encompasses other symptoms such as cognitive loss, sleep disturbances, loss of the ability to smell and gastrointestinal problems. PD is characterised by the loss of dopaminergic neurons of the substantia nigra (SN) pars compacta (SNpc), noradrenergic neurons in the locus coeruleus and the accumulation of insoluble protein inclusions known as Lewy bodies throughout the basal ganglia, which eventually

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Paul Adlard's research focuses on promoting healthy brain ageing. By gaining an understanding of the mechanisms that lead to age-related dysfunction we can subsequently apply this knowledge to provide a targeted and novel therapeutic strategy to maintain/improve brain health. The current focus of the lab is understanding the role that metals play in the cellular events and pathways that occur during both normal and pathological ageing (particularly Alzheimer's disease) that are related to synaptic plasticity and cognitive function.

spread from within the medulla oblongata through to the neocortex in six pathological stages.[3] A feature of PD is abnormal metal homeostasis within specific regions of the brain involved in the disease. Atypical brain metal concentrations in human PD brain tissue were first reported by Lhermitte *et al.*[4] in 1924, who commented on abnormal histological deposits of redox-active iron (Fe) in the globus pallidus of a 53-year old male with PD. Increases in both Fe and zinc (Zn) and a decrease in copper (Cu) levels have since been observed in PD-affected SN.[5,6] Serum Fe has been observed to decrease in PD patients,[7] though no change in serum Cu and Zn has been identified.[8] Elevated brain Fe is a generally accepted consequence of aging, with the more focal Fe accumulation occurring in regions of the brain considered at risk of neurodegeneration,[9] including those affected by PD. Elevated Fe levels in the SN has thus become an important target in PD research.[10] Hyperechogenicity of the SN, measurable through ultrasonography, has been associated with increased nigral Fe and microglial activation in PD.[11] Preliminary studies of a large cross-sectional group of subjects found that 8 otherwise healthy adults displaying hyperechogenicity had developed clinical PD symptoms in follow-up consultations, suggesting hyperechogenicity, and therefore elevated nigral Fe levels were present prior to physical manifestation of the clinical defined disease.[12] Thus, it is becoming more widely accepted that deriving the association between metal dyshomeostasis and PD progression is an important step in understanding both the cause of disease and developing therapeutics. Significant attention is being paid to examining metallobiology in both human PD and the various Parkinsonian animal models used to study disease aetiology. A successful Parkinsonian model must thus mimic the metal dyshomeostasis of idiopathic PD in addition to other typical pathological features in order to provide a complete model of disease. This review will focus upon metal metabolism in one of the primary research tools utilised for PD research: the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxin model.

Metals and MPTP toxicity: cause or effect?

J. William Langston's pioneering work in the 1980s[13] paved the way for the widespread use of MPTP as an *in vivo* neurotoxin that recapitulates some aspects of the neuronal loss observed in PD. The acute neurotoxicity was first described in detail after a number of drug users developed L-3,4-dihydroxyphenylalanine-(L-DOPA) responsive PD symptoms after administering the synthetic opioid 1-methyl-4-phenyl-4-propionoxypiperidine contaminated with 3% MPTP, which was confirmed by post mortem identification of lesions in the SN.[14] The subsequent symptoms, disease progression and pathology of these people was indistinguishable from the sporadic form of the disease and as such has initiated its use in research.[15] Jackson-Lewis and Przedborski[16] identified this, along with the relative ease of administration compared to other candidates and the consistent lesion as the major reasons MPTP is commonly chosen for PD research. The MPTP model still engenders controversy regarding the applicability of the results to the human condition, mainly as the toxin does not illicit all the symptoms or the progressive nature of the human disease. Irrespective of this, MPTP provides the most complete model of nigrostriatal cell death that can be studied *en masse* in the laboratory setting.

MPTP neurotoxicity arises from the formation of the toxic 1-methyl-4-phenylpyridinium (MPP^+) metabolite.[17] Monoamine

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oxidase (MAO) activity in glia converts MPTP to 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$), which rapidly dismutases into MPP^+.[18] Like rotenone ((2*R*,6a*S*,12a*S*)-1,2,6,6*a*,12,12*a*-hexahydro-2-isopropenyl-8,9-dimethoxychromeno[3,4-*b*]furo(2,3-*h*)-chromen-6-one) and paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride), MPP^+ accumulates in mitochondria, where it is a potent complex I inhibitor, interfering with the first step of the electron transport chain, decreasing cellular ATP production, inhibiting mitochondrial enzymes and increasing the synthesis of reactive oxygen species (ROS).[19–21] Importantly, complex I inhibition is a feature of MPP^+ toxicity mirrored in idiopathic PD.[22,23]

MPP^+ also stimulates increased ROS formation in the cytoplasm, independent of mitochondrial inhibition,[24] suggesting the mechanism of cell death is multifaceted. Cell damage resulting from ROS is promoted by the presence of redox-active metals, such as Fe, which mediate the formation of harmful hydrogen peroxide and hydroxyl radicals via Fenton chemistry.[25] Changes in metal levels and metabolism following MPTP-induced neurotoxicity are an important hallmark of the toxin that appears to mirror metal dyshomeostasis in humans, and taken together suggest the role of metals in dopaminergic cell death. The role of metals in MPTP-induced neuron loss was first discussed by Poirier *et al.*,[26] who found that MPTP and high concentrations of transition metals potentiate autoxidation of dopamine (DA) through increased production of reactive oxygen species (ROS). The fact that DA-rich neurons are more susceptible to cell death in both PD and MPTP-treated cells suggests that DA catabolism plays a major role. Autoxidation of DA occurs through two pathways: (i) MAO on the surface of mitochondria coverts DA to an aldehyde intermediate that is further metabolised to 3,4-dihydroxyphenylacetic acid, or DOPAC; and (ii) oxidation of the catechol moiety forms an electron-deficient DA quinone.[27] The redox-active properties of transition metals including Fe, Cu and Zn may potentiate the oxidation of DA and formation of toxic species associated with this degradation pathway. Furthermore, the MPTP-induced elevation of such metals could contribute to the rapid neuron death cascade observed following insult, accelerating the DA-mediated cell loss observed in idiopathic PD.

Redox active metals such as Mn have been shown to cause nigral cell death in a manner mimicking the action of neurotoxins like MPP^+.[28] Further, Mn inhibits the activity of tyrosine hydroxylase, the enzyme responsible for the conversion of L-tyrosine to L-DOPA thus decreasing striatal DA. Mn could also stimulate reduction of striatal DA concentrations directly through DA autoxidation. However, more recent studies[29] directly comparing Mn administration with MPTP insult suggests Mn alone does not produce comparable neurotoxicity, and that there is no potentiating effect of Mn when administered 3 weeks prior to MPTP administration. Mn exposure did increase astrocyte activity in the globus pallidus of treated C57BL/6 mice. Paradoxically, Mn administration for 7 days prior to MPTP insult demonstrated a capacity to *increase* DA by up to 60% compared to MPTP treatment alone.[30]

The conflicting data described here regarding the role of Mn in mitigating and/or accentuating MPTP toxicity highlights a significant limitation in generalising the effects of all redox active metals and the role they play in the toxin's mode of action. Redox active metals are implicated in ROS generation and ultimately cell death; therefore, pre-treatment of subjects with metals can significantly alter homeostasis before MPTP insult occurs, potentially complicating interpretation of MPTP as a model for cell death in PD. In their protocol for producing the MPTP mouse model of PD, Jackson-Lewis and Przedborski[16] insist on experimentally measuring the impact of drug pre-treatment on MPTP metabolism (such as assessment of MPP^+ levels) when investigating potential neuroprotective agents, suggesting that if MPTP toxicokinetics are altered in the pre-treated animal administration of the insult should be delayed until after MPTP/MPP^+ metabolism is established.

This has implications for studying the effects of metals on MPTP toxicity, as well as for understanding the properties of metals in arresting MPTP damage. Another example of this is the conflicting data regarding potential protective properties of Cu in MPTP treated mice. Cu is noted as a major source of free radicals in Alzheimer's disease,[31,32] and elevated Cu levels can increase cellular oxidative load.[33] There is some literature describing how Cu may attenuate MPTP-induced neuronal death. Mice with supplemented Cu^{2+} intake (through $CuSO_4$-spiked drinking water for 30 days prior) displayed dose-dependent resistance to intracerebroventricular MPP^+ administration. Cu treatment was only effective at reducing MPP^+-induced striatal lipid peroxidation and DA depletion at either a high Cu dose for chronically-exposed animals, or in animals receiving an acute intraperitoneal dose 24 hours prior to lesioning.[34] Similar evidence suggesting Cu interferes with induced toxicity was observed in rats given intraperitoneal $CuSO_4$ injections prior to MPP^+ administration, where attenuation of the neurotoxin was measured by reduced TH inactivation, less protein nitration and marked retention of striatal DA in the lesioned animals.[35] Again, it is difficult to directly associate Cu pre-treatment with any potential neuroprotection against MPTP and MPP^+ due to the myriad of cellular processes influenced by drastically altering Cu homeostasis. Elevated extracellular Cu has been shown to disrupt DA metabolism without any external neurotoxin in a neuroblastoma cell line through inhibition of transmembrane proton pumps required for active DA transport.[36] Duncan and White[37] suggested that oral Cu intake prior to neurotoxin exposure may mitigate the redox activity of Fe and MPTP (see following section) through competitive uptake in the intestinal tract. Priority should be given to investigating the effect of rapid influx of Cu on the neuron's metal trafficking systems, especially considering the known implications of altered Fe metabolism in human PD. Decreased Cu levels have been observed in MPTP-lesioned animals with no Cu-chelating pre-treatment or Cu supplementation. Both Cu and Mn have displayed marked decreases in striatal regions of MPTP-treated mice, with Cu exhibiting a near-halving of total content in the midbrain.[38]

The changes in Cu concentrations observed in MPTP treated cells are not necessarily reflective of Cu changes in human PD. In fact, the ambiguity in data describing post-lesioning Cu levels reflects the limited knowledge of Cu metabolism in idiopathic PD in humans.[6,8] Compared to Fe, relatively little is known about perturbations in Cu metabolism in PD. Pall *et al.*[39] observed an increase in cerebrospinal fluid Cu

concentrations in 24 idiopathic PD patients compared to a 34-strong control group, possibly explaining reduced ferroxidase activity from Cu-binding ceruloplasmin observed in a later study of PD cases.[40] Imaging of Cu distribution in MPTP lesioned mice brains found decreased Cu in periventricular regions up to 7 days after administration, which either returned to or exceeded pre-dosing levels 28 days following.[41] However, the role of Cu in PD pathogenesis may be independent of the cell death observed following MPTP intoxication; Cu has been shown to play a role in the aggregation of insoluble α-synuclein-based Lewy bodies,[42] a feature observed in human PD that is not mirrored in MPTP models.[43,44] If Cu-mediated Lewy body formation is indeed one of the major pathways through which altered Cu metabolism contributes to neuron loss in PD, interpretation of data describing changes in Cu levels following MPTP should take this into account. As with all metals, the various roles of Cu in numerous cellular processes do somewhat limit decisive conclusions that can be drawn from experimental data when comparing human disease states to animal models.

Does iron metabolism following MPTP insult reflect changes in PD?

Iron has been implicated more than any other redox active metal in PD pathogenesis. Since Lhermitte's early observations of 1924,[4] significant attention has been given to elucidating the role of Fe in neuron death. Typical features of generalised Fe overload in the cell closely mirror those observed in PD, including increased ROS production, decreased activity of antioxidant proteins and inhibition of mitochondrial enzymes, including complex I.[45] Importantly, numerous biochemical characteristics of Fe overload are also present following MPTP administration.

A proposed mechanism of Fe-mediated MPTP neurotoxicity is presented in Fig. 1. *In vitro* studies of MPTP in astrocyte cultures found that glial conversion to MPP^+ by MAO activity is the primary method of bioactivation, but redox-active Fe also contributes to the formation of the toxic metabolite.[46] MPP^+ is released into the cytosol and taken up into neurons via the dopamine transporter (DAT), where it accumulates and stimulates cellular dysfunction and death by mitochondrial failure (by way of inhibition of mitochondrial complex I) and increased oxidative stress (the theorised pathway involving Fe).[47] Thus, the role of Fe in MPTP-induced cell loss appears to be multi-dimensional: Fe is not only involved in the production of cytotoxic species through metal-mediated O_2^- production, but also in the generation of the toxic moiety MPP^+ from its parent MPTP molecule. Further evidence for the role of Fe in MPTP toxicity has been uncovered at the genetic level. Gene expression profiling of MPP^+-exposed MN9D dopaminergic cells found 51 genes that exhibited a significant response to the

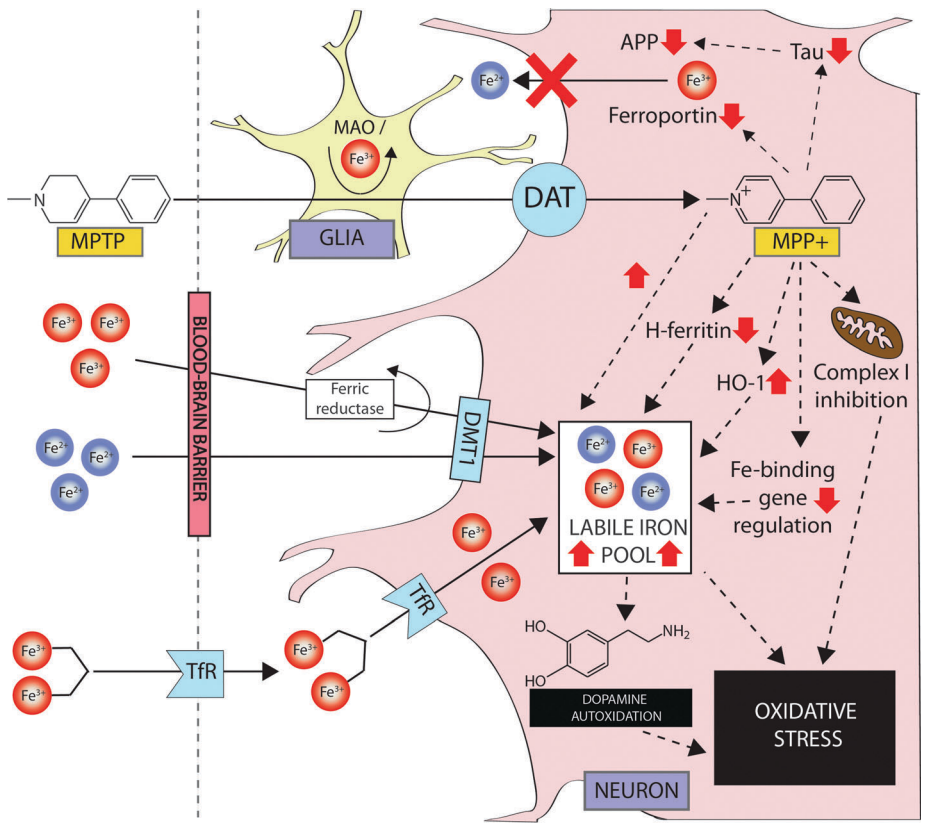


Fig. 1 Proposed mechanism for Fe-mediated neurotoxicity of MPTP. MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPP^+ = 1-methyl-4-phenylpyridinium, MAO = monoamine oxidase, APP = amyloid precursor protein, DAT = dopamine transporter protein, DMT1 = divalent metal transporter protein 1, TfR = transferrin receptor, HO-1 = heme oxygenase-1.

toxin compared to control, with known functions including apoptosis, cellular metabolism, signal transduction and oxidative stress. Using Gene Ontology for Function Analysis (GOFFA), a functional tree of gene expression related to Fe binding was deduced. All six of the identified genes were down regulated, four of which were associated with oxidative stress (*Cdo1, P4ha1, Rrm2, Tbxas1*),[48] highlighting the suspected role of Fe in increased oxidative damage arising from MPP^+ toxicity. Interestingly, many of these genes are also activated in idiopathic Parkinson's disease.[49–51]

MPP^+ is highly efficacious at stimulating DA release, resulting in a marked increase in striatal hydroxyl radical ($^{\bullet}OH$) concentrations that can be arrested by DA-depleting drugs such as reserpine. Additional hydroxyl species are sourced through MPP^+ induction of nitric oxide synthase mechanisms.[52] This efflux of dopamine increases MAO-A and -B activity, with increased DA deamination producing elevated levels of H_2O_2. Fe-mediated Fenton chemistry in the cell ensures continued production of hydroxyl radicals by oxidation of Fe^{2+} in the presence of H_2O_2, while constantly regenerating through reduction with the same H_2O_2 species. Increasing the amount of striatal Fe available increases the formation of $^{\bullet}OH$[53] and other products of dopamine autoxidation, contributing to the cell's overall oxidative load. Assuming a modest source of Fe is available, this reaction continues, overwhelming cellular antioxidant mechanisms and inducing cell death.

MPTP administration has demonstrated an effect on labile Fe pools by increasing levels of free Fe in the dopaminergic pathway. Both ferric (Fe^{3+}) and ferrous (Fe^{2+}) species were observed to increase in the SN of unilaterally lesioned African green[54] and macaque monkeys.[55,56] The source of changes in the labile Fe pool in PD and the oxidation state of Fe species involved is an obvious target for determining the aetiology of the disease. A potential candidate for elevated brain Fe levels is via the storage protein ferritin. Ferritin contains 24 subunits, consisting of H-units (heavy chain) with ferroxidase activity to convert toxic Fe^{2+} to manageable Fe^{3+} species, and L-units (light chain), which promote ferrihydrite formation for storage. Selective elevation of ferritin levels in transgenic mice has been shown to offer some degree of neuroprotection against MPTP-induced neuron death,[57] though interestingly chronic ferritin expression in dopaminergic neurons resulted in older animals showing more susceptibility to MPTP neurotoxicity.[58] Unilateral lesioning of monkeys with MPTP stimulated a bilateral increase in ferritin expression in the pallidum and SN pars reticulata.[59]

If ferritin's ability to appropriately handle Fe is compromised, the vast Fe stockpile (fully assembled ferritin can store up to 4500 Fe^{3+} ions) can present a major source of redox-active material. Mutation to the gene producing ferritin L-units produces ferritin species with a predisposition to causing oxidative damage through Fe mishandling.[60] Accumulation of ferritin following MPTP exposure does not necessarily reduce the labile Fe pool within the cell if the capacity of the protein to store Fe is reduced. Oxidative damage affecting Fe storage capacity of the ferritin molecule itself has been demonstrated *in vitro,* where incubation of apoferritin with the heme precursor 5-aminolevulinic acid resulted in a 61% decrease in Fe uptake following exposure.[61] MPTP lesioning of mice elicited a decrease in H-unit ferritin that resulted in an increase in labile Fe^{3+} in the SN.[62] Paraquat, a cation that displays similar structural properties to MPP^+, promotes complete release of Fe from ferritin, though electron spin resonance spectroscopy showed paraquat did not induce structural deformities in ferritin chains and that the protein regained the ability to reincorporate Fe into its structure.[63]

Ferritin levels in PD have not demonstrated a clear increase in expression, rather changes observed in idiopathic PD are somewhat ambiguous.[64] Nigral ferritin immunoreactivity has been described as decreasing (with a corresponding increase in total Fe, suggesting Fe storage capacity may be compromised)[6] and increasing (with corresponding increase in Fe^{3+}, suggesting ferritin expression may be in response to elevated Fe)[65,66] in PD. The significance of ferritin in Fe metabolism is clear, and the role it plays in cell death mechanisms found in both MPTP toxicity and idiopathic PD is undoubtedly of great importance in elucidating PD pathogenesis. However, it is uncertain if ferritin's role in PD progression is contributory to cell death or reactionary, as part of the cell's defence mechanism.

Interaction between ferritin and MPTP may still hold key information regarding the risk factors of elevated Fe in the aging brain. MRI analysis of ferritin in the human brain *in vivo* suggested ferritin levels increase in the caudate, putamen, globus pallidus, thalamus and hippocampus (but decreased in the frontal lobes) with age.[67] Ferritin accumulation in the SN of human PD brains appears confined to glia.[68] One theory supporting age-related changes in ferritin-accumulating cells and associated Fe toxicity is the ability of ferritin to release Fe^{2+} in response to elevated O_2^- levels.[69] Hence, accumulation of ferritin with age could indeed provide a viable source of Fe for the increasing labile Fe pool, entering neurons via microglial activation and uptake through divalent metal transporter-1 (DMT1) on the neuronal membrane. A lack of ferritin up-regulation in SN neurons of human PD cases may explain the increases in available brain Fe and heightened susceptibility to neuron loss with age.[70] Disruption of ferritin expression or activity in the neuron may limit the cell's practical defences against sudden Fe influx, increasing the labile Fe pool and causing oxidative damage. Thus, alterations in ferritin levels occurring with age have a direct effect on nigral Fe and susceptibility to Fe-related oxidative stress that occurs in the aging brain.

MPTP has been employed to study the possible influence of this progressive change in brain Fe. Histochemical study of Fe deposits in two age groups of macaque monkeys given a single unilateral MPTP injection found that dense, focal deposits of Fe were only observed in the SN of older (>7 years) monkey and no significant variation in MPTP-induced neuron loss was obvious between the two groups. The Fe deposits were observed bilaterally, with the authors suggesting this was indicative of Fe deposition prior to lesioning[71] though, as previously noted, unilateral MPTP lesioning in monkeys has resulted in increased bilateral ferritin expression.[59] Neonatal Fe feeding of mice has been used to model age-related changes in Fe,[72]

and in conjunction with noradrenaline denervation by *N*-[2-chloro-ethyl]-*N*-ethyl-2-bromobenzylamine (DPS4), near total abolition of spontaneous motor activity with no restorative effects observed after administration of L-DOPA was observed in Fe-loaded, MPTP treated mice.[73] This suggests that increasing amounts of brain Fe, as is the case with aging, can cause vulnerability in both the dopaminergic and noradrenergic pathways, with denervation of noradrenaline potentiating MPTP toxicity following Fe overload. Again, it is important to consider the implications pretreatment of animals might have on other cellular metal regulation systems prior to MPTP/MPP^+ metabolism being established. However, unlike the observed ambiguity surrounding Cu pretreatment, Fe loading of mice clearly enhances MPTP toxicity,[74] and when considered in conjunction with evidence of increased brain Fe with age presents a practical picture of how Fe contributes to oxidative damage in PD.

Dietary Fe control has also been used in mice to demonstrate its role in DA synthesis and susceptibility to oxidative damage through DA toxicity. Mice fed a Fe-deficient diet showed decreased striatal DA content compared to mice fed a normal diet, possibly due to insufficient Fe needed for TH synthesis. Interestingly, Fe deficient mice did not display DA reduction or motor deficiencies compared to control animals following acute MPTP exposure,[75] suggestive of the influence that an increased labile Fe pool may have on DA autoxidation.

Elevated free Fe may not necessarily be a direct cause of increased free radical production; rather unbound Fe may result from other pathways such as an increase in activity of heme oxygenase-1 (HO-1) in response to brain injury. HO-1 catalyses degradation of heme to biliverdin, which is subsequently metabolised to bilirubin, CO and free Fe. In PC-12 cells treated with MPP^+ HO-1 induction was observed to rapidly increase in line with ROS production. Inhibition of HO-1 by zinc protoporphyrin-IX resulted in a marked increase in ROS production, indicating HO-1 may play a cytoprotective role against MPP^+-induced toxicity.[76] This suggests elevated free Fe post-MPP^+ (and thus MPTP) exposure could stem from increased heme degradation. Conversely, Lee *et al.*[77] described increased HO-1 expression brought about by administration of an Fe-dependent prolyl hydroxylase enzyme inhibitor helped attenuate nigral Fe accumulation following MPTP exposure. 3,4-Dihydroxybenzoate upregulated neuronal expression of the HIF-1α gene, increasing cellular HO-1 levels and returning nigral Fe concentration to near control levels. Innamorato *et al.*[78] recently used MPTP-lesioned Nrf2- (master regulator of antioxidant response elements) and HO-1- (target gene of Nrf2) null mice to demonstrate that both knockout mice displayed equivalent Fe deposition in the SN compared to lesioned wild-type animals, suggesting HO-1 and associated antioxidant response elements do not contribute to Fe accumulation or trafficking in MPTP induced cell death.

Variation in tau protein and Fe concentrations were also observed in MPTP lesioned mice. Tau is a microtubule-associated protein that is linked to a variety of neurodegenerative disorders, termed tauopathies. Modifications to tau, such as phosphorylation and glycosylation are thought to play a major role in tau mediated neuronal toxicity.[79] A possible mechanism demonstrating tau involvement in Fe trafficking has been proposed, where tau contributes to amyloid precursor protein (APP) surface activity. APP has ferroxidase activity,[80] where it interacts with membrane ferroportin to shunt Fe from the cell. Loss of tau in primary cell cultures resulted in marked Fe accumulation. Similarly, in MPTP treated mice a decrease in tau levels was observed in the SN three days after acute dosage, remaining suppressed until day forty-five post treatment, with a corresponding increase in nigral Fe.[81] In human PD, oxidation of ceruloplasmin, a Cu-binding ferroxidase protein, has shown a similar effect of reducing cellular Fe export leading to increased Fe retention.[82]

Fe is transported into neurons through either delivery by transferrin (Tf) to membrane receptors or via DMT1.[83] DMT1 facilitates the rapid transfer of Fe from the extracellular spaces into the cytoplasm, as either free Fe^{2+} or by mediation of a ferric reductase enzyme.[84] Decreased ferroportin, in addition to increased levels of DMT1 have been observed in the SN dopaminergic neurons of MPTP-treated mice, with corresponding Fe accumulation, though not in similar neurons of the adjacent ventral tegmental area (VTA).[85] Increased expression of DMT1 has also been reported in the SNpc of human PD samples, in line with an observed elevation in DMT1 expression in MPTP intoxicated mice.[86] It is still unclear as to why DA neurons in the VTA of mice are resistant to MPTP challenge yet adjacent neurons in the SNpc are vulnerable, though recent evidence suggested that VTA neurons demonstrate a functional genetic response to MPTP insult including ion/metal regulatory genes (*PANK2* and *Car4*) that is not observed in SNpc DA neurons.[87]

Increased Fe in at-risk areas of the brain has been extensively described in both idiopathic PD and MPTP models. Indeed, Fe accumulation is a common theme in various neurological disorders with parkinsonian pathology, as reviewed by Berg and Hochstrasser,[88] as well as generalised inflammation in PD.[89] While it is apparent that elevated Fe contributes to MPTP-mediated neuron loss, the precise mechanism by which Fe stimulates oxidative damage is still not clear; in fact it is probable that perturbations to Fe regulatory systems within the cell contribute to the formation of toxic species through multiple pathways. Commonality between Fe accumulation and oxidative stress would suggest Fe involvement in neuron loss is not necessarily a causative factor in PD; however altered iron metabolism has shown promise as a target for novel therapeutics, justifying targeting cellular Fe handling as an import field of study. Regardless, most reports have described Fe-related factors in isolation, due mostly to the difficulties in observing Fe metabolism as a whole. Improving techniques will ensure that Fe and MPTP toxicity, and thus PD pathogenesis will remain a keenly investigated topic for some time.

MPTP stimulates a cellular metallomic response

Changes to redox-active metal levels in the PD brain may not directly contribute to elevated levels of oxidative stress; metals

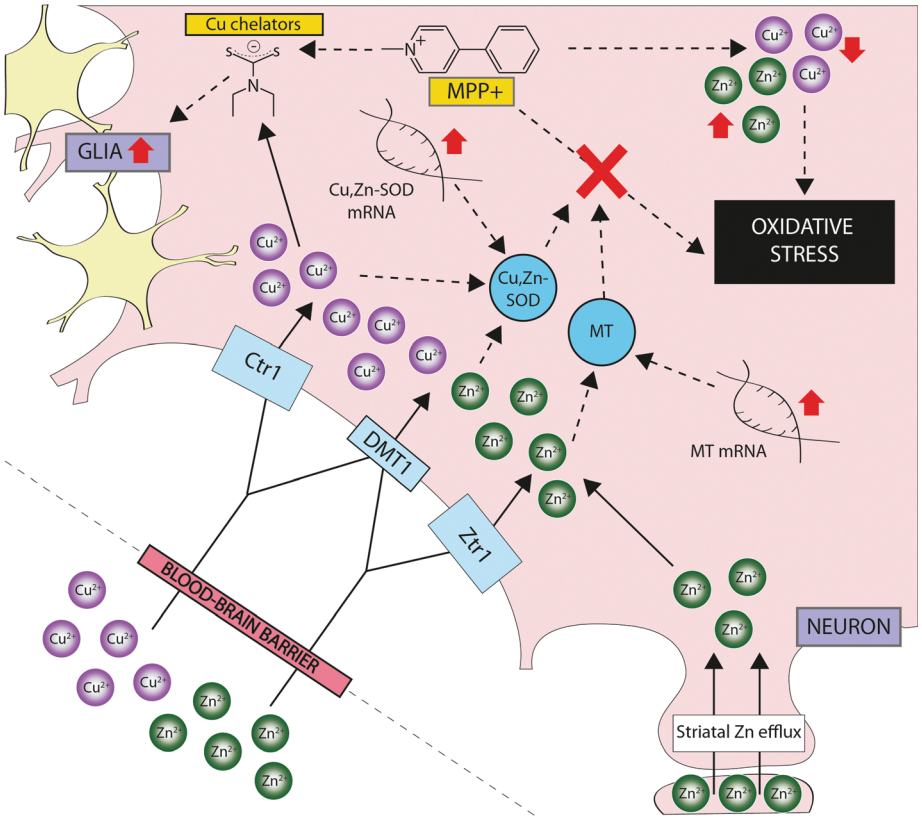


Fig. 2 Proposed mechanism for Cu- and Zn-mediated cellular response to MPTP insult. MPP^+ = 1-methyl-4-phenylpyridinium, Ctr1 = copper transporter 1, Ztr1 = zinc transporter 1, DMT1 = divalent metal transporter 1, Cu,Zn-SOD = Cu,Zn superoxide dismutase, MT = metallothionein.

such as Cu and Zn are also key functional components of antioxidant proteins (see Fig. 2). The changes in metal levels observed in MPTP are most likely intertwined: altered Fe levels induce increased oxidative stress, leading to an influx of metal-binding proteins (such as superoxide dismutases – SOD) that scavenge the evolved free radicals, mitigating cell damage. Major proteins with antioxidant properties include Cu, Zn- and Mn-SOD and Cu/Zn binding metallothioneins (MTs).

Cellular Zn homeostasis has been implicated as a factor that may be involved in neurodegenerative processes and diseases, with Zn ionophores showing efficacy in restoring cognition in animal models of Alzheimer's disease.[90,91] Increasing concentrations of Zn^{2+} with age may contribute to increased mitochondrial ROS formation.[92] It is thus not surprising that Zn has exhibited dyshomeostasis in MPTP models of PD. Mice exposed to acute doses of MPTP possessed degenerating neurons in the SNpc that showed an accumulation of cytosolic Zn compared to saline-treated controls,[93] though increased brain Zn may be more generally indicative of brain trauma.[94] Mice administered moderate amounts of Zn did not display depleted DA levels, though co-administration of Zn with MPTP significantly lowered DA, suggesting a possible potentiating effect of Zn on MPTP toxicity.[95] Zn may, however, be indicative of a cellular response to MPTP injury involving endogenous antioxidant proteins like Cu,Zn-SOD. 90% of brain Zn is protein-bound, the remaining 10% of labile Zn is confined to presynaptic vesicles.[96] Rojas *et al.*[97] reported that, following MPP^+ insult, the number of Zn-positive terminals decreased in the mouse striatum and increased in the stratum radiatum and stratum oriens regions of the CA2 zones of the hippocampus. This efflux of Zn could be indicative of a rapid mobilisation of ionic Zn preceding synthesis of MT[98] and Cu,Zn-SOD, both antioxidant proteins known to scavenge free radicals.[99] Both of these proteins have shown biological importance in PD more broadly, and MPTP-induced toxicity specifically.

SOD-group enzymes are ubiquitous proteins that utilise a redox-active metal to dismutase O_2^- to O_2 through reduction, or to H_2O_2 through oxidation with the presence of H^+. In humans, SOD exists as either Mn- or Cu,Zn species, both of which are found in the brain and show age-related changes.[100] Synthetic O_2^- mimetics have shown reduced paraquat-induced neuronal toxicity in both cell cultures and adult mice.[101] One hypothesised mechanism of MPTP action involves selective nitration of TH. This process is mediated by production of O_2^- that in turn produces peroxynitrite ($ONOO^-$), nitrating the enzyme and rendering it inactive. In mice overexpressing Cu,Zn-SOD this effect was not observed, suggesting blocking $ONOO^-$ formation by scavenging O_2^- formation can help arrest MPTP toxicity.[102] MPTP administration itself may stimulate increased SOD activity; both Cu,Zn-SOD and Mn-SOD demonstrated a twofold jump in activity in the VTA of treated mice after 7 days of chronic exposure. Interestingly, only Mn-SOD

displayed increased activity in the SN region.[103] Increased nigral Mn-SOD activity was confirmed in additional studies, along with an observed decrease in SN Cu,Zn-SOD activity following MPTP lesioning. Pretreatment of mice with 17β-estradiol, a sex hormone, increased Cu,Zn-SOD and Mn-SOD immunoreactive neurons post-insult, though not reactive glia.[104] Both Cu,Zn-SOD and Mn-SOD null mice display heightened sensitivity to MPTP lesioning, whereas mutation to both *SOD1* (Cu,Zn) and *SOD2* (Mn) genes demonstrate resistance to the toxin compared to control.[20]

In human PD, an increase in Mn-SOD mRNA was observed in line with decreased Cu,Zn-SOD expression in the SN,[105] agreeing with decreased Cu,Zn-SOD and increased Mn-SOD immunoreactivity in the SN of MPTP treated mice.[106] Mn-SOD accounts for around 10% of intracellular SOD isoforms and is a predominately mitochondrial enzyme. Increased expression of Mn-SOD is likely indicative of altered oxidative load arising from mitochondrial dysfunction. In MPTP toxicity, O_2^- released from mitochondria reacts with nitric oxide (NO) to form $ONOO^-$. Inhibition of NO synthases prevents MPTP toxicity in baboons;[107] thus increased Mn-SOD activity chaperoning errant O_2^- could similarly limit formation of this reactive nitrogen species (RNS).

MPTP lesioning of common marmosets demonstrated loss of Cu,Zn-SOD mRNA throughout the basal ganglia, most marked in the SNpc. This loss of a major antioxidant enzyme (in conjunction with depletion of glutathione peroxidase in both the SNpc and SNr) has also been observed in human PD patients,[108] further supporting the hypothesis that metal dyshomeostasis in MPTP-mediated cell death is indeed reflective of PD etiology. Little information is available directly comparing Cu,Zn-SOD activity and total Cu and Zn levels in either MPTP models or human PD. Analysis of blood taken from PD patients found red cell Cu,Zn-SOD activity was increased, as was red cell Cu and Zn, in addition to plasma Cu levels compared to control donors.[109] It is difficult, however, to infer the significance of altered circulating Cu,Zn-SOD activity compared to expression in the brain. Correlating Cu,Zn-SOD and total metal levels is challenging, considering that these metals are also active components of other metal-binding proteins. Cu,Zn-SOD is a very active Cu and Zn binder, and any loss of capacity to uptake free Cu and Zn may increase intracellular metal levels and thus redox activity.[110] However, the role of other proteins with affinity to Cu and Zn increasing uptake of metals in direct response to decreased Cu,Zn-SOD has not been studied and is deserving of attention.

Metallothioneins are a group of 0.5–14 kDa proteins containing approximately 25–30% cysteine residues[111] that have shown potential protective features in a range of genetic PD animal models.[112] The large sulfur-component of MTs results in a strong affinity to group 11 and 12 metals, including endogenous Cu and Zn and heavy metals such as cadmium (Cd) and mercury (Hg). These cysteine residues are also capable of chaperoning ROS, removing the threat of cellular damage through reduction of the harmful species. Like SOD, MTs are thought to play a role in mitigating oxidative damage through attenuation of $ONOO^-$ synthesis.[113] While metal ions do not play a direct role in ROS or RNS removal by MTs, the mere presence of elevated metal concentration can induce MT expression. In SK-N-SH cell lines grown in Zn-rich media increased MT expression help prevent cell death from methamphetamine toxicity.[114,115] Induction of MT expression by exposure to Cd and dexamethasone (a known MT induction factor) five hours prior to MPTP insult resulted in increased DA levels compared to non-induced animals.[116] However, there is some contention regarding the role of MT isoforms following MPTP neurotoxicity. Rojas and Klaassen[117] reported that the DA denervation by MPTP was not attenuated in MT knock-out mice. MT expression was, however, observed to decrease in the striatum of mice in response to a MPP^+ challenge;[118] this observation was supported by data demonstrating a reduction in MT-1 mRNA.[119,120] Unfortunately, relatively little information is present in literature describing changes in MT levels in the PD brain. Further study into how MTs are expressed and distributed in the human dopaminergic pathway is required.

In addition to its role in promoting oxidative stress through changes to the labile Fe pool, other metalloenzymes also rely upon Fe for activity. TH uses O_2, tetrahydrobiopterin and Fe as cofactors in its role in DA synthesis. Loss of TH activity precludes DA conversion from L-DOPA, accounting for marked DA decrease following MPTP administration. Monkeys given an MPTP insult displayed decreased TH mRNA compared to unlesioned animals.[121] In human PD, surviving dopaminergic neurons have shown similar decreases in TH mRNA levels.[122]

Metal chelators arrest MPTP and MPP^+ neurotoxicity

If the hypothesised changes in the labile Fe pool are a direct cause of increased oxidative stress in PD (and the MPTP model), it would thus stand to reason that modulation of redox active metals like Fe may aid in reducing the impact of sudden metal dyshomeostasis.

3-Hydroxypyridine-4-one (deferiprone) has shown *in vitro* neuroprotective properties against a range of oxidative stress stimuli, including MPP^+, amyloid beta (Aβ), H_2O_2 and Fe^{3+} in neuroblastoma SHSY-5Y cells.[123] Active metal chaperones like the orally bioavailable and blood–brain barrier-permeable 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol) and associated 8-hydroxyquinoline analogues have shown potential as therapeautics for several neurodegenerative disorders,[124] even so far as to demonstrate applicability in restoring cognition in animal models of Alzheimer's disease[90] and improving executive function in human clinical trials.[125] In clioquinol-treated animals given acute MPTP doses, increases in markers of oxidative stress and corresponding decreases in cellular antioxidants normally seen following MPTP leisoning were not observed.[57] 5-(*N*-Methyl-*N*-propargyaminomethyl) 8-hydroxyquinoline, or M30, is a Fe-ionophore that also shows MAO-A and -B inhibitory properties. Acute and chronic dosing of animals with M30 results in the expected hallmarks of MAO inhibition, including increased DA, serotonin and noradrenaline levels, and

corresponding decreases in monoamine metabolites. Administration of M30 at the time of MPTP insult resulted in attenuated DA depletion.[126] M30 may attenuate the effects of MPTP when concurrently administered in two possible ways: by either inhibiting MAO-B mediated conversion of MPTP to MPP^+ in glia; or by chelating free Fe required for MPTP oxidation, both of which disrupt MPTP metabolism. Oral administration of M30 for 14 days following MPTP insult was shown to offer some degree of restitution to nigrostriatal dopaminergic neurons, with increased striatal DA levels and a restoration of transferrin receptors (TfR) in the SNpc to within 20% of control levels. Increased DA levels are again indicative of MAO inhibition, and increased TfR-positive cells observed in M30-only treated animals were found in similar numbers in MPTP treated animals, suggesting Fe-chelation by M30 affects Fe transfer in and out of cells via Tf.[127] The MAO inhibitory properties of 8-hydroxyquinoline compounds stem from the *N*-propargyl group common to clinical anti-Parkinson's drugs rasagiline and selegiline.[128] Selegiline has shown significant effectiveness in both protecting against MPTP toxicity (as does rasagiline)[129] and slowing PD progression in human trials.[130] While MAO inhibition may prevent both MPTP to MPP^+ conversion in animal models, as well as limiting DA autoxidation in human PD, interaction with metal metabolism may be present. Selegiline has been suggested to both promote SOD expression, and limit Fe-mediated DA oxidation in several models of PD, including MPTP,[131] which may reflect a partial mode of action for the compound in humans independent of MAO inhibition.

R-Apomorphine is a MAO inhibitor and dopamine D_1–D_2 receptor agonist that has demonstrated similar effectiveness to L-DOPA as a treatment for PD in humans.[132] In addition to its affinity to dopamine receptors, *R*-apomorphine is also an effective free radical scavenger and Fe-chelator that, when concurrently administered to mice with MPTP, showed possible neuroprotective features suggestive of antioxidant properties, as opposed to impeding MPP^+ formation through MAO inhibition.[133] Ginsenoside Rg1, an active component of ginseng root has shown potential beneficial properties in pretreated mice exposed to MPTP. Rg1 treated animals displayed lower Fe-positive cell densities following MPTP administration, with decreased DMT1 and increased ferroportin expression, indicative of a shift towards pre-toxin Fe trafficking mechanisms.[134] Similar effects were observed in Rg1 pre-treated MES23.5 cells exposed to MPP^+.[135]

The Fe^{3+} chelator deferrioxamine (also known as desferal and desferrioxamine) has shown conflicting data regarding its role in either potentiating or preventing MPTP toxicity. In MPP^+ treated rats, deferrioxamine was shown to enhance the formation of hydroxyl radicals in the striatum.[136] Conversely, pretreatment of SK-N-SH dopaminergic neurons in culture with deferrioxamine and coenzyme Q10 (an antioxidant enzyme) was shown to reduce the impact of Fe loading.

Administration of diethyldithiocarbamate, a Cu chelator, to mice treated with MPTP resulted in potentiation of MPTP neurotoxicity with corresponding DA depletion and increased astrocyte activity at the site of lesion.[137] Cu-chelation does not necessarily result in MPTP potentiation; D-penicillamine (used to chaperone Cu in Wilson's disease) did not increase striatal DA depletion as diethyldithiocarbamate did; instead it showed some, albeit modest, prevention of MPTP-induced dopamine loss.[138] For further information, a range of Cu complexing agents was recently review by Duncan and White.[37]

Chaperoning of metals via chelating or ionophoric compounds has shown great potential as viable therapeutics for treating neurodegenerative diseases, which is reflected by the range of compounds studied within the context of MPTP-induced neuron loss (Table 1). Thus, the importance of properly elucidating the role of metals in PD and the suitability of MPTP as a model for metal-based neurodegeneration is integral to the development of next generation chelators and ionophores.

Metals and alternative parkinsonian toxins

Widespread use of MPTP to model PD is due not only to its clinically indistinguishable recapitulation of parkinsonism in humans and non human primates, but also its comparatively simpler method of administration compared to other neurotoxins.[16] However, distinct differences in the mode of toxicity of MPTP and alternative nigral insults cannot be disregarded when discussing the appropriateness of MPTP as a model for PD, particularly in relation to relating disease progression to metal metabolism.

6-Hydroxydopamine (6-OHDA) is a catecholamine with selective toxicity shown towards both DA and noradrenergic neurons.[139] Administration of 6-OHDA is somewhat more complicated; requiring stereotaxic injection of 6-OHDA into the nigra itself, medial forebrain bundle or caudate-putamen to produce the desired lesion of the nigrostriatal pathway.[140] A major advantage of 6-OHDA over MPTP is the reported ability of the toxin to produce limited effects on the contralateral side, and a controllable loss of dopamine in the ipsilateral hemisphere.[141,142] Controlled administration of 6-OHDA to only the nigrostriatal pathway, whilst leaving the mesolimbic pathway unaffected produces a lesion that is reflective of the pattern of cell loss observed in idiopathic PD.[143]

Bilateral elevation in nigral Fe following unilateral 6-OHDA administration has been observed, though significant retention of Fe at the site of injection was also still observed 21 days post-lesion.[144] Three-dimensional reconstruction of Fe distribution surrounding the SN using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) imaging found that this artefact arising from the lesioning procedure did not contribute to nigral Fe levels,[145] though most common analytical techniques for measuring metals lack the spatial specificity to resolve these sources of Fe. Ultrasound analysis of Fe in the SN of lesioned rats has also shown an increase Fe levels.[146] Significant unilateral reduction in striatal Fe and Cu and increase in periventricular Cu, measured by LA-ICP-MS, has also been reported 42 days after 6-OHDA administration.[147]

The effect of 6-OHDA on metal metabolism in the nigrostriatal pathway does mirror MPTP in some key features. In cell culture down-regulation of ferroportin following 6-OHDA insult

Table 1 Metal chelators used in MPTP neurotoxicity research

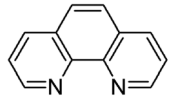
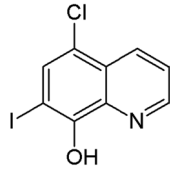
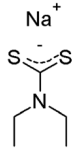
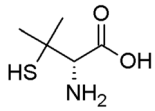
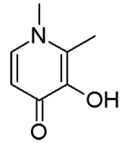
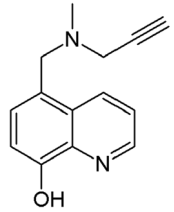
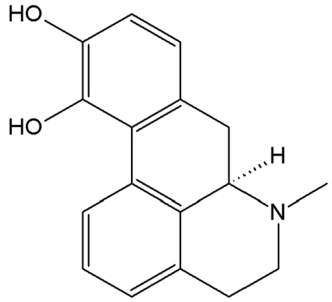
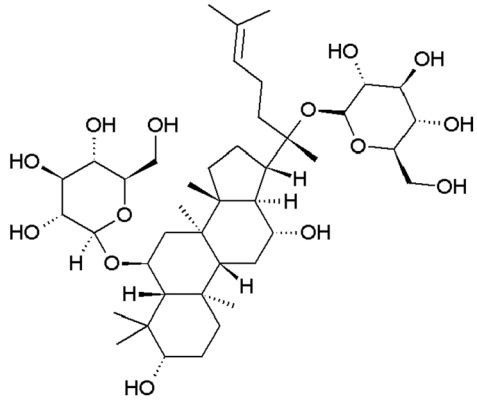
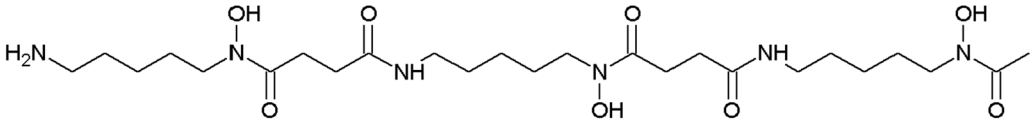
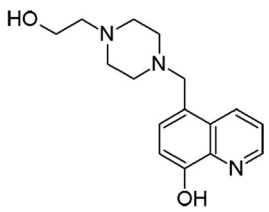
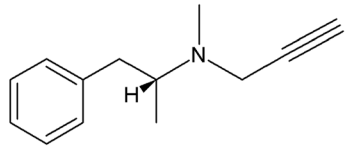
Structure	Name (IUPAC nomenclature)	Metal affinity	Ref.
	Phenanthroline (1,10-phenanthroline)	Low (Fe)	46
	Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline)	Low (Fe, Cu, Zn)	57,176
	Sodium diethyldithiocarbamate (sodium (diethylcarbamothioyl)sulfanide)	High (Cu)	137,177
	D-Penicillamine ((2*S*)-2-amino-3-methyl-3-sulfanyl-butanoic acid)	Medium (Cu)	138,178
	Deferiprone (3-hydroxypyridine-4-one)	High (Fe), low (Cu, Zn)	123,179,180
	M30 (5-(*N*-methyl-*N*-propargyamino-methyl)8-hydroxyquinoline)	Low (Fe)	126,181
	*R*-Apomorphine ((6*aR*)-6-methyl-5,6,6*a*,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline-10,11-diol)	High (Fe)	133

Table 1 (*continued*)

Structure	Name (IUPAC nomenclature)	Metal affinity	Ref.
	Gensenoside Rg1 (6,20-bis(β-D-glucopyranosyl)-(3β,6α,12β,20*S*)-3,6,12,20-tetrahydroxydammar-24-ene)	Low (Fe)	134,135
	Deferioxamine, desferrioxamine, deferal (*N*′-{5-[acetyl(hydroxy)amino]pentyl}-*N*-[5-({4-[(5-aminopentyl)(hydroxy)amino]-4-oxobutanoyl}amino)pentyl]-*N*-hydroxysuccinamide)	High (Fe)	46,136,176,182,183
	VK-28 (5-[[4-(2-hydroxyethyl)-1-piperazinyl]methyl]-8-quinolinol)	High (Fe)	155,176
	Selegiline ((*R*)-*N*-methyl-*N*-(1-phenylpropan-2-yl)prop-1-yn-3-amine)	Unknown	129,131

may contribute to Fe accumulation,[148,149] as may a similar up-regulation of DMT-1.[150] 6-OHDA also elicits a similar up-regulation of MT levels following exposure.[151] Some interesting differences between the two toxins do exist; cell culture studies of Fe^{3+} and Cu^{2+} potentiation of 6-OHDA in SH-SY5Y cells found Cu accelerated 6-OHDA auto-oxidation and promoted metal-mediated DNA damage to a greater degree than Fe alone.[152] Iron release from ferritin following 6-OHDA administration appears to subsequently accelerate autoxidation of the toxin.[153] SOD-1 itself directly inhibits 6-OHDA autoxidation by up to 96% *in vitro*.[154] Several metal chelators show similar effects in arresting toxicity as those used in MPTP research, including VK-28, an 8-hydroxyquinoline analogue,[155] and Rg1.[156] Desferrioxamine-pretreated rats showed significant protection against 6-OHDA-induced DA loss,[157] as did deferiprone.[158]

As mentioned previously, rotenone and paraquat (1,1′-dimethyl-4,4′-bipyridinium) are two commercial pesticides with structurally similarities to MPTP and MPP^+. The primary mechanism of rotenone toxicity also involves complex I inhibition, and pathological features of rotenone neurotoxicity include the presence of Lewy body-like cytoplasmic inclusions containing ubiquitin and α-synuclein,[159] which is an important feature of idiopathic PD not reflected in the MPTP paradigm. Paraquat does not directly interact with the mitochondrial electron transport chain, instead it partakes in redox cycling to produce the same increase in oxidative stress.[160] Relatively sparse information is available regarding the role of metals in rotenone and paraquat toxicity and how it relates to PD. Ceruloplasmin-null mice exposed to a low dose of rotenone over 28 days showed a significant effect on motor behaviour, pathology and oxidative stress markers compared to wild types,[161] suggestive again of antioxidant properties of the Cu ferroxidase protein. Rotenone also increases H-ferritin mRNA and protein synthesis in NIH3T3 fibroblasts and SH-SY5Y neuroblastoma cells.[162] Direct comparison of paraquat and MPP^+ toxicity in cell culture found that both molecules have a similar deleterious impact on cellular mRNA and protein expression related to intracellular iron transport. Paraquat toxicity is sometimes coupled with administration of complex III inhibitor maneb (manganese ethylene-bis-dithiocarbamate) to produce a modest, yet consistent lesion of the nigrostriatal pathway with minimal damage to surrounding regions.[163]

Lactacystin is a relatively new alternative to MPTP for modelling PD in animals, stemming from concern regarding the failure of MPTP to reproduce all pathological features of PD. Lactacystin is a potent proteosomal inhibitor that, when injected directly into the SN reduces striatal DA and induces motor dysfunction in rats, as well as replicating Lewy-body formation in the cytoplasm by impairing the ubiquitin proteasome system (UPS),[164,165] though it lacks the consistency of the lesion produced by MPTP. Iron regulatory gene profiling of cells exposed to lactacystin found evidence that the toxin promotes TfR expression, but not DMT-1 and mitochondrial Fe regulatory proteins as with MPP^+.[166] Lactacystin-exposed MES23.5 cells also demonstrated H-unit ferritin and increased labile Fe,[167] mirroring the effects of protracted MPTP exposure. Genetic overexpression of H-unit ferritin was shown to reduce lactacystin-mediated DA depletion and neuron loss.[168] As is the case with MPTP, several Fe chelators have shown some degree of protection and rescue in animals exposed to lacatcystin, including the 8-hydroxquinilone M30[169] and desferrioxamine,[170] suggesting Fe is also integral in the mode of action of lacatacystin.

Chronic co-administration of MPTP and probenecid (MPTP/P; 4-(dipropylsulfamoyl)benzoic acid) is often used to enhance nigrostriatal damage by inhibiting the urinary and neuronal excretion of toxic MPTP metabolites.[171,172] MPTP/P retains the hallmarks of traditional MPTP lesioning, whilst also producing proteinaceous inclusions with α-synuclein and ubiquitin immunoreactivity in both the SN and cortex.[173] This emerging model presents an intriguing possibility to study the metallobiology of PD in a system exhibiting both Fe elevation and metal–protein aggregation. Copper was recently shown to play a role in ubiquitin aggregation *in vitro*.[174] Whilst ubiquitin accumulation is not unique to Parkinson's disease,[175] this toxin model provides a more complete picture of both PD pathology, as well as metal-mediated neurodegeneration.

Conclusion

MPTP remains the most commonly used model for preclinical testing of new therapies for PD, as well as playing an important role in investigating the mechanisms of dopaminergic neuron loss.[16] Most research into MPTP metallobiology tends to examine factors in isolation, rather than investigating the effect of altered metal homeostasis on the multitude of intersecting pathways that are relevant to the pathogenesis of PD. As such, there are several examples where interpretation of changes in metal content following MPTP administration may not be truly indicative of the toxin's mode of action, and thus not representative of idiopathic PD.

This does not, however, discredit the use of MPTP as a viable PD model for studying metal-mediated cellular response. Significant evidence points to an apparent dual mechanism of toxicity for MPTP, through both complex I inhibition and increasing the labile Fe pool in the cell. These changes in harmful Fe levels appear to mirror the alterations in Fe observed in human PD cases, as do the changes in expression of important metal-binding enzymes, including Cu,Zn-SOD and Mn-SOD. Several therapeutic agents that have shown potential in treating PD have a mechanism of action that is also attributed to an affinity for redox-active metals, which has been shown in MPTP models of disease.

A major barrier to building a comprehensive picture of metallobiology in PD stems from the ubiquity of the very metals implicated in the disease, in that their varied roles and functions are difficult to study *in toto*. Viewing each metal-mediated process in isolation cannot account for the knock-on effects of altered metal homeostasis. Further development of more holistic systems biology approaches to studying metal metabolism will help better understand not only MPTP as a model for PD, but cell metallobiology as a whole.

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