EDITORIAL

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Monitoring increased iron levels in multiple sclerosis using MRI

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Clinical symptoms & pathophysiology of multiple sclerosis in relation to iron accumulation

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease of the CNS characterized by a varied and unpredictable time course [1]. Though the specific cause is unknown, it appears to involve both genetic susceptibility and triggers such as a virus, dysregulated metabolism or environmental factors [2,3]. In most patients, MS consists of initial episodes of reversible neurological deficit, followed by progressive deterioration that can become severe and result in significant patient disability. In particular, evolution of the disease produces a spectrum of clinical features that may include motor weakness, sensory and gait disturbances, vision loss and cognitive changes [1].

Conventional MRI, using standard T_1 - and T_2 -weighted imaging has an important role in diagnosing MS [4]. However, it is now generally accepted that conventional MRI measures of MS disease status, such as single time point lesion load and lesion volume, have not correlated well with clinical symptoms or demonstrated significant

predictive power for determining disease progression [5,6]. By contrast, post-mortem histopathological studies have consistently revealed free iron is present in brain tissue affected by MS [7]. Its presence makes it a potential candidate as an imaging biomarker. Not only are increased iron levels related to MS neurodegeneration, they also have a distinct signature on certain MRI contrasts [8–11].

Further support for the use of iron as a biomarker is provided by our understanding of the cellular-level, MS inflammatory response. The autoimmune targets in MS are believed to be cellular components of the CNS, normally inaccessible due to their location behind the blood–brain barrier. During disease initiation, entry of autoimmune cells (T cells, B cells and monocytes) through the blood–brain barrier is triggered by cytokine and chemokine signals [2]. The inflammatory, autoimmune cells involved contain ferritin iron, an essential compound in normal cellular function. Because of the unique MRI signal produced by ferritin iron, MRI can be used to evaluate the cellular-level infiltrates that play a substantial role in the MS.

KEYWORDS

• iron • MRI • multiple sclerosis

“An integrative approach using MRI could ... lead to a better understanding of multiple sclerosis pathogenesis.”

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“One particular area of the multiple sclerosis brain where iron accumulates more readily than others is the subcortical nuclei.”

One particular area of the MS brain where iron accumulates more readily than others is the subcortical nuclei [11–14]. Elevated iron levels have been reported in subcortical nuclei in both clinically isolated syndrome (CIS) [12] and clinically definite MS patients [11,13]. Excessive levels of subcortical iron exert oxidative stress and result in cell death [15]. Consequently, monitoring the accumulation of iron in the subcortical nuclei and linking this to clinical disability is an emerging area of major interest in MRI of MS.

MRI techniques for visualization of iron in MS

Over the last two decades, several quantitative MRI techniques for monitoring iron in MS have been examined. These include: R_2 (spin echo transverse relaxation rate)- [11,16,17] and R_2^* (gradient echo transverse relaxation rate)-based relaxometry [9,10]; magnetic field correlation imaging (MFCI) [8]; and quantitative susceptibility mapping (QSM) [12,13,18].

Because of its insensitivity to nonlocal background magnetic fields, R_2 has the potential to offer reliable and reproducible measurements of subcortical iron. At higher main magnetic fields (≥ 3 T), subcortical R_2 measurements may be particularly useful because the R_2 value of iron compounds increases linearly with main magnetic field strength [19]. Indeed, Lebel *et al.* [11] have demonstrated increased R_2 in selected subcortical nuclei of RRMS patients compared with age and gender matched controls. In the pulvinar nucleus, increased R_2 values were correlated with extended disability status scale (EDSS) score. Recently, Walsh *et al.* [14] have also demonstrated a strong correlation between subcortical R_2 *in vivo* and optical density of iron staining obtained from postmortem histopathology.

Currently however, whole-brain imaging is generally not performed with R_2 mapping due to the associated longer acquisition times. If challenges related to the longer acquisition times and increased radiofrequency power deposition (particularly at higher main magnetic fields) can be addressed, this method will become more readily applicable for monitoring whole-brain iron in MS.

The use of R_2^* as a biomarker of iron is attractive because of its relative ease and efficiency of acquisition using a standard multi-echo, gradient echo sequence. Khalil *et al.* have recently demonstrated in two separate studies [9,10] that R_2^* in subcortical nuclei is increased in MS and CIS

patients compared with control subjects. Notably, subcortical R_2^* levels were also increased in MS compared with both CIS and healthy controls. As well, using stepwise linear regression, subcortical R_2^* values were correlated with age, disease duration, EDSS and mental processing speed.

These results are encouraging. However, care must be taken in the interpretation of R_2^* values in brain tissue due to the significant impact of nontissue specific magnetic fields produced by susceptibility boundaries in the brain [20,21]. Additionally, interpretation of the R_2^* signal becomes more challenging in cases where multiple tissue components (e.g., myelinated axons, microglia and oligodendrocytes) exist within an imaging voxel. In such a scenario, a simple mono-exponential decay model of R_2^* is generally insufficient [22–24]. Future clinical studies using R_2^* to monitor longitudinal iron accumulation in subcortical nuclei should help improve our understanding of the utility of this biomarker in predicting the MS progression. Further work should also examine the relationship between subcortical iron and more common imaging-based measures of MS such as lesion load and brain atrophy. Investigating these relationships has the potential to more clearly define consistent, whole-brain imaging biomarkers of MS.

Compared to R_2 and R_2^* mapping, MFCI advantageously removes the effect of dipole–dipole interactions and macroscopic, inhomogeneous magnetic fields which can make quantitation more difficult [25,26]. Moreover, because the MFC parameter scales quadratically with main magnetic field strength [25,26], its acquisition using high-field MRI is an attractive option. MFC values generally also have a larger dynamic range compared with R_2 and R_2^* values [25,26]. As a result, this method may be useful for mapping subtle differences in iron concentration which are not detected using other iron imaging techniques. Ge *et al.* [8] have demonstrated significant increases in MFC of RRMS patients compared with matched control subjects in the globus pallidus, putamen and thalamus. The increases in the thalamus and globus pallidus were associated with white matter (WM) lesion load. Moderate correlations were also found between MFC in the thalamus and neuropsychological test scores [8].

To date, however there have been few MFCI studies of MS due to the more complicated pulse sequence used with its acquisition (an asymmetric spin-echo sequence with echo-planar imaging

readout). Future research, focusing on larger MS patient cohorts with varying disease subtypes, is necessary to more fully understand the utility of this method. A comparison of MFCI-derived iron measures to those obtained from relaxometry and QSM would also be instructive.

QSM is a relatively new, MRI-based, iron mapping method that has shown an increased sensitivity to changes in subcortical iron associated with normal ageing [27] and MS disease-related processes [13]. The increased sensitivity has been hypothesized to result from the ability of QSM to measure the mean magnetic field perturbation in an imaging voxel, as opposed to the signal relaxation of protons in water molecules [28]. The mean magnetic field perturbation can be more directly linked to the amount of iron in tissue [27]. Additionally, QS maps can be efficiently derived from standard gradient echo imaging data. For example, Langkammer *et al.* [13] have demonstrated elevated QS values in subcortical nuclei of CIS and clinically definite MS patients compared with controls. Subcortical nuclei QSM values were higher with increasing EDSS and lower with both normalized total volume of gray matter and cortical gray matter volume [13].

Nevertheless, the use of QSM is still hampered by challenges related to proper processing of the MRI phase information [29–31]. Equally relevant, the specific tissue structures and chemical compounds which contribute to the QS signal within a voxel are not completely understood. With more advanced biophysical models and improved QS map calculation, however, QSM has the potential to serve a significant role in the efficient detection of whole-brain distributions of iron in MS.

On balance, although iron-sensitive MRI has already shown potential as a clinical biomarker of MS, its exact utility for predicting onset, tracking disease progression or monitoring therapy is yet to be determined. Present iron imaging techniques offer complementary information to the standard scans conventionally used in MS diagnosis. With additional refinement, they have the potential to assume a more substantial role in both tracking MS progression and stratifying MS disease subtypes.

Clinical & diagnostic significance of tracking iron accumulation using MRI

Regardless of whether increased iron levels are a cause or a consequence of the MS pathological

process, their detection may, in future, be used to measure the effects of treatment and predict disease progression. Several candidate mechanisms have been proposed for the role of iron in MS. The most general of these posits that, when iron becomes unchelated or is metabolically dysregulated, cell damage occurs due to the catalysis of free radical oxidations induced by free iron species [15,32]. In particular, lipid peroxidation reactions catalyzed by iron are believed to be one of the key initiators of cell death in MS [15]. In these reactions, the unsaturated lipids in cell membranes react with free radical oxygen species to produce toxic lipid hydroperoxides, which contribute to DNA damage and result in cell death [15].

Current understanding of the underlying cellular sources of iron-induced tissue damage has led to research investigating iron chelators and biochemical inhibitors of lipid peroxidation for MS therapy [33–35]. Although these therapies have demonstrated effectiveness in animal models [36,37], in clinical trials with progressive MS patients they have failed to demonstrate significant improvement in patient symptoms [38,39].

From a diagnostic standpoint, longitudinal imaging studies of iron may allow clinicians to pre-empt worsening clinical symptoms by knowing when to administer therapy. QSM has already recognized there is a progressive increase in iron levels in newly formed, contrast-enhancing WM lesions [18]. This has been attributed to iron-bearing cell infiltration [18]. As well, non-enhancing WM lesions have characteristically shown an accentuated iron and ferritin rim with a reduction in iron at the lesion core [40]. Such MRI signatures are believed to signify an acute inflammatory and demyelinating MS response. Administering therapy catered to the inflammatory phases of the MS response could, accordingly, be facilitated by using iron-sensitive MRI. However, for this to be practical, the cost–effectiveness and specificity of iron-imaging methods need to be further addressed.

Considering iron accumulation in separate brain regions (e.g., WM lesions, subcortical nuclei and normal-appearing WM) has been a major focus of current MRI research in MS. Future studies should examine the relationship between iron accumulations in different structures across the whole brain. This would offer an improved depiction of the full disease process and help clinicians understand which parts of

“Future studies should examine the relationship between iron accumulations in different structures across the whole brain.”

the brain are most significantly affected in the various MS subtypes.

Conclusion

Is iron solely a byproduct of the MS inflammatory process? Or does it play a causal role in the progressive demyelination and axonal loss? Is increased iron storage a function of oligodendrocyte impairment and subsequent iron release? These questions remain unanswered. An integrative approach using MRI could help answer the above questions and lead to a better understanding of MS pathogenesis. In doing so, it could

also provide increased insight into the efficacy of such novel MS therapies as iron chelators and inhibitors of iron-related oxidative stress.

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