Iron in multiple sclerosis: roles in neurodegeneration and repair

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Abstract

MRI and histological studies have shown global alterations in iron levels in the brains of patients with multiple sclerosis (MS), including increases in the iron stored by macrophages and microglia. Excessive free iron can be toxic, and accumulation of iron in MS has generally been thought to be detrimental. However, iron maintains the integrity of oligodendrocytes and myelin, and facilitates their regeneration following injury. The extracellular matrix, a key regulator of remyelination, might also modulate iron levels. This Review highlights key histological and MRI studies that have investigated changes in iron distribution associated with MS. Potential sources of iron, as well as iron regulatory proteins and the detrimental roles of excessive iron within the CNS, are also discussed, with emphasis on the importance of iron within cells for oxidative metabolism, proliferation and differentiation of oligodendrocytes, and myelination. In light of the beneficial and detrimental properties of iron within the CNS, we present considerations for treatments that target iron in MS. Such treatments must balance trophic and toxic properties of iron, by providing sufficient iron levels for remyelination and repair while avoiding excesses that might overwhelm homeostatic mechanisms and contribute to damage.

Introduction

Multiple sclerosis (MS) is an inflammatory and degenerative condition characterized by damage to the myelin sheath that surrounds axons, as well as to oligodendrocytes that produce myelin. The cause of MS continues to be elusive, but several factors that lead to neurodegeneration have been identified, including inflammatory molecules, protease dysfunction, glutamate excitotoxicity, and mitochondrial abnormalities. Dysregulation of iron metabolism, and the resultant cytotoxicity, is increasingly being implicated in MS. For example, areas of hypointensity (darkness) seen on T2-weighted or T2*-weighted MRI in patients with MS might be caused by iron deposits, as iron is paramagnetic and causes a loss of phase coherence among hydrogen atoms.[1] Aside from conventional MRI, specialized MRI methods such as susceptibility-weighted imaging, quantitative susceptibility mapping and R2* (the reciprocal of T2*)—which are very sensitive to iron contained in deoxyhaemoglobin, ferritin and haemosiderin[2]—have corroborated postmortem histological evidence of elevated iron content in the brains of patients with MS.

In this Review, we provide an overview of the roles of iron in cellular processes that are relevant to MS, and then discuss the potential pathogenic and reparative roles that iron modulation might have in this condition.

Iron accumulation in MS

MRI has been used to investigate iron deposition in the brains of living patients with MS. Elevated iron content has been detected using susceptibility MRI in deep grey matter structures, and is associated with increased disability and grey matter atrophy (Table 1). In support of these MRI results, neuropathology at autopsy reveals substantial degeneration of deep grey matter structures, which corresponds with iron accumulation and oxidative damage.[3] Moreover, by comparing measures from the same individual before and after death, changes indicative of iron accumulation detected by susceptibility MRI have been shown to correlate with increased iron content revealed by postmortem histopathology[4] or X-ray fluorescence (Figure 1).[5] In the vicinity of lesions, susceptibility MRI has revealed *in vivo* changes that are suggestive of iron deposition (Table 2) in areas that demonstrate myelin loss, focal iron deposits or both at autopsy.[6,7]

Histopathological analysis of brain tissue provides important subcellular information on iron regulation in MS. In a healthy adult brain, iron is mainly found in oligodendrocytes, and the discovery of similar levels of iron accumulated in microglia and other macrophages in patients with MS suggests that a pathogenic process is occurring.[7]

Cytotoxicity of iron build-up in the CNS

Iron's ability to exchange electrons and switch between ferrous (Fe^{2+}) and ferric (Fe^{3+}) states—known as redox switching—makes it important in many biochemical processes. Iron redox switching is integral to numerous aspects of nervous system function, such as the synthesis of neurotransmitters and DNA.[8] Iron is also a component of the cytochrome haem group involved in

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Key points

- Multiple sclerosis (MS) involves profound destruction of oligodendrocytes and myelin
- MRI and histological studies suggest that iron levels are dysregulated in MS: iron accumulates in grey matter and is depleted in normal-appearing white matter
- Iron accumulation promotes cytotoxicity through a variety of mechanisms, including chemical reactions leading to oxidative stress, increased proinflammatory cytokine levels, glutamate toxicity, and impaired DNA repair
- Iron is a cofactor for a variety of enzymes involved in maintaining the health of oligodendrocytes and myelin, and may be a crucial component of remyelination
- Treatments for iron excess in MS must address not only the increased iron levels within grey matter, but also the requirement for iron in remyelination and repair
- The extracellular matrix, a key regulator of remyelination, may also modulate iron availability

xenobiotic metabolism, and of proteins that are crucial for energy production via the electron transport chain.[9] Iron's redox-switching ability also means it can be a dangerous catalytic element.

Under healthy conditions, iron is bound to a ligand; free (unbound) iron can participate in reactions that produce toxic reactive oxygen species.[10] Ferrous iron (also known as redox-active iron) can react with hydrogen peroxide via the Fenton reaction, producing the extremely reactive hydroxyl radical (Figure 2).[10,11] The hydroxyl radical can oxidize membrane lipids, denature proteins, damage DNA, increase intracellular free calcium concentrations, and cause cell dysfunction and death.[12,13] The reactivity of this free radical is essentially limited by its rate of diffusion. After producing a hydroxyl radical, ferrous iron is oxidized to ferric iron. Redox cycling of iron can then occur when a reducing equivalent, such as superoxide, reacts with ferric iron to regenerate redox-active iron.[14]

In cell cultures, elevated iron levels can directly damage DNA by interfering with the function of DNA repair enzymes.[15] Mitochondrial DNA is more sensitive to damage than is nuclear DNA,[16] and although mitochondria are generators of reactive oxygen species even under normal conditions, owing to oxidative metabolism, damaged mitochondria have increased leakage of electrons, and generate excessive reactive oxygen species.[17] Patients with MS show evidence of widespread mitochondrial damage,[18,19] and the consequent increase in production of reactive oxygen species can promote iron-mediated oxidative damage that leads to progressive genomic damage and further impairment of mitochondrial function.[20]

Owing to the extremely reactive nature of the hydroxyl radical, the sites of iron reactions and hydroxyl radical production are important considerations for determining potential cytotoxic effects. In cell culture, iron overload is toxic to neurons and oligodendrocytes, but astrocytes seem to be resistant to this toxicity.[21] The increased resistance of astrocytes to reactive oxygen species resulting from iron excess might be attributable to their superior ability to upregulate antioxidants, such as glutathione, compared with neurons and oligodendrocytes.[22–24]

The high rate of oxidative metabolism and high intracellular iron concentrations in oligodendrocytes,[7,25–27] as well as the intensive protein and lipid synthesis necessary to maintain the lipid-rich myelin sheath, might contribute to the increased susceptibility of these cells to oxidative stress.[24,28] The ability of iron to promote

Table 1 Evidence of global iron perturbation in MS

Disease setting	Observation	Detection method	Evidence
MS, CIS	Iron deposits in deep grey matter structures	Susceptibility MRI (R2*, phase-contrast, SWI, quantitative susceptibility mapping)	Changes suggestive of iron deposition in the caudate,[112–115] putamen,[112,114,115] globus pallidus,[112–116] pulvinar nucleus of the thalamus,[113,115,116] thalamus[113] and substantia nigra[113,116]
MS, CIS, RRMS, SPMS	Iron deposition is associated with disease progression in MS Less iron deposition in CIS than in RRMS and SPMS	Susceptibility MRI (R2*, SWI)	Significantly higher basal ganglia R2* values in RRMS than in CIS, suggestive of greater iron deposition in RRMS[117] Abnormal phase changes in subcortical deep grey matter, suggestive of iron accumulation, are greater in SPMS than in RRMS[118]
MS	Iron accumulation strongly correlates with disability progression	MRI (T2-weighted, R2*, phase-contrast), EDSS scores	Worsening EDSS scores correlate with MRI findings suggestive of iron deposition in the caudate,[119] putamen,[119] thalamus,[116,119] pulvinar nucleus of the thalamus,[113,116] red nucleus,[113,116] substantia nigra[113] and globus pallidus[113]
MS	Iron accumulation correlates with grey matter atrophy	Susceptibility MRI (R2*,SWI)	Increased R2* values in the putamen, globus pallidus and caudate correlate significantly with overall grey matter atrophy[117] Changes in phase correlate significantly with atrophy in the caudate, pulvinar nucleus of the thalamus, caudate and putamen[118]
Chronic MS	Iron levels are reduced in NAWM and correlate with disease duration	Histopathology, densitometry	Significantly decreased iron load in NAWM[34] Loss of oligodendrocytes and reduction in hephaestin[34]
MS	Tissue iron and/or deoxyhaemoglobin and/or myelin reduced in NAWM	Susceptibility MRI (R2')	Reduced R2' values in NAWM of patients with MS compared with controls[120]

Abbreviations: CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; NAWM, normal-appearing white matter; RRMS, relapsing–remitting MS; SPMS, secondary progressive MS; SWI, susceptibility-weighted imaging.

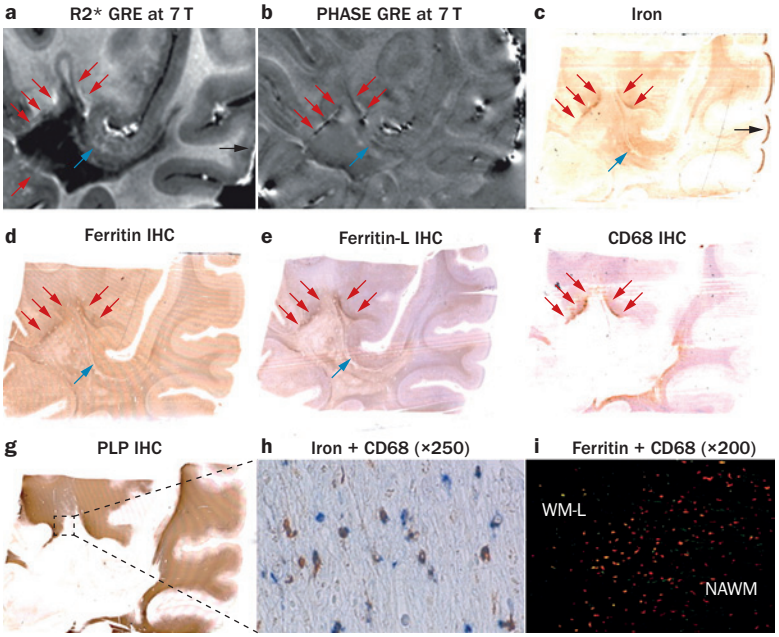

Figure 1 Iron deposits in MS revealed through imaging and histopathology. The rim of a white matter lesion (red arrows) in a patient with secondary progressive MS revealed by postmortem **a**–**b** susceptibility MRI (R2*, phase-contrast), and **c**–**f** tissue staining for iron and ferritin, which colocalize with staining for microglia and macrophages (CD68$^+$ cells). Blue arrows highlight a region of iron accumulation located perivascularly. **g**–**i** PLP staining indicates that the same area has mild to moderate demyelination; the demyelinated area shows overlapping staining for iron (brown) and CD68$^+$ microglia and macrophages (blue). Similarly, double immunofluorescence reveals colocalization (yellow) of ferritin (green) and CD68 (red). Abbreviations: GRE, gradient recalled echo; IHC, immunohistochemistry; NAWM, normal-appearing white matter; PLP, proteolipid protein; WM-L, white matter lesions. © Bagnato, F. *et al.* Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. *Brain* (2011), **134** (12), 3599–3612, by permission of Oxford University Press.[6]

Table 2 Evidence of local iron perturbation in MS

Disease setting	Observation	Methods	Evidence
MS	Iron staining in macrophages and microglia, around sites of inflammation and near demyelinated plaques	Susceptibility MRI (R2*, phase-contrast), histopathology	High R2* and low phase values in lesion rims, correlate with ferritin and iron deposits that colocalize with microglia[7]
MS	Late active lesions have increased iron load, whereas inactive or remyelinated lesions show iron loads similar to or reduced compared with controls	Histopathology, densitometry	Highest levels of iron at the edges of classic active lesions; no iron deposits in remyelinated plaques[34] Iron deposits correlate with ferritin, detected in macrophages and microglia[34]
Chronic MS	Lesions show dramatic loss of iron and myelin	Susceptibility MRI (R2*, phase-contrast), histopathology	Reductions in R2* correspond with dramatic losses of iron and myelin Negative phase values correspond to focal iron deposition and myelin loss[6]
EAE	Iron deposition in CNS, specifically around vessels and in areas of inflammation	MRI (susceptibility-weighted, T2-weighted), histopathology	Iron deposition‡ in the brain[44,121] and lumbar spinal cord[43,121] Iron deposits colocalize with microglia[44,121] and astrocytes[121]

‡Areas of dark or hypointense signal. Abbreviations: EAE, experimentally induced autoimmune encephalomyelitis; MS, multiple sclerosis.

glutamate release might also contribute to oligodendrocyte death by inducing excitotoxicity via *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors on these cells.[29–32] In addition, oligodendrocyte progenitor cells (OPCs), which are implicated in remyelination in MS, have heightened susceptibility to metabolic stress compared with oligodendrocytes.[33] In lesion environments, therefore, iron-mediated oxidative stress will have implications not only for oligodendrocyte cytotoxicity, but also for attempts at repair. As noted above, in patients with MS, iron accumulates in macrophages and microglia around the rim of lesions, and these iron-laden cells show signs of dystrophy.[34] The acidic lysosomes in macrophages and microglia would enable redox-active iron to be present in high concentrations, and destruction of iron-loaded macrophages and microglia would release redox-active iron into the extracellular space.

Iron might also induce cytotoxicity in MS by activating immune cells and affecting their polarization. Macrophages (including microglia) respond to diverse stimuli by becoming polarized towards different phenotypes. The classic M1 phenotype of activated macrophages is proinflammatory, promoting type 1 T-helper cell responses and amplifying immune responses, whereas the M2 phenotype is associated with increased debris clearance and is considered regulatory and anti-inflammatory.[35,36] Iron overload in macrophages promotes a proinflammatory M1 activation state.[37,38]

The presence of activated immune cells—which is observed in MS, especially in the relapsing–remitting stage—is a key source of reactive oxygen species that can amplify iron-mediated oxidative stress.[39] Furthermore, myelin accumulation in macrophages and microglia might impair the ability of these cells to accumulate intracellular iron: in MS lesions, iron is predominantly stored in M1-polarized, nonphagocytic macrophages and microglia that do not contain intracellular myelin basic protein, and iron accumulation enhances their proinflammatory state.[38] This effect presents intriguing consequences for MS, as large numbers of lipid-laden macrophages and microglia are present in actively demyelinating lesions, which, therefore, have reduced capacity for iron uptake. As a result, extracellular iron accumulates and can be taken up by other cell types, where it causes cytotoxicity.

Causes of iron accumulation

The possible causes of iron accumulation and deposition in the CNS include degeneration of oligodendrocytes and myelin, infiltration of immune cells into sites of neurodegeneration, release of haem following vascular haemorrhage, dysregulation of iron transport proteins and/or other regulatory molecules, and other pathologies. Which of these mechanisms might be involved in MS is not yet known.

Oligodendrocytes are the most metabolically active cells in the brain (probably owing to their role in myelination), and contain an abundance of iron-requiring enzymes that are important for oxidative metabolism. In addition, oligodendrocytes are rich in ferritin-bound

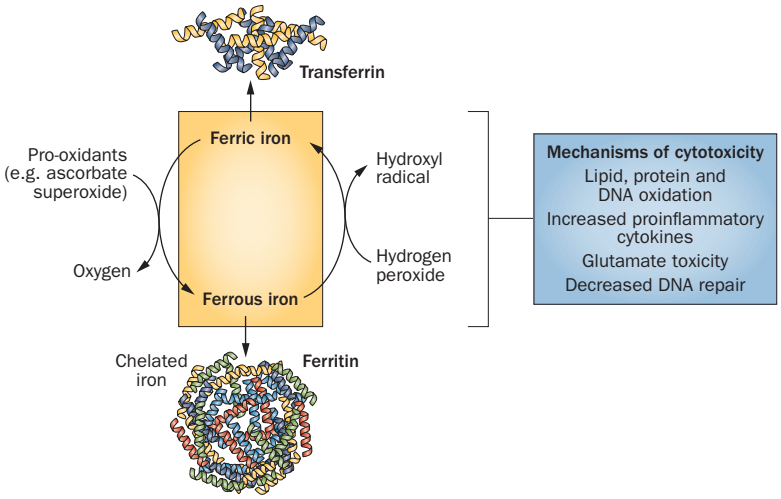

Figure 2 Iron homeostasis and metabolism. Reaction of ferrous iron with hydrogen peroxide produces hydroxyl radicals via the Fenton reaction. Ferric iron can then be recycled back into ferrous iron by pro-oxidants, which enables iron to act as a catalyst for hydroxyl radical production. Iron accumulation promotes cytotoxicity through a variety of mechanisms, including oxidative stress, increased proinflammatory cytokines, glutamate toxicity and impaired DNA repair.

iron.[7,25–27] The profound destruction of oligodendrocytes and myelin sheaths in actively demyelinating MS lesions is one obvious potential source of the iron accumulation observed in activated macrophages and microglia.[34]

Perturbation of iron transport and regulatory proteins might also explain the changes in iron distribution in MS. Immune cells such as monocytes, which infiltrate the CNS during MS pathogenesis and progression, might represent sources of iron, as these cells are involved in systemic iron homeostasis and sequester iron in the periphery in response to inflammatory microenvironments, and iron is present in infiltrating macrophages around the rim of active lesions (Figure 1).[7,34,38]

Vascular damage or interference with the blood–brain barrier—leading to lysis of erythrocytes and degradation of haemoglobin—represents another potential source of excess iron deposition in patients with MS.[40] In support of this concept, histological examinations have revealed the presence of iron around blood vessels,[7,34,41] suggesting the occurrence of microhaemorrhages. MS lesions frequently have a central vein running through them, and iron staining has revealed haemosiderin deposits (a breakdown product of haemoglobin) within and outside white matter lesions in patients with MS.[7,41] Moreover, haemoglobin is a highly reactive molecule, and the nonenzymatic degradation of haemoglobin and haem represents another source of reactive species production and oxidative stress.[42]

Relevance of animal models

Similar imaging and histological observations have been made in mice with experimental autoimmune encephalomyelitis (EAE)—which mimics the inflammatory and demyelinating features of MS—and in patients with MS. Specifically, hypointense areas on susceptibility-weighted imaging[43] or T2*-weighted MRI[44] in mice with EAE correlate with iron deposits, some of which are observed in microglia.[44] In the susceptibility-weighted imaging study, the areas of iron deposition also demonstrated demyelination,[43] similar to that seen using phase imaging in patients with MS.[6]

Despite evidence from MRI and histopathological studies that iron accumulation occurs in both patients with MS and mice with EAE, the results of a study published in 2014 suggested that not all animal models of MS show evidence of iron accumulation.[45] In the few instances where iron deposition was present, it was observed in macrophages around lesions. The researchers also reported that rodents tend to display little to no global iron accumulation during ageing, unlike humans.[45] These observations suggest that most animal models of MS have much lower iron loads and lower levels of oxidative stress than patients with MS.[45] These findings may challenge the relevance of such models for studying the roles of iron and iron-targeted therapies in MS.

Iron depletion in MS

It is interesting to note that in patients with MS, while iron content is elevated in deep grey matter structures and in the vicinity of lesions, iron levels are reduced in the normal-appearing white matter (NAWM), and the extent of iron depletion correlates with disease duration.[34] Furthermore, iron content is low in remyelinated plaques,[34] suggesting that dynamic shuttling of iron continues throughout the MS disease process. These observations reveal that the iron dysregulation associated with MS is not merely global iron accumulation, but a redistribution of iron levels between different areas of the brain.

Roles of iron in cell health and function

Although iron accumulation has a variety of cytotoxic effects, iron is important for normal cell function and repair, so areas of iron deficiency may undergo pathogenetic changes. For example, the high rate of oxidative metabolic activity in oligodendrocytes,[46] and the requirement of iron for enzymes involved in oxidative metabolism as well as for the synthesis and maintenance of myelin, suggest that iron is critical for oligodendrocyte activity and integrity (Figure 3). For example, it is interesting to note that in leukocortical lesions in MS, remyelinating oligodendrocytes are less frequently observed in white matter than in grey matter.[47] A variety of factors might inhibit the activity of oligodendrocytes in white matter, but the iron deficiency observed in NAWM is likely to be a contributory factor.

Iron is needed for a variety of functions that are crucial for cellular health and normal cell cycle progression. Iron has an integral role in energy production as a cofactor for the cytochrome oxidase system and mitochondrial respiratory complexes I–IV.[48] Enzymes involved in metabolism and biosynthesis that require iron to function include glucose-6-phosphate 1-dehydrogenase (G6PD), dioxygenase, succinate dehydrogenase and NADH dehydrogenase.[46] G6PD is especially important for the pentose phosphate shunt, which provides NADPH for myelin fatty acid synthesis.[46]

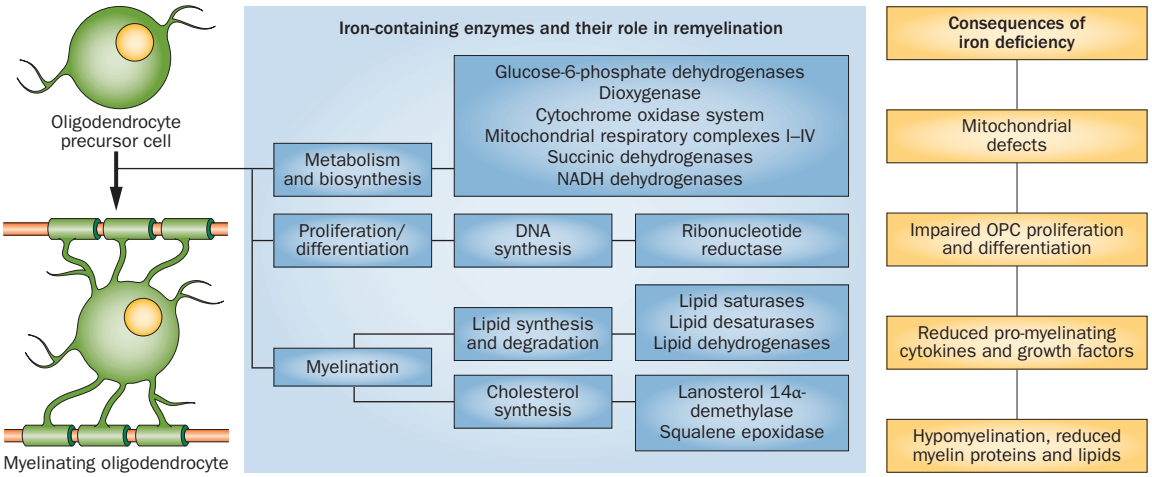

Figure 3 The role of iron-containing enzymes in remyelination. Remyelination requires the recruitment of oligodendrocyte precursor cells to demyelinated lesions followed by their maturation to myelin-forming oligodendrocytes. Iron is required in a variety of enzymes that influence myelin formation, including those that regulate metabolism, proliferation and differentiation of cells of the oligodendrocyte lineage, and the deposition of myelin membranes (blue boxes). Iron deficiency is associated with impairments in oligodendrocyte well-being and remyelination (yellow boxes). This Figure includes only a partial list of iron-requiring enzymes involved in remyelination.

Iron is also directly involved in myelin formation. During embryonic development, the highest rate of iron uptake into the brain coincides with onset of myelination[66] and, therefore, accumulation of iron in oligodendrocytes is probably an important prerequisite for myelination. Iron is also a cofactor in several enzymes involved in cholesterol and lipid synthesis, such as lanosterol 14α-demethylase (a member of the cytochrome P450 superfamily), which catalyses major steps in cholesterol synthesis.[49] Cholesterol availability is a crucial prerequisite for myelin growth.[50] Other enzymes crucial for myelin synthesis and turnover include squalene monooxygenase (involved in cholesterol synthesis), lipid saturases and desaturases (such as acyl-CoA desaturase), and enzymes involved in lipid degradation, including lipid dehydrogenases (Figure 3).[46,51,52]

Consequences of iron deficiency

Following a demyelinating insult, remyelination can occur spontaneously, entailing the generation, maturation and differentiation of OPCs into myelin-forming oligodendrocytes (Figure 3). However, insufficiency of trophic factors and/or abundance of nonpermissive molecules in MS lesions lead to remyelination failure and chronically demyelinated axons that are susceptible to degeneration. During remyelination, OPCs are recruited to a lesion, proliferate, and differentiate into mature oligodendrocytes that can remyelinate denuded axons. Considering the requirement for iron-containing enzymes in all these processes, iron levels in oligodendrocytes probably have an important influence on remyelination and, therefore, on neuronal repair. Iron-deficient oligodendrocytes and reduced iron availability—whether through iron chelation[53] or impaired iron export from astrocytes[54]—lead to reductions in OPC proliferation, oligodendrocyte differentiation and remyelination following injury. The effects of iron on proliferation and differentiation probably depend on the stage of maturation of oligodendrocytes, as well as on their levels of intracellular iron: OPCs undergo differentiation when exposed to high levels of iron, but increased iron exposure does not affect the maturation of mature (postmitotic) oligodendrocytes.[55]

Rats subjected to iron deficiency during development exhibit impaired OPC proliferation and differentiation, as well as decreased expression of myelin proteins.[56] Transient infections occurring before peak myelination in neonatal mice lead to neuronal iron sequestration, altered iron homeostasis in the brain, impaired oligodendrogenesis, and hypomyelination.[57] Iron deficiency also leads to altered myelin composition characterized by reduced levels of myelin cholesterol, phospholipids, galactolipids and myelin proteins, as well as altered lipid rafts.[58]

Normal iron levels might also be indirectly important for repair by acting on other brain cells that have roles in remyelination. Although excessive iron promotes inflammatory responses by inducing an M1 activation state in macrophages and microglia, some components of neuroinflammation that are also driven by iron have beneficial consequences for remyelination. M1 activated macrophages and microglia can express proinflammatory cytokines, such as tumour necrosis factor (TNF) and IL-1β, which are important for remyelination. TNF directly promotes OPC proliferation and remyelination, possibly by acting through its receptor TNFR2.[59] IL-1β promotes astrocyte proliferation, and its inactivation impairs oligodendrocyte differentiation and remyelination.[60] TNF and IL-1β also prompt astrocytes to secrete the growth factors fibroblast growth factor 2 and insulin-like growth factor 1 (IGF-1), respectively.[54] IGF-1 prevents oligodendrocyte apoptosis,[61] fosters the growth and differentiation of OPCs, and is required for oligodendrocyte development and myelination.[62] Macrophages can also secrete ferritin, which is taken up

by oligodendrocytes, prolongs their survival, and favours the formation of new oligodendrocytes.[52,63,64]

Considering that iron levels are crucial both for maintaining proper function of enzymes involved in OPC proliferation, differentiation and myelination and for the production of proinflammatory cytokines and growth factors that promote remyelination, the dysregulation of iron metabolism in MS will not only promote cytotoxicity, but also inhibit the subsequent repair processes. The ratio of M1:M2 macrophages and microglia also affects iron availability, owing to their different handling of intracellular and extracellular iron.[65] M1 macrophages have low levels of the iron exporter ferroportin and high levels of ferritin, which sequesters iron and reduces its availability to other cells.[66] By contrast, M2 macrophages have high levels of ferroportin and can supply iron to proliferating cells, which might be an important step in repair.[66]

The global decrease of iron in NAWM in patients with MS might be protective against inflammation-mediated demyelination and oxidative stress,[34] but might also result in iron-deficient oligodendrocytes. Whether the decrease in the fraction of total iron stored within oligodendrocytes is due to a reduction in oligodendrocyte number, or whether oligodendrocytes are becoming iron-starved, perhaps as a consequence of other cells becoming iron-laden, is not yet clear. Given the importance of iron for cellular health and for myelin synthesis by oligodendrocytes, the question of whether oligodendrocytes in NAWM are becoming iron-deficient is important. The potential role of iron in repair processes is also an important consideration when devising treatments for conditions of iron overload in MS.

Iron regulatory factors

Iron regulatory proteins manage the delicate balance between the need for iron and the damage that may ensue from perturbed iron levels. Dysregulated iron metabolism can result from deficits in the proteins responsible for transport and storage of iron, or from iron excess that overwhelms these regulatory mechanisms, and can lead to increased free ferrous iron and potential oxidative damage.

Ferritin is the main intracellular iron-binding protein. This molecule sequesters iron, rendering it nonreactive and unable to bind to hydrogen peroxide, thus preventing the Fenton reaction from occurring.[67] In normal adult white matter, stored iron in oligodendrocytes and myelin is predominantly bound to ferritin.[34,68] Neurons also contain an endogenous supply of iron bound to ferritin.[69] Under normal intracellular conditions, a small amount of iron that is not bound to proteins is also present, which comprises the labile iron pool.[70,71] In the labile pool, iron is bound to low-molecular-weight compounds, and is considered chelatable (or bioavailable) owing to its ability to react with endogenous reactive oxygen species in several cellular compartments such as the nucleus, cytosol, lysosomes and mitochondria; these regions are all potential sites of hydroxyl radical production and damage.[14,71]

Transferrin is the protein responsible for shuttling iron into cells by binding to dedicated receptors. One mechanism for transport of iron across the blood–brain barrier involves the binding of soluble transferrin-bound iron to transferrin receptors on brain endothelial cells.[72] Iron efflux into the CNS can also occur via astrocyte endfeet. Astrocytes express both ferroportin and ceruloplasmin (a ferroxidase), which cooperate in iron export.[73,74] Whereas systemic iron metabolism is controlled at the transcriptional level, cellular iron levels are controlled by post-transcriptional mechanisms. Iron levels can be effectively sensed by cytoplasmic iron regulatory proteins 1 and 2, which respond to changing iron levels by post-transcriptionally regulating mRNAs that encode proteins involved in iron uptake, utilization, storage and export.[75] The expression of iron regulatory proteins is also sensitive to other intracellular conditions including hypoxia and reactive oxygen species: hypoxia-inducible factors (HIFs) can regulate the expression of iron-related proteins, including divalent metal transporter 1, haem-oxygenase-1, transferrin and ceruloplasmin.[76] Iron transport proteins[77] and the role of iron regulatory proteins in maintenance of iron homeostasis[75,78] have been comprehensively reviewed elsewhere.

Iron regulatory proteins are important to consider in MS because they represent a possible mechanism to explain global iron dysregulation via their influence on iron shuttling between cells, iron localization, and availability of proteins, such as ferritin, to bind and limit iron toxicity. Knowledge of the changing temporal and spatial relationships between iron levels and the function of iron regulatory proteins in patients with MS is currently lacking, but would provide valuable insight into iron dysregulation associated with MS, and might uncover potential therapeutic interventions to manage iron levels.

Iron in the extracellular matrix

One unexplored target for the treatment of dysregulated iron metabolism in MS is the extracellular matrix (ECM). Some evidence suggests that the ECM might act as a reservoir of nonreactive iron that both protects against iron-mediated damage and promotes cellular repair. Perineuronal nets (PNNs) are lattice-like networks of ECM molecules, including the highly polyanionic chondroitin sulphate proteoglycans (CSPGs), which are connected to hyaluronan and stabilized by link proteins, tenascin C and tenascin R.[79] PNNs extend from astrocyte processes to ensheath neuron cell bodies and proximal dendrites, and may act as a glial–neuron interface.[80,81] Owing to their highly negative charge, PNNs are able to bind cations, including iron; indeed, PNNs have a higher iron-binding capacity than any other tissue in the brain.[82]

Mossbauer spectroscopy can determine the valence state of iron, and this technique has shown that ferric iron binds to PNNs that are in close proximity to neurons.[82] In Alzheimer disease, neurons that are closely associated with PNNs are less affected than are PNN-free neurons by neurofibrillary degeneration or accumulation of lipofuscin, a pigment consisting of crosslinked protein

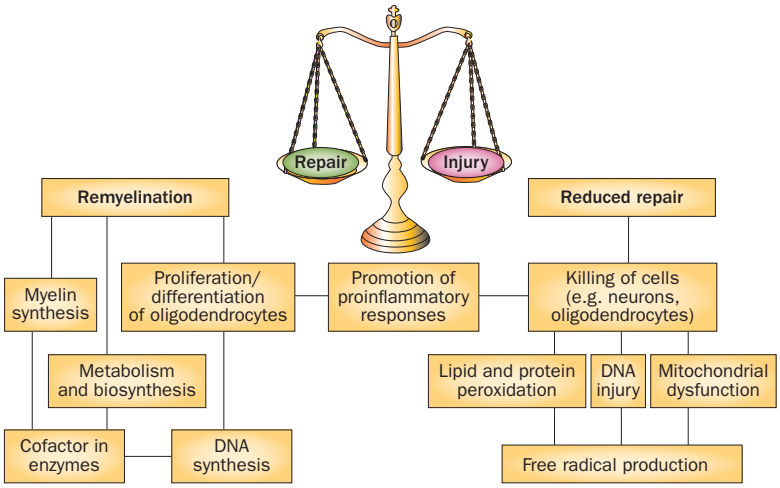

Figure 4 Balancing the toxic and trophic properties of iron. This balance is important for the development of potential iron-targeted therapies for multiple sclerosis. Iron is required in processes that are important for repair, including myelination, but the dysregulation or accumulation of iron can lead to hydroxyl radical production and injury.

and lipids formed by iron-induced oxidative damage.[83] PNN-coated neurons have increased intracellular iron levels[84] as well as decreased evidence of degeneration after iron-induced oxidative stress.[85] Certain components of PNNs are important for protection from iron-induced neuronal damage,[86] and levels of one such component, the CSPG aggrecan, increase following iron injection.[86] Upregulation of CSPGs as a protective mechanism against iron toxicity could explain the accumulation of these proteoglycans in the vicinity of MS lesions,[87] as iron deposits are observed at the edges of active lesions.[34]

The molecular composition of the ECM enables it to act as a diffusion barrier for many different ions. For example, CSPGs represent a large negatively charged surface area that can potentially interact with other charged molecules. Disrupting the formation of CSPGs at the perinodal matrix decreases nerve conduction velocities and facilitates extracellular diffusion of ions, suggesting that the ECM acts as a barrier to ion diffusion in white matter.[88] This mechanism could potentially be exploited to reduce iron toxicity by limiting the spread of iron through the brain from sites of neurodegeneration.

The ECM's ability to act as an extracellular repository of redox-active iron might serve an important neuroprotective function in diseases in which extracellular iron overload is caused by overwhelmed homeostatic defence mechanisms. However, the potential benefit of the ECM acting to scavenge extracellular free iron—thereby reducing local oxidative stress—must be balanced against its other known effects on repair. Certain ECM components, such as CSPGs, reduce OPC process extension and might also act as a barrier to OPC migration.[89] By contrast, other ECM components promote remyelination, for example, laminins, which guide the proliferation, survival and maturation of cells of the oligodendrocyte lineage.[90,91] The various CSPGs probably have different affinities for extracellular iron. Moreover, CSPGs have variable sulphation patterns on their hexosamine side chains, which influence the ability of these molecules to bind growth factors, adsorb to neurons and prevent NMDA-mediated neuronal death,[92] and might also alter their ability to bind extracellular iron. Further studies are required to determine whether specific ECM components can effectively scavenge redox-active iron, and whether they could be modified, both in space and time, to promote repair and remyelination.

Considerations for treatment

Iron chelators are beneficial in conditions of iron overload, in which the excess iron is free to react and facilitate production of reactive oxygen species, as well as to promote proinflammatory activity. Iron deficiency has a beneficial effect—specifically, attenuation of symptoms—in mice with EAE.[93] The protection against EAE conferred by iron deficiency might be due to immunosuppression, in particular, disruption of T-cell proliferation and lymphokine release,[94,95] both of which are iron-dependent and required for the EAE disease process. The reduction in EAE symptoms that is associated with iron deficiency could also be explained by the iron-dependent activation of the HIF pathway, through inhibition of the ubiquinone degradation pathway.[96,97] Increased levels of HIF-1α could result in translation of protective factors, including erythropoietin.

Iron chelation therapy does not seem to be beneficial in patients with MS. Three studies of the iron chelator deferoxamine in patients with this condition showed little evidence of improvement: Expanded Disability Status Scores either worsened or showed no change in many patients, although all three studies were limited by low patient numbers.[98–100] These studies have been reviewed in detail elsewhere.[101]

Treatment options for MS must balance the trophic versus toxic properties of iron: treatments should not only prevent increases in iron levels that could overwhelm homeostatic mechanisms and contribute to cytotoxicity and inflammation, but also maintain adequate levels of iron for oligodendrocyte differentiation, remyelination and repair (Figure 4). A preliminary clinical study found that patients with secondary progressive MS benefited from the combination of iron depletion and erythropoietin.[102] This benefit might stem from reduced iron in tissues, reduced haemoglobin concentrations, and increased levels of erythropoietin, all of which have neuroprotective effects. Another important consideration when devising treatments that target iron metabolism or oxidative injury in the CNS is that these therapies must be able to cross the blood–brain barrier in sufficient quantities to be effective. This consideration is particularly important in progressive MS, in which the blood–brain barrier disturbance that is prominent in the relapsing–remitting phase of disease seems to be mild or absent.[103]

Considering the extreme potential for damage associated with increased production of hydroxyl radicals by free iron, antioxidants represent a logical therapeutic option for preventing free radical damage in patients

with MS. Potential antioxidants can be derived from either endogenous sources (for example, glutathione, catalase, superoxide dismutase, quinone oxidoreductase-like protein 1, uric acid, lipoic acid or bilirubin) or exogenous sources (such as omega-3 fatty acids, statins, antioxidant vitamins or polyphenols).[104] However, despite their theoretical advantage, clinical evidence that antioxidants are beneficial in patients with MS is scarce. Nutrition studies do not consistently show that diets high in antioxidants are beneficial in terms of ameliorating MS progression,[105] and in large prospective clinical studies, intake of vitamins C and E was not associated with a reduced risk of MS.[106]

Antioxidant therapy should counter the oxidative stress resulting from high iron levels and prevent lipid and protein oxidation, but the greatest limitation of this approach is that molecules that act as antioxidants in certain situations can act as pro-oxidants in others. The brain contains high levels of ascorbate (vitamin C), which, in the absence of transition metals, has important antioxidant properties.[107] In the presence of unliganded iron, however, ascorbate promotes the production of free radicals by reducing ferric iron and recycling it back to the redox-active state (Figure 2).[14,107,108] The distinction between molecules that normally act as antioxidants and molecules that still act as antioxidants in the presence of free iron is an important consideration when designing treatments to combat oxidative stress in MS. Another challenge is to ensure consistent availability of antioxidants over time, and at concentrations that will be effective at sites of injury. Extensive removal of oxidants may also have off-target adverse effects, as some of these molecules—albeit at low levels—are involved in crucial signalling pathways.

Conclusions

The primary cause of MS has remained enigmatic, and the factors involved in neurodegeneration in MS are not fully understood. Although substantial evidence documents a role for iron in the neurodegenerative processes associated with MS, whether iron is a byproduct of the disease process or a key component that promotes neurodegeneration is currently unknown. Determination of the role of iron in MS might help to bring the scientific community closer to revealing the causes of neurodegeneration, leading to more-effective treatments for patients with MS.

Elucidation of the source and pathogenetic mechanisms of iron deposition in MS will also be important for other disorders associated with iron accumulation, including Alzheimer disease, Huntington disease and Parkinson disease.[109,110] Iron-derived reactive species are also implicated in vascular disorders such as atherosclerosis, microangiopathic haemolytic anaemia, vasculitis and reperfusion injury.[111] Although demyelination is not a feature of these diseases or other neurodegenerative diseases involving iron dysregulation, important insights into these conditions might be gained from studying the influence of iron on neurotoxicity and mechanisms of repair in MS.

The complexity and multifactorial nature of degenerative disorders associated with abnormal iron regulation also suggests that multiple interventions are required. For example, treatments for iron accumulation (such as iron-chelating therapies) are beneficial for neuroprotection, but might also impair repair processes, including remyelination. Thus, management of these diseases requires an understanding of the complex cellular cascades that lead to, and are susceptible to, iron accumulation.

Review criteria

PubMed was searched for articles in English using the following terms: “iron and multiple sclerosis”, “iron and oligodendrocytes or remyelination”, and “iron and extracellular matrix”. Relevant references found within the identified papers were also included.

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Author contributions

All authors researched data for the article, made substantial contributions to discussions of the content, and wrote and edited the manuscript before submission.