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Review

Iron and multiple sclerosis

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ABSTRACT

Iron is essential for normal cellular functioning of the central nervous system. Abnormalities in iron metabolism may lead to neuronal death and abnormal iron deposition in the brain. Several studies have suggested a link between brain iron deposition in normal aging and chronic neurologic diseases, including multiple sclerosis (MS). In MS, it is still not clear whether iron deposition is an epiphenomenon or a mediator of disease processes. In this review, the role of iron in the pathophysiology of MS will be summarized. In addition, the importance of conventional and advanced magnetic resonance imaging techniques in the characterization of brain iron deposition in MS will be reviewed. Although there is currently not enough evidence to support clinical use of iron chelation in MS, an overview of studies of iron chelation or antioxidant therapies will be also provided.

1. Introduction

Iron is critical for normal brain functioning. However, aberrant iron metabolism and abnormal iron deposition in the brain are associated with many neurologic disorders including multiple sclerosis (MS) (Stankiewicz and Brass, 2009). Several possible causes of abnormal iron deposition in MS have been postulated including blood-brain barrier dysfunction, decreased iron clearance because of axonal dysfunction and inflammation, or dysregulation of iron transport proteins because of inflammation (Stankiewicz et al., 2007). Abnormal iron homeostasis may contribute to the neurodegeneration seen in MS.

Magnetic resonance imaging (MRI) studies have shown that excessive iron accumulates in the gray matter (GM) of MS patients, mainly in the basal ganglia. Recently, the use of advanced MRI techniques and ultra–high-field MRI has aided characterization of iron deposits in both the GM and white matter (WM) of patients with MS. However, further MRI optimization will enhance our understanding of iron's role in MS pathophysiology. Combined histopathologic MRI studies have begun to elucidate the role of iron deposition in MS pathology. More investigation in MS is required to clarify whether either chelation or antioxidant treatments represent a viable therapeutic alternative.

In this article, we will provide an overview of research related to the normal iron homeostasis and role of iron in the pathophysiology of MS. Recent data from key original reports regarding the role of conventional and advanced MRI techniques in the assessment of iron deposition in MS will be explored. We will also focus on the most relevant therapeutic intervention studies with respect to both animal and human models that have added to the current understanding of iron toxicity in MS.

2. Iron metabolism

The maintenance of proper iron concentrations in the body is vital to optimal functioning. Iron is involved in many crucial processes including myelin production, oxygen transport, glucose metabolism, synthesis of neurotransmitters, and DNA replication (Pinero and Connor, 2000). It is, therefore, unsurprising that iron deficiency at birth can impair normal cognitive development. Iron deficiency later in life may lead to cognitive impairment, attentional problems, or restless leg syndrome (Beard, 2003). Conversely, too much iron can also cause neurologic disease. Neuroferritinopathy, a disorder of movement characterized by extrapyramidal symptoms, is thought to be because of a mutation in ferritin light chain gene 1 resulting in ferritin and iron accumulation in the brain (Mancuso et al., 2005).

Normally, elimination of iron from the body is fairly consistent and occurs from either bleeding or shedding of cells from the skin or gut. Consequently, iron concentration in the body is regulated by control of iron intake from food (Fig. 1).

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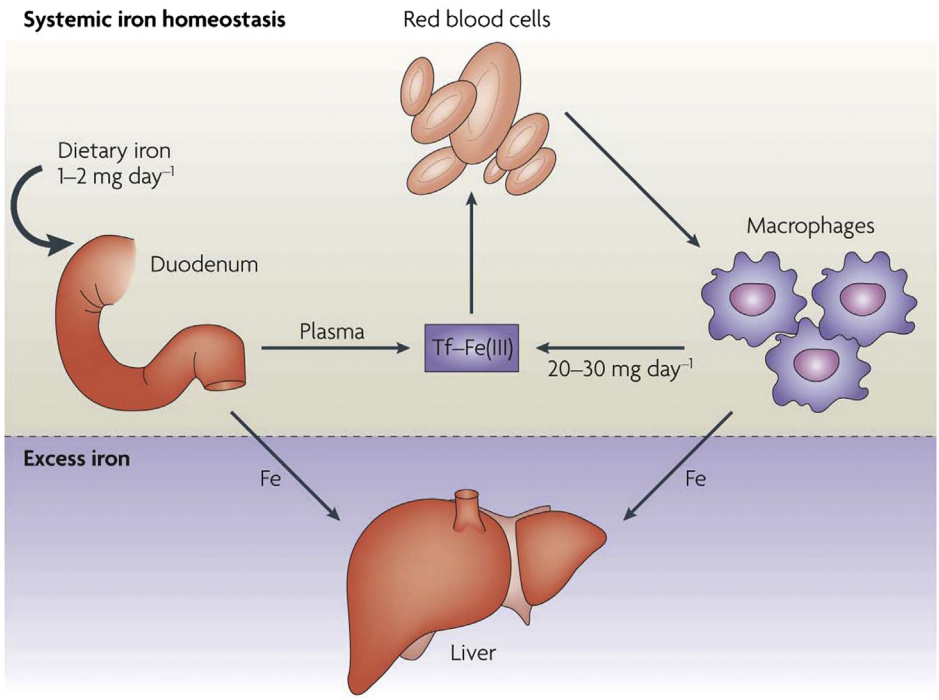

Fig. 1. Dietary iron (1–2 mg per day) is absorbed by cells of the duodenum before being exported into plasma where it binds to transferrin (Tf). Tf-bound iron is delivered to tissues and cells (primarily to reticulocytes) where it is incorporated in hemoglobin. Old erythrocytes (red blood cells) are phagocytosed by macrophages, which degrade hemoglobin and recycle iron back into plasma (20–30 mg per day) where it again binds Tf. If iron is absorbed or released into the plasma at a higher level than the iron-binding capacity of Tf, then the excess non–Tf-bound iron is deposited in parenchymal tissues (such as the liver). Reprinted from De Domenico et al. (2008) with permission from the publisher.

3. Iron trafficking in the body

3.1. Entry through the gut

Iron is absorbed via the gut, most often in the duodenum through the crypts of Lieberkühn. For iron to enter the bloodstream, it must pass through the gut cells. On the intestinal luminal side, mechanisms involving ferrireductase duodenal cytochrome b activity and divalent metal transporter 1 (DMT1) are involved in entry into the enterocyte (De Domenico et al., 2008). Export from the basolateral side of the enterocyte involves ferroportin and hephaestin. Hepcidin and hemochromatosis protein 2 are thought to interact to determine how much iron is admitted to the bloodstream via a feedback loop (Pantopoulos et al., 2012). In the bloodstream, iron circulates in the blood bound to transferrin (Tf) (Fig. 2).

3.2. Cellular iron transport and homeostasis

Transferrin receptor (TfR) and Tf mediate cellular uptake. Tf and TfR have a high binding affinity for one another. These binding complexes are taken up by cells via clathrin-coated pits. After acidification in the endosome, iron is released from Tf and is exported into the cytoplasm by DMT1 (De Domenico et al., 2008). Dissociated Tf and TfR are exported back to the cell surface, and Tf reenters the bloodstream (Dautry-Varsat et al., 1983). Regulation of cellular iron levels are achieved through feedback loops involving iron-response proteins and iron-response elements that are involved in translation of ferritin, TfR, and other regularly proteins. Further details can be found in an excellent review by Rouault (2006a).

4. Iron trafficking and distribution in the brain

4.1. Brain iron transit

Iron intake in the brain is also rigorously regulated and interacts with other metals such as copper (Skjørringe et al., 2012). It can enter the brain through the blood-brain barrier and blood-cerebrospinal fluid barrier, which exists between the blood and the choroid plexus (Skjørringe et al., 2012). Most commonly, iron-bound Tf in the blood is captured by TfR found on brain endothelial cells, and this complex is then endocytosed. Once iron has crossed into the central nervous system, it is released from Tf into the interstitium (Benarroch, 2009). Brain iron homeostasis is tightly regulated, and this balance is achieved in a similar way to peripheral control of intracellular iron (Rouault and Cooperman, 2006b). Currently, the mechanisms for iron egress from the brain are poorly understood. Brain iron is likely reabsorbed back into the bloodstream via the cerebrospinal fluid through the subarachnoid space (Benarroch, 2009) (Fig. 3).

4.2. Normal cellular and structural distribution of iron in the brain

Starting at low levels at birth, brain iron concentration increases rapidly over the first 2 decades of life and then rises more slowly thereafter (Hallgren and Sourander, 1958). Oligodendrocytes and astrocytes acquire solely non–Tf-bound iron, whereas neurons have both TfRs and DMT1 (Moos et al., 2007). Although monocytes and macrophages contain iron to promote free radicals as part of respiratory burst activity, brain microglia rarely contain iron. Iron can also be stored in the central nervous system bound not only to stable ferritin but also to more reactive substances like neuromelanin and hemosiderin. In the normal brain, the highest

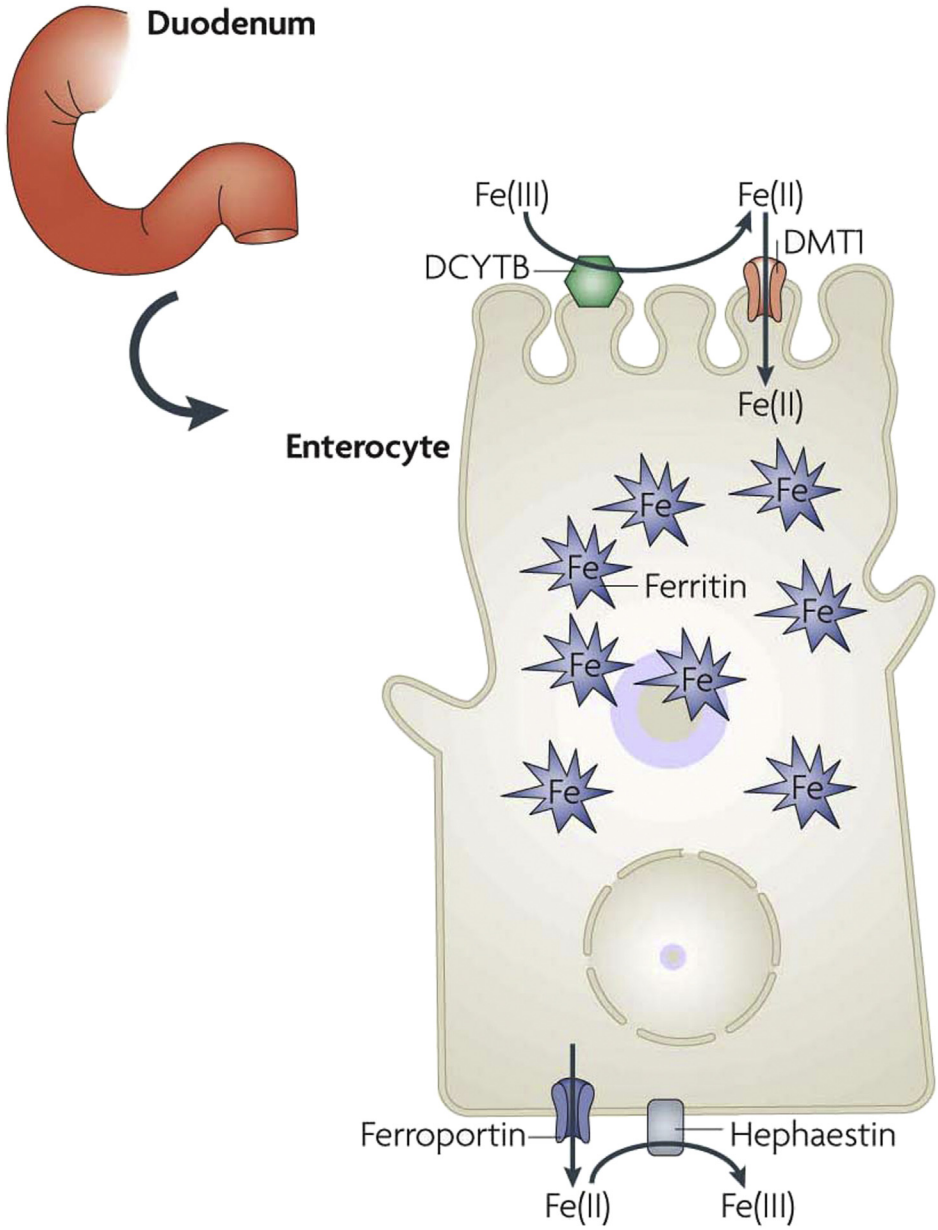

Fig. 2. Ferric iron, Fe(III), in the diet is converted to ferrous iron, Fe(II), by a ferroreductase duodenal cytochrome b that is located on the apical surface of enterocytes of the duodenal mucosa. Fe(II) is then transported into enterocytes through the divalent metal transporter (DMT1). Fe(II) in enterocytes can be incorporated into the cytosolic iron-storage molecule ferritin or can be transported across the basolateral surface of enterocytes into the plasma by ferroportin. Fe(II) is subsequently converted to Fe(III) by a membrane-associated ferroxidase, hephaestin. Reprinted from De Domenico et al. (2008) with permission from the publisher.

concentrations of iron are found in the globus pallidus, caudate nucleus, putamen, dentate nucleus, red nucleus, and substantia nigra (Aoki et al., 1989).

5. Imaging brain iron deposition in multiple sclerosis

MRI is a powerful tool to detect and quantify brain iron deposition in vivo. Among all the brain's essential metals, brain iron concentrations are normally high enough to affect the MRI signal (Stankiewicz et al., 2007). Iron can be found as heme iron, such as in hemoglobin, or non-heme iron. Unlike heme iron, failure to achieve homeostasis of non-heme iron metabolism has been associated with neurodegeneration (Madsen and Gitlin, 2007). Although MRI is not able to accurately differentiate between heme iron and non-heme iron in vivo, it is suggested that ferritin and hemosiderin are the only types of non-heme iron responsible for MRI signal changes (Haacke et al., 2005; Schenck and Zimmermann, 2004). Other non-heme iron forms, such as the free liable iron pool or Tf-bound iron, do not seem to influence the MRI signal because of their low concentration (Haacke et al., 2005; Schenck and Zimmermann, 2004). Brain iron accumulation shortens the longitudinal (T1) and transverse (T2) relaxation times of the mobile protons in the brain resulting in signal loss or hypointensity on T2-weighted images (T2 hypointensity) and hyperintensity on T1-weighted images (T1 hyperintensity). Building on these principles, several pulse sequences and quantitative MRI techniques have been developed and employed in detection and quantification of iron in the brain. The use of high-field (3 T) and ultra–high-field (>3 T) MRI has further facilitated the detection of brain iron in both GM and WM. In the following

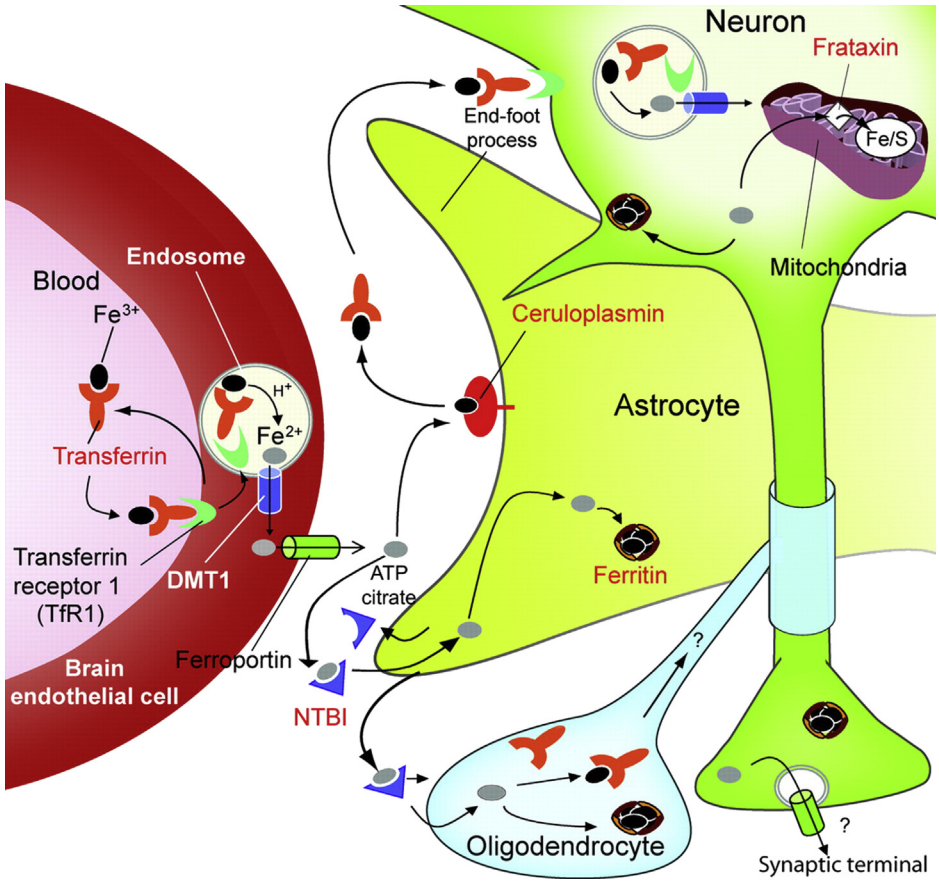

Fig. 3. Overview of iron homeostasis in the central nervous system. The transferrin receptor 1 (TfR1) in the luminal membrane of brain endothelial cells binds ferric iron (Fe^{3+})–loaded Tf and internalizes this complex in endosomes, where Fe^{3+} is reduced to ferrous iron (Fe^{2+}). Ferrous iron may be transported to the cytosol by the endosomal divalent metal transporter 1 (DMT1) and then exported into the extracellular fluid by action of ferroportin. An alternative hypothesis (not shown) is that the Tf-TfR1 receptor complex may be transported from the luminal to the abluminal surface followed by iron release. Ceruloplasmin, expressed in astrocyte end-foot processes, oxidizes newly released Fe^{2+} to Fe^{3+}, which binds to Tf. Tf is the main source of iron for neurons. Fe^{2+} may also bind to adenosine triphosphate (ATP) or citrate released from astrocytes and be transported in the form of non–transferrin-bound iron (NTBI), which is the source of iron to oligodendrocytes and astrocytes. Oligodendrocytes synthesize Tf, which may have a role in intracellular iron transport along their processes. In the cytosol, the storage protein ferritin sequesters and reduces levels of free iron. Mitoferrin (not shown) transports iron into the mitochondria, where the chaperone frataxin facilitates the biosynthesis of iron-sulfur (Fe/S) clusters. Reprinted from Benarroch (2009) with permission from the publisher.

sections, the role of iron in the pathophysiology of MS and its link to neuroinflammation, neurodegeneration, and clinical status is described.

5.1. GM iron deposition

A common finding in patients with MS on spin-echo T2-weighted clinical MRI scans is diffuse hypointensity of the cortical and deep GM areas (e.g., the red nucleus, thalamus, dentate nucleus, lentiform nucleus, caudate, and rolandic cortex) versus age-matched normal controls (Fig. 4).

T2 hypointensity in GM has been shown to be associated with brain atrophy, disability progression, and cognitive impairment in patients with MS (Bakshi et al., 2000, 2001, 2002; Bermel et al., 2005; Brass et al., 2006a, 2006b; Ceccarelli et al., 2009, 2010, 2011; Drayer et al., 1987a, 1987b; Neema et al., 2007, 2009, 2012; Tjoa et al., 2005; Zhang et al., 2007, 2010). T2 hypointensity, as a sign of excessive GM iron deposition, has been shown to be present in all MS subtypes (Bakshi et al., 2000, 2001, 2002; Bermel et al., 2005; Brass et al., 2006a, 2006b; Ceccarelli et al., 2009, 2010, 2011; Drayer et al., 1987a, 1987b; Neema et al., 2007, 2009; Tjoa et al., 2005; Zhang et al., 2007, 2010). Although iron has emerged as a surrogate marker for neurodegeneration in MS, it still remains unclear whether iron deposition is simply an epiphenomenon resulting from brain tissue degeneration or if it directly contributes to brain damage in MS.

Recent T2 intensity studies involving early and milder forms of MS (Ceccarelli et al., 2009, 2010, 2011) have begun to bridge gaps in knowledge regarding the role of iron deposition in the pathophysiology of the disease. In patients with clinically isolated syndrome (CIS), lower T2 intensity in the caudate nucleus was found compared with healthy controls (Ceccarelli et al., 2010). In patients with established MS, T2 hypointensity best predicted disability progression over time and was a better marker of disability than other measures such as whole brain atrophy and T2-lesion volume (Neema et al., 2009). In contrast, in CIS patients, T2 hypointensity of deep GM was not associated with subsequent evolution to clinically definite MS (Ceccarelli et al., 2010). Further evidence that iron could accumulate early in the disease course has been provided by studies in pediatric MS patients (Ceccarelli et al., 2011). Like CIS, selective T2 hypointensity was found in the caudate nucleus of pediatric MS patients with a sparing of other deep GM structures (Ceccarelli et al., 2011). Furthermore, despite their favorable clinical course and the lower overall brain tissue damage, similar T2 intensity was found in benign MS patients compared with secondary progressive MS patients (Ceccarelli et al., 2009). Taken together,

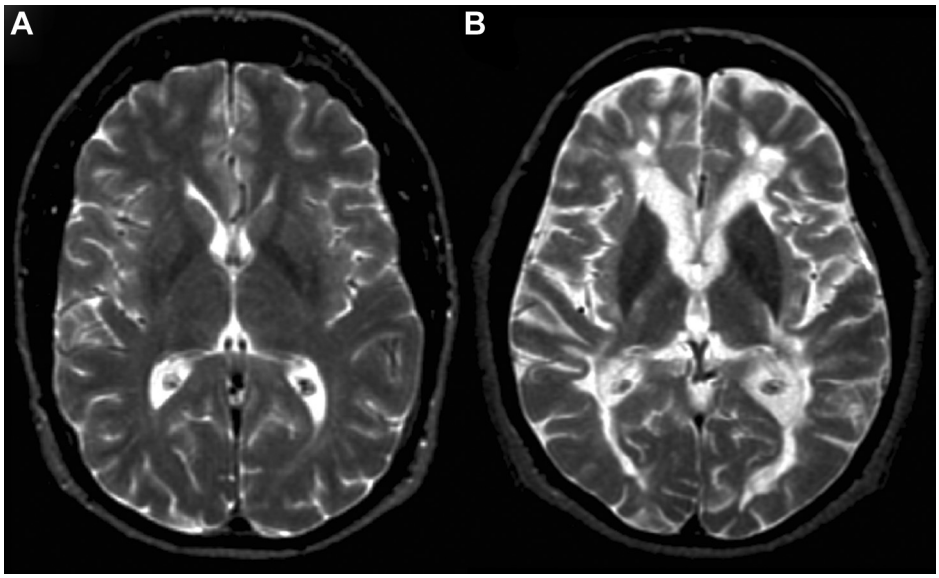

Fig. 4. T2 hypointensity in multiple sclerosis (MS): T2-weighted fast spin-echo axial magnetic resonance imaging scans obtained at 1.5 T of a healthy control (A) in the fifth decade and an age-matched patient with relapsing-remitting MS (B). In the patient with MS, note bilateral hypointensity of various deep gray matter areas, including the thalamus, lentiform nucleus, and caudate compared with the healthy control. This T2 hypointensity most likely represents pathologic iron deposition. The patient also has brain atrophy. Reprinted from Neema et al. (2012) with permission from the publisher.

these findings suggest that brain iron accumulation occurs early in the disease course and increases with disease duration. Few studies have looked at the effect of treatment on brain T2 signal change (Bermel et al., 2005; Pawate et al., 2012). A recent longitudinal year long pilot study of natalizumab treatment of MS patients (Pawate et al., 2012) showed a treatment effect on limiting progression of T2 hypointensity in GM, suggesting that impeding the inflammatory cascade could lead to decreased iron deposition in the brain.

Although T2 intensity studies have provided important clues regarding iron accumulation in the brain of MS patients, the effects of various other confounding factors, such as water content, make the T2 intensity method a nonspecific indicator for estimating iron levels (Neema et al., 2007). Because of their increased sensitivity to iron-related susceptibility changes, new advanced quantitative MRI techniques, and related postprocessing analysis, combined with the use of high-field and ultra–high-field strength MRI, are currently being employed to further define the role of iron in MS (Bagnato et al., 2013; Ge et al., 2007; Habib et al., 2012; Hagemeier et al., 2012a, 2013a, 2013b; Hammond et al., 2008; Khalil et al., 2009, 2011a, 2011b; Lebel et al., 2012; Pitt et al., 2010; Ropele et al., 2011; Rumzan et al., 2013; Walsh et al., 2014; Zivadinov et al., 2012). To date, most of these MRI studies have significantly improved the detection of abnormal iron deposition in cortical and deep GM and confirmed its clinical relevance in MS (Bagnato et al., 2013; Ge et al., 2007; Habib et al., 2012; Hagemeier et al., 2012a, 2013a, 2013b; Hammond et al., 2008; Khalil et al., 2009, 2011a, 2011b; Lebel et al., 2012; Pitt et al., 2010; Ropele et al., 2011; Rumzan et al., 2013; Walsh et al., 2014; Zivadinov et al., 2012). Interestingly, using 7-T MRI and quantitative magnetic susceptibility mapping (Al-Radaideh et al., 2013), iron deposition in several deep GM areas, including the caudate, putamen, pallidus, and pulvinar nucleus, has been shown in CIS patients, further supporting and extending previous findings (Ceccarelli et al., 2010). Recently, a study using susceptibility-weighted filtered phase imaging found that GM iron-related changes may precede GM atrophy in CIS patients suggesting that abnormal iron deposition may be an early surrogate of the disease (Hagemeier et al., 2012a).

Although the measures of GM iron deposition hold promise as clinically relevant biomarkers, more work is necessary before these techniques find use in clinical practice to inform care for individual patients.

5.2. WM iron deposition

Pathologic studies have shown that iron accumulation in the WM is mainly present at the level of MS lesions (Craelius et al., 1982; Hametner et al., 2013) and near the veins (Adams, 1988). However, MRI detection of iron deposition in the WM is still challenging because of the confounding effect on the MRI signals of edema, inflammation, gliosis, and myelin content (Langkammer et al., 2010; Neema et al., 2007; Walsh et al., 2013; Yao et al., 2012). The application of multimodal approach of advanced iron-sensitive MRI techniques, such as phase imaging, R2*, and ultra–high-field scanners, has enhanced the current understanding of WM iron deposition in MS. In particular, with the use of phase imaging, it is possible to visualize phase hypointense WM lesions that are likely high in iron content and often missed by conventional MRI sequences (Eissa et al., 2009; Haacke et al., 2009; Hagemeier et al., 2012b; Hammond et al., 2008). Various phase hypointense WM MS lesion patterns have also been identified such as nodular, ring, and scattered shapes, suggesting that iron deposition could be inside or at the edge of phase WM lesions in MS (Eissa et al., 2009; Haacke et al., 2009; Hagemeier et al., 2012b). Such phase WM lesions have also been observed even at the earliest clinical stages of MS (Hagemeier et al., 2012b). Although the interplay between various cellular structures is thought to be responsible for the diverse hypointense patterns in the WM lesions (Bagnato et al., 2011; Bian et al., 2013; Mehta et al., 2013), the mechanisms behind these have yet to be elucidated. In addition, a reliable MRI method to characterize the presence of iron in WM is currently unavailable (Walsh et al., 2013; Yao et al., 2012). Future combined histopathologic-multimodal MRI studies will shed light on the mechanisms underlying iron-related MRI changes seen in WM, which in turn may provide insights into understanding the link between WM damage and clinical status.

6. Iron toxicity

Bound iron is considered "safe," but free iron is more likely to exchange electrons with nearby molecules and produce free radicals. Normal metabolic processes in the mitochondria form hydrogen peroxide as the result of molecular reduction of oxygen. Hydrogen peroxide alone is not particularly toxic, but in the presence of free iron ($Fe2+$), a free radical ($\cdot OH$) is formed when free iron donates an electron to hydrogen peroxide via the Fenton reaction. This free radical can interact with oxygen and other molecules in the brain to form more free radicals propagating a deleterious positive feedback loop. Hydroxyl radicals can attack proteins, deoxynucleic acids, and lipid membranes. This process can disrupt cellular integrity and function eventually leading to cell apoptosis (Halliwell, 2006). Oxidative stress has been implicated in the pathogenesis of MS, potentially contributing to both demyelination and axonal damage (Gilgun-Sherki et al., 2004).

7. Protection from iron-mediated damage

7.1. Endogenous protection

Experimental work suggests that the body has natural processes in place to stem potentially damaging free-radical formation. Both glutathione and vitamin E have been implicated as protective. Glutathione in a reduced state interacts with hydrogen peroxide and other organic peroxides to yield an oxidized form of glutathione, water, and alcohol. Removal of hydrogen peroxide leaves less potential for interaction with free iron and less subsequent production of hydroxyl radicals. Cells able to survive potentially to toxic iron show increased glutathione production (Aguirre et al., 2006). A form of vitamin E (alpha-tocopherol) can act as an antioxidant by donating a proton to reactive oxygen species, thus making them more stable and less reactive (Crichton et al., 2002). Experimental work finds that cerebellar granule cell cultures and hippocampal neurons treated with iron in the presence of either alpha-tocopherol or an analogue were better preserved (de Jesus Ferreira et al., 2005; Van der Worp et al., 1999).

7.2. Exogenous protection

Because iron may contribute to the pathogenesis of MS, human and animal investigations have been performed to ascertain whether binding iron (chelation) or reducing iron associated free-radical damage (antioxidants) might represent viable treatment strategies.

7.2.1. Chelation

Iron chelators may attenuate free-radical toxicity by binding circulating free iron and preventing this iron from participating in redox reactions. Iron chelation therapy has been tried in the experimental autoimmune model of MS with some success. A study conducted in experimental autoimmune encephalomyelitis (EAE) induced by spinal cord homogenates showed a response to desferrioxamine (Bowern et al., 1984). Two other studies investigated desferrioxamine in a myelin basic protein-induced EAE model. Willenborg et al. (1988) found no response if the desferrioxamine was administered in a preclinical phase, whereas Pedchenko and LeVine (1999) found that if the desferrioxamine was administered later, during a time of clinical symptoms, it effectively attenuates the disease. The short half-life of desferrioxamine, potential critical window for effect, or other free-radical scavenging effects outside iron binding might account for the discrepant studies. Pedchenko and Levine (1999) also reported that dexrazoxane, another iron chelator similar to desferrioxamine but with a longer half-life also attenuated the course of EAE, but its effect was less robust than that seen with desferrioxamine. Also, rats given dexrazoxane in conjunction with mitoxantrone experienced more improvement on clinical indices than rats treated solely with mitoxantrone (Weilbach et al., 2004).

Only 1 human trial using desferrioxamine has been conducted in a small group of secondary progressive MS patients (Lynch et al., 2000). Nine patients received up to 8 courses of desferrioxamine, and although the drug was well tolerated, little effect was seen on disability. Specifically, a single patient improved, 3 patients were unchanged, and 5 patients worsened. These disappointing results call into question whether treatment with desferrioxamine (or iron chelation more generally) could be an effective treatment strategy for MS. It is worth noting, however, that this trial was small and that the enrolled patients had more advanced disease. Patients with higher disability typically have been refractory to treatment in MS drug trials.

It is clear that iron deposition occurs in MS and that iron chelation can work in the EAE model. It remains to be seen, however, whether chelation therapy can be a successful treatment strategy in MS.

7.2.2. Antioxidants

Antioxidants might help counteract iron's role in disease by protecting the body from oxidative stresses. A study of MS plaques revealed decreased levels of antioxidants and increased free-radical activity (Langemann et al., 1992). MS patients also exhibit increased levels of oxidative stress markers compared with normal controls, with correlation seen between disability and higher levels of oxidative stress markers (Oliveira et al., 2012).

Many antioxidants have been suggested to be neuroprotective through reduction of free-radical formation including vitamin A, vitamin C, vitamin E, coenzyme Q10, green tea extract, nitric oxide, selegiline (monoamine oxidase inhibitor with antioxidant properties), and Ginkgo biloba.

A full accounting of antioxidants that have been successful in attenuating the EAE model of MS is outside the scope of this review, but many have succeeded. Commonly recognized antioxidants that work on an EAE model include vitamin A (Massacesi et al., 1987), vitamin C (Spitsin et al., 2002), lipoic acid (Marracci et al., 2002), resveratrol (Shindler et al., 2010), blueberries (Xin et al., 2012), green tea (Aktas et al., 2004), and curcumin (Xie et al., 2009). Although these antioxidants can show an effect in animals, it is not always clear that this effect is because of reduction of free radicals. For example, experimental work with green tea extract demonstrates that this compound affects T cells (Wang et al., 2012). It is also worth noting that some antioxidants such as idebenone have failed to provide a beneficial effect in the EAE model (Fiebiger et al., 2013).

Few human trials of antioxidants have been performed. Perhaps, the most visible drug with potential antioxidant effect in the MS space is the recently Food and Drug Administration–approved dimethyl fumarate. Although argument remains about how the drug is having its treatment effect on MS, experiments suggest that the drug works on a free-radical scavenging pathway (Nrf2) and has antioxidant effects (Linker et al., 2011). Another drug, inosine, has been trialed in 2 separate MS cohorts. This precursor drug is metabolized to the antioxidant uric acid. Although successful in an EAE model, inosine has shown mixed results in relapsing-remitting MS patients. Gonsette et al. (2010) did not show a benefit from the drug. Markowitz et al. (2009) found that MS patients with higher serum urate levels did benefit from inosine compared with their pretreatment state. Renal calculi were seen in both the Gonsette (3.8%) and Markowitz (25%) trials. Double-blind placebo-controlled studies with increased enrollment and longer observation periods

are needed before conclusions about long-term efficacy and safety of antioxidants can be made.

8. Conclusions

We have reviewed the physiology of iron metabolism and the role of abnormal brain iron deposition in MS. We have seen that although iron is vital for normal neuronal processes, abnormal iron accumulation may cause neurodegeneration through lipid peroxidation and cell death in the brain. Human MRI studies reported an association between abnormal brain iron deposition and clinical dysfunction in MS patients. It remains to be elucidated whether iron deposition is a marker or mediator of the destructive cascade in MS. Future studies incorporating newer pulse sequences, multimodal MRI in conjunction with histopathologic assessments, and novel postprocessing techniques should shed light on mechanisms responsible for abnormal iron deposition and on its role in the pathogenesis of MS. Animal models of MS have shown a neuroprotective effect by either iron chelation or antioxidants; however, in MS patients, the effectiveness of these pharmacologic modification is still debatable and requires further investigation.

Disclosure statement

None of the authors have a conflicts of interest.

References

Adams, C.W., 1988. Perivascular iron deposition and other vascular damage in multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 51, 260–265.

Aguirre, P., Mena, N., Tapia, V., Rojas, A., Arrendondo, N., Núñez, M.T., 2006. Antioxidant responses of cortex neurons to iron loading. Biol. Res. 39, 103–104.

Aktas, O., Prozorovski, T., Smorodchenko, A., Savaskan, N.E., Lauster, R., Kloetzel, P.M., Infante-Duarte, C., Brocke, S., Zipp, E., 2004. Green tea epigallocatechin-3-gallate mediates T cellular NF-kappa B inhibition and exerts neuroprotection in autoimmune encephalomyelitis. J. Immunol. 173, 5794–5800.

Al-Radaideh, A.M., Wharton, S.J., Lim, S.Y., Tench, C.R., Morgan, P.S., Bowtell, R.W., Constantinescu, C.S., Gowland, P.A., 2013. Increased iron accumulation occurs in the earliest stages of demyelinating disease: an ultra-high field susceptibility mapping study in clinically isolated syndrome. Mult. Scler. 19, 896–903.

Aoki, S., Okada, Y., Nishimura, K., Barkovich, A.J., Kjos, B.O., Brasch, R.C., Norman, D., 1989. Normal deposition of brain iron in childhood and adolescence: MRI imaging at 1.5T. Radiology 172, 381–385.

Bagnato, F., Hametner, S., Welch, E.B., 2013. Visualizing iron in multiple sclerosis. Magn. Reson. Imaging 31, 376–384.

Bagnato, F., Hametner, S., Yao, B., van Gelderen, P., Merkle, H., Cantor, F.K., Lassmann, H., Duyn, J.H., 2011. Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. Brain 134, 3602–3615.

Bakshi, R., Benedict, R.H., Bermel, R.A., Caruthers, S.D., Puli, S.R., Tjoa, C.W., Fabiano, A.J., Jacobs, L., 2002. T2 hypointensity in the deep gray matter of patients with multiple sclerosis: a quantitative magnetic resonance imaging study. Arch. Neurol. 59, 62–68.

Bakshi, R., Dmochowski, J., Shaikh, Z.A., Jacobs, L., 2001. Gray matter T2 hypointensity is related to plaques and atrophy in the brains of multiple sclerosis patients. J. Neurol. Sci. 185, 19–26.

Bakshi, R., Shaikh, Z.A., Janardhan, V., 2000. MRI T2 shortening ('black T2') in multiple sclerosis: frequency, location, and clinical correlation. Neuroreport 11, 15–21.

Beard, J., 2003. Iron deficiency alters brain development and functioning. J. Nutr. 133, 1468S–1472S.

Benarroch, E.E., 2009. Brain iron homeostasis and neurodegenerative disease. Neurology 72, 1436–1440.

Bermel, R.A., Puli, S.R., Rudick, R.A., Weinstock-Guttman, B., Fisher, E., Munschauer 3rd, F.E., Bakshi, R., 2005. Prediction of longitudinal brain atrophy in multiple sclerosis by gray matter magnetic resonance imaging T2 hypointensity. Arch. Neurol. 62, 1371–1376.

Bian, W., Harter, K., Hammond-Rosenbluth, K.E., Lupo, J.M., Xu, D., Kelley, D.A., Vigneron, D.B., Nelson, S.J., Pelletier, D., 2013. A serial in vivo 7T magnetic resonance phase imaging study of white matter lesions in multiple sclerosis. Mult. Scler. 19, 69–75.

Bowern, N., Ramshaw, I., Clark, I., Doherty, P., 1984. Inhibition of autoimmune neuropathological process by treatment with an iron chelating agent. J. Exp. Med. 160, 1532–1543.

Brass, S.D., Benedict, R.H., Weinstock-Guttman, B., Munschauer, F., Bakshi, R., 2006a. Cognitive impairment is associated with subcortical magnetic resonance imaging grey matter T2 hypointensity in multiple sclerosis. Mult. Scler. 12, 437–444.

Brass, S.D., Chen, N.K., Mulkern, R.V., Bakshi, R., 2006b. Magnetic resonance imaging of iron deposition in neurological disorders. Top Magn. Reson. Imaging 17, 31–40.

Ceccarelli, A., Filippi, M., Neema, M., Arora, A., Valsasina, P., Rocca, M.A., Healy, B.C., Bakshi, R., 2009. T2 hypointensity in the deep gray matter of patients with benign multiple sclerosis. Mult. Scler. 15, 678–686.

Ceccarelli, A., Rocca, M.A., Neema, M., Martinelli, V., Arora, A., Tauhid, S., Ghezzi, A., Comi, G., Bakshi, R., Filippi, M., 2010. Deep gray matter T2 hypointensity is present in patients with clinically isolated syndromes suggestive of multiple sclerosis. Mult. Scler. 16, 39–44.

Ceccarelli, A., Rocca, M.A., Perego, E., Moiola, L., Ghezzi, A., Martinelli, V., Comi, G., Filippi, M., 2011. Deep grey matter T2 hypo-intensity in patients with paediatric multiple sclerosis. Mult. Scler. 17, 702–707.

Craelius, W., Migdal, M.W., Luessenhop, C.P., Sugar, A., Mihalakis, I., 1982. Iron deposits surrounding multiple sclerosis plaques. Arch. Pathol. Lab. Med. 106, 397–399.

Crichton, R.R., Wilmet, S., Legssyer, R., Ward, R.J., 2002. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J. Inorg. Biochem. 91, 9–18.

Dautry-Varsat, A., Ciechanover, A., Lodish, H.F., 1983. pH and the recycling of transferrin during receptor-mediated endocytosis. Proc. Natl. Acad. Sci. U.S.A 80, 2258–2262.

De Domenico, I., McVey Ward, D., Kaplan, J., 2008. Regulation of iron acquisition and storage: consequences for iron-linked disorders. Nat. Rev. Mol. Cell Biol. 9, 72–81.

de Jesus Ferreira, M.C., Crouzin, N., Barbanel, G., Cohen-Solal, C., Récasens, M., Vignes, M., Guiramand, J., 2005. A transient treatment of hippocampal neurons with alpha-tocopherol induces a long-lasting protection against oxidative damage via a genomic action. Free Radic. Biol. Med. 39, 1009–1020.

Drayer, B., Burger, P., Hurwitz, B., Dawson, D., Cain, J., 1987a. Reduced signal intensity on MR images of thalamus and putamen in multiple sclerosis: increased iron content? AJR Am. J. Roentgenol. 149, 357–363.

Drayer, B.P., Burger, P., Hurwitz, B., Dawson, D., Cain, J., Leong, J., Herfkens, R., Johnson, G.A., 1987b. Magnetic resonance imaging in multiple sclerosis: decreased signal in thalamus and putamen. Ann. Neurol. 22, 546–550.

Eissa, A., Lebel, R.M., Korzan, J.R., Zavodni, A.E., Warren, K.G., Catz, I., Emery, D.J., Wilman, A.H., 2009. Detecting lesions in multiple sclerosis at 4.7 tesla using phase susceptibility-weighting and T2-weighting. J. Magn. Reson. Imaging 30, 737–742.

Fiebiger, S.M., Bros, H., Grobosch, T., Janssen, A., Chanvillard, C., Paul, F., Dörr, J., Millward, J.M., Infante-Duarte, C., 2013. The antioxidant idebenone fails to prevent or attenuate chronic experimental autoimmune encephalomyelitis in the mouse. J. Neuroimmunol. 262, 66–71.

Ge, Y., Jensen, J.H., Lu, H., Helpern, J.A., Miles, L., Inglese, M., Babb, J.S., Herbert, J., Grossman, R.I., 2007. Quantitative assessment of iron accumulation in the deep gray matter of multiple sclerosis by magnetic field correlation imaging. AJNR Am. J. Neuroradiol. 28, 1639–1644.

Gilgun-Sherki, Y., Melamed, E., Offen, D., 2004. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J. Neurol. 251, 261–268.

Gonsette, R.E., Sindic, C., D'hooghe, M.B., De Deyn, P.P., Medaer, R., Michotte, A., Seeldrayers, P., Guillaume, D., 2010. Neuroprotection in multiple sclerosis: the Association of Inosine and Interferon ASIIMS study group. Boosting endogenous beta in relapsing- remitting Multiple Sclerosis (ASIIMS) trial. Mult. Scler. 16, 455–462.

Haacke, E.M., Cheng, N.Y., House, M.J., Liu, Q., Neelavalli, J., Ogg, R.J., Khan, A., Ayaz, M., Kirsch, W., Obenaus, A., 2005. Imaging iron stores in the brain using magnetic resonance imaging. Magn. Reson. Imaging 23, 1–25.

Haacke, E.M., Makki, M., Ge, Y., Maheshwari, M., Sehgal, V., Hu, J., Selvan, M., Wu, Z., Latif, Z., Xuan, Y., Khan, O., Garbern, J., Grossman, R.I., 2009. Characterizing iron deposition in multiple sclerosis lesions using susceptibility weighted imaging. J. Magn. Reson. Imaging 29, 537–544.

Habib, C.A., Liu, M., Bawany, N., Garbern, J., Krumbein, I., Mentzel, H.J., Reichenbach, J., Magnano, C., Zivadinov, R., Haacke, E.M., 2012. Assessing abnormal iron content in the deep gray matter of patients with multiple sclerosis versus healthy controls. AJNR Am. J. Neuroradiol. 33, 252–258.

Hagemeier, J., Heininen-Brown, M., Poloni, G.U., Bergsland, N., Magnano, C.R., Durfee, J., Kennedy, C., Carl, E., Weinstock-Guttman, B., Dwyer, M.G., Zivadinov, R., 2012b. Iron deposition in multiple sclerosis lesions measured by susceptibility-weighted imaging filtered phase: a case control study. J. Magn. Reson. Imaging 36, 73–83.

Hagemeier, J., Weinstock-Guttman, B., Bergsland, N., Heininen-Brown, M., Carl, E., Kennedy, C., Magnano, C., Hojnacki, D., Dwyer, M.G., Zivadinov, R., 2012a. Iron deposition on SWI-filtered phase in the subcortical deep gray matter of patients with clinically isolated syndrome may precede structure-specific atrophy. AJNR Am. J. Neuroradiol. 33, 1596–1601.

Hagemeier, J., Weinstock-Guttman, B., Heininen-Brown, M., Poloni, G.U., Bergsland, N., Schirda, C., Magnano, C.R., Kennedy, C., Carl, E., Dwyer, M.G., Minagar, A., Zivadinov, R., 2013a. Gray matter SWI-filtered phase and atrophy are linked to disability in MS. Front. Biosci. (Elite Ed) 5, 525–532.

Hagemeier, J., Yeh, E.A., Brown, M.H., Bergsland, N., Dwyer, M.G., Carl, E., Weinstock-Guttman, B., Zivadinov, R., 2013b. Iron content of the pulvinar nucleus of the thalamus is increased in adolescent multiple sclerosis. Mult. Scler. 19, 567–576.

Hallgren, B., Sourander, P., 1958. The effect of age on the non-haemin iron in the human brain. J. Neurochem. 3, 41–45.
Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97, 1634–1658.
Hametner, S., Wimmer, I., Haider, L., Pfeifenbring, S., Brück, W., Lassmann, H., 2013. Iron and neurodegeneration in the multiple sclerosis brain. Ann. Neurol. 74, 848–861.
Hammond, K.E., Metcalf, M., Carvajal, L., Okuda, D.T., Srinivasan, R., Vigneron, D., Nelson, S.J., Pelletier, D., 2008. Quantitative in vivo magnetic resonance imaging of multiple sclerosis at 7 Tesla with sensitivity to iron. Ann. Neurol. 64, 707–713.
Khalil, M., Enzinger, C., Langkammer, C., Tscherner, M., Wallner-Blazek, M., Jehna, M., Ropele, S., Fuchs, S., Fazekas, F., 2009. Quantitative assessment of brain iron by R(2)* relaxometry in patients with clinically isolated syndrome and relapsing-remitting multiple sclerosis. Mult. Scler. 15, 1048–1054.
Khalil, M., Langkammer, C., Ropele, S., Petrovic, K., Wallner-Blazek, M., Loitfelder, M., Jehna, M., Bachmaier, G., Schmidt, R., Enzinger, C., Fuchs, S., Fazekas, F., 2011a. Determinants of brain iron in multiple sclerosis: a quantitative 3T MRI study. Neurology 77, 1691–1697.
Khalil, M., Teunissen, C., Langkammer, C., 2011b. Iron and neurodegeneration in multiple sclerosis. Mult. Scler. Int. 2011, 606807.
Langemann, H., Kabiersch, A., Newcombe, J., 1992. Measurement of low-molecular-weight antioxidants, uric acid, tyrosine and tryptophan in plaques and white matter from patients with multiple sclerosis. Eur. Neurol. 32, 248–252.
Langkammer, C., Krebs, N., Goessler, W., Scheurer, E., Ebner, F., Yen, K., Fazekas, F., Ropele, S., 2010. Quantitative MR imaging of brain iron: a postmortem validation study. Radiology 257, 455–462.
Lebel, R.M., Eissa, A., Seres, P., Blevins, G., Wilman, A.H., 2012. Quantitative high-field imaging of sub-cortical gray matter in multiple sclerosis. Mult. Scler. 18, 433–441.
Linker, R.A., Lee, D.H., Ryan, S., van Dam, A.M., Conrad, R., Bista, P., Zeng, W., Hronowsky, X., Buko, A., Chollate, S., Ellrichmann, G., Brück, W., Dawson, K., Goelz, S., Wiese, S., Scannevin, R.H., Lukashev, M., Gold, R., 2011. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 134, 678–692.
Lynch, S.G., Fonseca, T., Levine, S.M., 2000. A multiple course trial of desferrioxamine in chronic progressive multiple sclerosis. Cell Mol. Biol. 46, 865–869.
Madsen, E., Gitlin, J.D., 2007. Copper and iron disorders of the brain. Annu. Rev. Neurosci. 30, 317–337.
Mancuso, M., Davidzon, G., Kurlan, R.M., Tawil, R., Bonilla, E., Di Mauro, S., Powers, J.M., 2005. Hereditary ferritinopathy: a novel mutation, its cellular pathology, and pathogenetic insights. J. Neuropathol. Exp. Neurol. 64, 280–294.
Markowitz, C.E., Spitsin, S., Zimmerman, V., Jacobs, D., Udupa, J.K., Hooper, D.C., Koprowski, H., 2009. The treatment of multiple sclerosis with inosine. J. Altern. Complement. Med. 15, 619–625.
Marracci, G.H., Jones, R.E., McKeon, G.P., Bourdette, D.N., 2002. Alpha lipoic acid inhibits T cell migration into the spinal cord and suppresses and treats experimental autoimmune encephalomyelitis. J. Neuroimmunol. 13, 104–114.
Massacesi, L., Abbamondi, A.L., Giorgi, C., Sarlo, F., Lolli, F., Amaducci, L., 1987. Suppression of experimental allergic encephalomyelitis by retinoic acid. J. Neurol. Sci. 80, 55–64.
Mehta, V., Pei, W., Yang, G., Li, S., Swamy, E., Boster, A., Schmalbrock, P., Pitt, D., 2013. Iron is a sensitive biomarker for inflammation in multiple sclerosis lesions. PLoS One 8, e57573.
Moos, T., Rosengren Nielsen, T., Skjorringe, T., Morgan, E.H., 2007. Iron trafficking inside the brain. J. Neurochem. 103, 1730–1740.
Neema, M., Arora, A., Healy, B.C., Guss, Z.D., Brass, S.D., Duan, Y., Buckle, G.J., Glanz, B.I., Stazzone, L., Khoury, S.J., Weiner, H.L., Guttmann, C.R., Bakshi, R., 2009. Deep gray matter involvement on brain MRI scans is associated with clinical progression in multiple sclerosis. J. Neuroimaging 19, 3–8.
Neema, M., Ceccarelli, A., Jackson, J., Bakshi, R., 2012. Magnetic resonance imaging in multiple sclerosis. In: Weiner, H., Stankiewicz, J. (Eds.), Multiple Sclerosis: Diagnosis and Therapy. Wiley-Blackwell, West Sussex, England, pp. 136–162.
Neema, M., Stankiewicz, J., Arora, A., Dandamudi, V.S., Batt, C.E., Guss, Z.D., Al-Sabbagh, A., Bakshi, R., 2007. T1- and T2-based MRI measures of diffuse gray matter and white matter damage in patients with multiple sclerosis. J. Neuroimaging 17, 16S–21S.
Oliveira, S.R., Kallaur, A.P., Simão, A.N., Morimoto, H.K., Lopes, J., Panis, C., Petenucci, D.L., da Silva, E., Cecchini, R., Kaimen-Maciel, D.R., Reiche, E.M., 2012. Oxidative stress in multiple sclerosis patients in clinical remission: association with the expanded disability status scale. J. Neurol. Sci. 321, 49–53.
Pantopoulos, K., Porwal, S.K., Tartakoff, A., Devireddy, L., 2012. Mechanisms of mammalian iron homeostasis. Biochemistry 51, 5705–5724.
Pawate, S., Wang, L., Song, Y., Sriram, S., 2012. Analysis of T2 intensity by magnetic resonance imaging of deep gray matter nuclei in multiple sclerosis patients: effect of immunomodulatory therapies. J. Neuroimaging 22, 137–144.
Pedchenko, T.V., LeVine, S.M., 1999. Desferrioxamine suppresses experimental allergic encephalomyelitis induced by MBP in SJL mice. J. Neuroimmunol. 84, 188–197.
Pinero, D., Connor, J., 2000. Iron in the brain: an important contributor in normal and diseased states. Neuroscientist 6, 435–453.
Pitt, D., Boster, A., Pei, W., Wohleb, E., Jasne, A., Zachariah, C.R., Rammohan, K., Knopp, M.V., Schmalbrock, P., 2010. Imaging cortical lesions in multiple sclerosis with ultra-high-field magnetic resonance imaging. Arch. Neurol. 67, 812–818.
Ropele, S., de Graaf, W., Khalil, M., Wattjes, M.P., Langkammer, C., Rocca, M.A., Rovira, A., Palace, J., Barkhof, F., Filippi, M., Fazekas, F., 2011. MRI assessment of iron deposition in multiple sclerosis. J. Magn. Reson. Imaging 34, 13–21.
Rouault, T.A., 2006a. The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat. Chem. Biol. 2, 406–414.
Rouault, T.A., Cooperman, S., 2006b. Brain iron metabolism. Semin. Pediatr. Neurol. 13, 142–148.
Rumzan, R., Wang, J.J., Zeng, C., Chen, X., Li, Y., Luo, T., Lv, F., Wang, Z.P., Hou, H., Huang, F., 2013. Iron deposition in the precentral grey matter in patients with multiple sclerosis: a quantitative study using susceptibility-weighted imaging. Eur. J. Radiol. 82, e95–e99.
Schenck, J.F., Zimmerman, E.A., 2004. High-field magnetic resonance imaging of brain iron: birth of a biomarker? NMR Biomed. 17, 433–445.
Shindler, K.S., Ventura, E., Dutt, M., Elliott, P., Fitzgerald, D.C., Rostami, A., 2010. Oral resveratrol reduces neuronal damage in a model of multiple sclerosis. J. Neuroophthalmol. 30, 328–339.
Skjørringe, T., Møller, L.B., Moos, T., 2012. Impairment of interrelated iron- and copper homeostatic mechanisms in brain contributes to the pathogenesis of neurodegenerative disorders. Front. Pharmacol. 3, 169.
Spitsin, S.V., Scott, G.S., Mikheeva, T., Zborek, A., Kean, R.B., Brimer, C.M., Koprowski, H., Hooper, D.C., 2002. Comparison of uric acid and ascorbic acid in protection against EAE. Free Radic. Biol. Med. 33, 1363–1371.
Stankiewicz, J., Panter, S.S., Neema, M., Arora, A., Batt, C.E., Bakshi, R., 2007. Iron in chronic brain disorders: imaging and neurotherapeutic implications. Neurotherapeutics 4, 371–386.
Stankiewicz, J.M., Brass, S.D., 2009. Role of iron in neurotoxicity: a cause for concern in the elderly? Curr. Opin. Clin. Nutr. Metab. Care 12, 22–29.
Tjoa, C.W., Benedict, R.H., Weinstock-Guttman, B., Fabiano, A.J., Bakshi, R., 2005. MRI T2 hypointensity of the dentate nucleus is related to ambulatory impairment in multiple sclerosis. J. Neurol. Sci. 234, 17–24.
Van der Worp, H.B., Thomas, C.E., Kappelle, L.J., Hoffman, W.P., de Wildt, D.J., Bär, P.R., 1999. Inhibition of iron-dependent and ischemia-induced brain damage by the alpha tocopherol analogue MDL74,722. Exp. Neurol. 155, 103–108.
Walsh, A.J., Blevins, G., Lebel, R.M., Seres, P., Emery, D.J., Wilman, A.H., 2014. Longitudinal MR imaging of iron in multiple sclerosis: an imaging marker of disease. Radiology 270, 186–196.
Walsh, A.J., Lebel, R.M., Eissa, A., Blevins, G., Catz, I., Lu, J.Q., Resch, L., Johnson, E.S., Emery, D.J., Warren, K.G., Wilman, A.H., 2013. Multiple sclerosis: validation of MR imaging for quantification and detection of iron. Radiology 267, 531–542.
Wang, J., Ren, Z., Xu, Y., Xiao, S., Meydani, S.N., Wu, D., 2012. Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4+ T-cell subsets. Am. J. Pathol. 180, 221–234.
Weilbach, F.X., Chan, A., Toyka, K.V., Gold, R., 2004. The cardioprotector dexrazoxane augments therapeutic efficacy of mitoxantrone in experimental autoimmune encephalomyelitis. Clin. Exp. Immunol. 135, 49–55.
Willenborg, D.O., Bowern, N.A., Danta, G., Doherty, P.C., 1988. Inhibition of allergic encephalomyelitis by the iron chelating agent desferrioxamine: differential effect depending on type of sensitizing encephalitogen. J. Neuroimmunol. 17, 127–135.
Xie, L., Li, X.K., Funeshima-Fuji, N., Kimura, H., Matsumoto, Y., Isaka, Y., Takahara, S., 2009. Amelioration of experimental autoimmune encephalomyelitis by curcumin treatment through inhibition of IL-17 production. Int. Immunopharmacol. 9, 575–581.
Xin, J., Feinstein, D.L., Hejna, M.J., Lorens, S.A., McGuire, S.O., 2012. Beneficial effects of blueberries in experimental autoimmune encephalomyelitis. J. Agric. Food Chem. 60, 5743–5748.
Yao, B., Bagnato, F., Matsuura, E., Merkle, H., van Gelderen, P., Cantor, F.K., Duyn, J.H., 2012. Chronic multiple sclerosis lesions: characterization with high-field-strength MR imaging. Radiology 262, 206–215.
Zhang, Y., Metz, L.M., Yong, V.W., Mitchell, J.R., 2010. 3T deep gray matter T2 hypointensity correlates with disability over time in stable relapsing-remitting multiple sclerosis: a 3-year pilot study. J. Neurol. Sci. 297, 76–81.
Zhang, Y., Zabad, R., Wei, X., Metz, L., Hill, M., Mitchell, J., 2007. Deep grey matter "black T2" on 3 tesla magnetic resonance imaging correlates with disability in multiple sclerosis. Mult. Scler. 13, 880–883.
Zivadinov, R., Heininen-Brown, M., Schirda, C.V., Poloni, G.U., Bergsland, N., Magnano, C.R., Durfee, J., Kennedy, C., Carl, E., Hagemeier, J., Benedict, R.H., Weinstock-Guttman, B., Dwyer, M.G., 2012. Abnormal subcortical deep-gray matter susceptibility-weighted imaging filtered phase measurements in patients with multiple sclerosis: a case-control study. Neuroimage 59, 331–339.