The Presence and Role of Iron in Mild Traumatic Brain Injury: An Imaging Perspective

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Abstract

Mild traumatic brain injury (mTBI), although often presenting without the gross structural abnormalities seen in more severe forms of brain trauma, can nonetheless result in lingering cognitive and behavioral problems along with subtle alterations in brain structure and function. Repeated injuries are associated with brain atrophy and dementia in the form of chronic traumatic encephalopathy (CTE). The mechanisms underlying these dysfunctions are poorly understood. There is a growing body of evidence that brain iron is abnormal after TBI, and brain iron has also been implicated in a host of neurodegenerative disorders. The purpose of this article is to review evidence about the function of iron in the pathophysiology of mTBI and the role that advanced imaging modalities can play in further elucidating said function. MRI techniques sensitive to field inhomogeneities provide supporting evidence for both deep gray matter non-heme iron accumulation as well as focal microhemorrhage resulting from mTBI. In addition, there is evidence that iron may contribute to pathology after mTBI through a number of mechanisms, including generation of reactive oxygen species (ROS), exacerbation of oxidative stress from other sources, and encouragement of tau phosphorylation and the formation of neurofibrillary tangles. Finally, recent animal studies suggest that iron may serve as a therapeutic target in mitigating the effects of mTBI. However, research on the presence and role of iron in mTBI and CTE is still relatively sparse, and further work is necessary to elucidate issues such as the sources of increased iron and the chain of secondary injury.

Key words: iron; MRI; TBI

Introduction

IN RECENT YEARS, increasing attention is being paid to the lingering and serious health problems that can result from mild traumatic brain injury (mTBI). Patients may exhibit cognitive and behavioral symptoms > 1 year post-injury,¹ and repeated incidents of mTBI are linked to serious deficits, generalized brain atrophy, and neuronal loss.^{2,3} mTBI often presents without any of the more apparent structural abnormalities seen in moderate and severe brain injury;¹ however, there is a growing body of evidence that shows that single concussive episodes are associated with subtle alterations in brain structure^{4–9} and function.^{10–13} mTBI differs from moderate and severe TBI in that large focal lesions do not contribute to the mechanism of injury in mTBI. The complex cognitive, behavioral, and affective deficits that mTBI patients have, suggest diffuse or global injury to the brain and brain systems. The mechanisms underlying observed structural changes and symptoms of mTBI are not known. In particular, elucidating secondary injury mechanisms that contribute to ongoing injury after mTBI is of interest, as such mechanisms could provide novel targets for treatment.

Recent investigations both in animal models and humans have implicated abnormal iron in the pathogenesis of the disorder.^{13–17} Iron plays a critical role in the metabolic processes of the human brain as a cofactor for numerous proteins. However, tight regulation is critical, as iron is a transition metal and participates in redox reactions to form reactive oxygen species (ROS) that in turn can cause oxidative stress. Elevated iron and oxidative stress are implicated in the pathology of diverse acute and chronic brain injuries including ischemia,¹⁸ hemorrhage,¹⁹ and neurodegenerative diseases such as Alzheimer's dementia²⁰ (AD) and Parkinson's disease (PD).²⁰ Chronic traumatic encephalopathy (CTE), a neurodegenerative disease associated with repetitive mTBI, shares a number of clinical and neuropathological characteristics with AD,³ including abnormal non-heme iron deposition in subcortical neurofibrillary tangles (NFTs).¹⁷ Along with compelling animal data, increased brain iron in humans following mTBI appears to be present using some new MRI techniques.

Iron as a source of pathology in mTBI has implications for how the disorder is evaluated and treated. This article summarizes the current work evaluating abnormal brain iron metabolism in mTBI and its role in the pathology and treatment thereof. Particular

attention is paid to imaging techniques as a powerful, noninvasive way to quantify brain iron.

Non-heme Iron and Heme Iron in mTBI

Non-heme iron

Brain iron abnormally deposited as a result of mTBI may belong to two categories of physiologic iron: non-heme iron and heme iron. Non-heme iron comprises all iron not bound to heme groups, for example, iron bound to proteins such as ferritin and transferrin as well as free iron. Abnormal non-heme iron is present in a variety of neurodegenerative conditions, and is associated with abnormal or dysfunctional iron transport pathways.²¹ There is a small but growing body of evidence that non-heme iron is increased after mTBI. In particular, in patients who have sustained multiple mTBIs, there is histological evidence¹⁷ of significant non-heme iron deposition similar to that seen in AD. Postmortem examination of two patients with dementia pugilistica (now called CTE) using laser microprobe mass analysis revealed¹⁷ high levels of iron within NFTs, and neuronal nuclei of the hippocampus and inferior temporal cortex. A similar distribution of elevated iron was observed in AD patients also examined in the study.¹⁷ In AD patients, it is known that non-heme iron within NFTs is redox active.^{22,23} Presumably this would also be the case in CTE, as the tau isoforms are identical to those of AD.³

Even in single-episode head trauma, there is evidence for increased non-heme iron. Using a controlled cortical impact mouse model, hypointensity on T2-weighted images has been confirmed histologically to correspond to abnormal non-heme iron accumulation in the thalamus. The authors, Onyszchuk et al., postulated that degeneration of thalamic neurons may result in release of free iron.¹⁶ Related observations have been made in human mTBI subjects.¹⁵ Because of its paramagnetism, iron serves as an endogenous tissue MR contrast agent. This has led to the

development of exciting MRI techniques to quantify and characterize tissue iron distribution based on its effect on the transverse relaxation of the NMR signal.^{24–27} We believe increasing amounts of brain iron as seen using MRI will be a sensitive method to detect subsequent injury.

Magnetic field correlation (MFC), an MR technique that utilizes an asymmetric spin echo sequence to probe local field inhomogeneity, has been recently developed and applied to quantifying *in vivo* iron.^{15,25–27} It is able to show differences in magnetic susceptibility between mTBI patients and control subjects believed to reflect brain iron (Fig. 1).

Technically, MFC is closely related to the non-monoexponential T2* relaxation. The MFC value is a measure of the variance of local Larmor precession frequency in a given imaging voxel. As the Larmor frequency is proportional to the value of the local magnetic field, MFC is a measure of the voxel-wise field variance.

The total MFC value is composed of two components: macroscopic and microscopic. The macroscopic component measures field inhomogeneities occur on a scale commensurate with or greater than voxel size. These originate from inter-voxel differences in susceptibility such as from tissue/tissue interfaces, air cavities, and artifacts resulting from imperfect shim. The microscopic component measures field inhomogeneities at a length scale much smaller than a voxel. These are induced by susceptibility differences occurring on the length scale of the order of several microns, which is the cellular scale, of particular interest when studying cellular level iron. It is of note that Jensen et al.²⁷ showed excellent correlation between MFC_{micro} and non-heme, cellular level iron as measured on autopsy specimens, and, furthermore, found that the dominant MFC component in most brain regions was the microscopic MFC.²⁷ Raz et al.¹⁵ used MFC to study mTBI patients, and found higher values in the thalamus and global pallidus compared with controls at an average of 18 months after injury. MFC is not dependent on intrinsic tissue T2 relaxivity,

FIG. 1. Parametric microscopic magnetic field correlation (MFC) maps of the deep gray matter structures superimposed on anatomic T1-weighted image in a normal control (**A**) and an age-matched 44 year-old mild traumatic brain injury (mTBI) patient (**B**) 5 days after injury show markedly higher microscopic MFC in the mTBI subject (MFC units = s^{-2}), believed to be the result of brain iron.

FIG. 2. Statistically significant differences between 24 patients (18 males, 6 females; average age=34, age range=18–56) ~1–2 months after mild traumatic brain injury as defined by the American Congress of Rehabilitation Medicine compared with matched normal controls show increased total magnetic field correlation (MFC) and MFC_{micro} in the thalamus believed to represent iron deposition. MFC_{micro} quantifies intravoxel field homogeneities; cellular-level iron would be expected to affect this component of MFC. MFC_{macro} quantifies field homogeneities occurring on a scale commensurate to or greater than the size of a voxel, cellular-level iron would not be expected to affect this component. $MFC_{Thalamus}$ is the sum of MFC_{micro} and MFC_{macro} [unpublished data].

providing further evidence that increased microscopic MFC reflects elevated levels of brain iron distributed at a cellular, rather than a molecular, level.¹⁵ Figure 2 shows differences in thalamic total MFC and MFC_{micro} between 24 patients with mTBI 1–2 months after injury and 24 control subjects, believed to be caused by differences in non-heme iron.

Heme iron

Heme iron refers to iron bound to heme cofactors within proteins such as hemoglobin. Following intracranial hemorrhage, the phagocytosis of red blood cells can lead to the focal deposition of the heme iron in the form of hemosiderin.²⁸ Hemosiderin is an insoluble iron storage complex that is believed to result from the degradation of ferritin,²⁴ and the presence of hemosiderin complexes indicates prior hemorrhage.²⁹

Although frank hemorrhage is common in moderate and severe TBI, it occurs to a much lesser degree in mTBI,³⁰ and patients with mTBI usually have normal CT and conventional MR imaging. However, there is evidence indicating the presence of microhemorrhage in some cases of mTBI. A case report of a patient with a history of mTBI, who died later of unrelated causes, reported evidence of previous hemorrhage in the form of hemosiderin-laden macrophages in the frontal and temporal lobes on histopathological examination.³¹ Recent studies utilizing newer imaging techniques, including susceptibility weighted imaging (SWI),^{32–35} likewise demonstrated that in a subset of mTBI patients, small hemorrhages occur. SWI combines the phase and magnitude of T2* gradient echo imaging (GRE) to accentuate net susceptibility effects and, therefore, to enhance detection of macroscopic field inhomogeneities such as from focal deposits of hemosiderin (Fig. 3).^{36,37}

However, as the SWI phase includes the effect of all susceptibility sources, it cannot distinguish between effects of iron distributed on a cellular level and macroscopic field inhomogeneities. Hence, this technique is complementary to the analysis of the relaxational effects, such as the T2* decay and the MFC. In combination with greater magnet strength, which also increases susceptibility effects,^{30,38} SWI proves to be significantly more sensitive

to hemorrhage than conventional T2* GRE.^{32,39} For example, a study of adolescents with moderate TBI showed a sixfold increase in hemorrhage detection.³⁹ In mTBI, small hemorrhages not observed using conventional T2-weighted images have been reported using SWI^{33,34} with the majority of such hemorrhages occurring in

FIG. 3. Maximum intensity projection in the axial plane of susceptibility weighted image in a 27-year-old boxer showing focal area of presumed hemorrhage in the left frontal white matter (arrow).

white matter in a distribution reminiscent of diffuse axonal injury.^{32–34} However, these abnormalities are present in only a subset of patients. Others lack detectable lesions even with the most sensitive imaging modalities. A recent article by Toth et al. found no traumatic lesions in 14 mTBI patients using SWI. The fact that an especially sensitive method such as SWI may also be ineffective in detecting lesions in mTBI underscores the importance of MFC and other iron-imaging methods.⁴⁰ An ongoing study in active National Football League players reports that 0 of 18 players evaluated had focal microhemorrhage.⁴¹ Altogether, these data suggest that a subgroup of mTBI patients do have areas of focal parenchymal hemorrhage, although the incidence is unknown.

Other MRI techniques of interest

The application of advanced MR imaging modalities to the detection of iron in mTBI has, to date, been limited and is summarized previously. In addition, a number of other MR imaging techniques are worth commenting on regarding detection and quantification of non-heme and heme iron, although a complete review of brain iron imaging is beyond the scope of this article. Several different techniques have been used to study other conditions that implicate abnormal iron deposition including AD and PD. MRI transverse relaxation rates $R2^{42–45}$ and $R2^*^{43,46–48}$ correlate with brain iron concentration, although they are affected by tissue fluid content. Field dependent relaxation rate increase imaging (FDRI)^{42,49,50} and quantitative susceptibility mapping (QSM)^{51–55} are more recent advances in brain iron imaging. FDRI utilizes imaging at two different field strengths, allowing the calculation of a parameter independent of tissue liquid content, which correlates with reported values of brain iron.⁴⁴ In QSM imaging, a quantitative map of intrinsic tissue susceptibility is calculated. QSM values strongly correlate with reported values of gray matter iron.^{51–53} These techniques hold promise as potential tools in the further study of iron's role in mTBI.

Heme-oxygenase 1 (HO-1)

Heme iron and non-heme iron can, of course, affect one another. In particular, increased heme iron may result in increased non-heme iron.^{37,56} Direct injection of blood into rat basal ganglia results in persistent threefold increase in non-heme iron concentration, believed to be caused by cellular lysis releasing iron and upregulation of HO-1 in surrounding inflammatory glial cells.⁵⁷ HO-1 degrades heme-bound iron into components, including free iron. As such, it is postulated that rises in HO-1 concentration increase heme degradation and contribute to iron overload.⁵⁷ In a controlled cortical impact rat model of TBI, expression of HO-1 was found to be increased in glial cells surrounding the impact site 2–4 days after injury.⁵⁸ The presence of HO-1 overexpression in mTBI and CTE is incompletely investigated. Chronic HO-1 upregulation as is seen in neurodegenerative disorders such as AD and PD⁵⁹ has been shown to promote unregulated accumulation of pathological iron in astroglia.^{59–61} There is evidence of a common pathway involving oxidative stress, glial HO-1 expression, mitochondrial iron sequestration, and mitochondrial insufficiency found in AD, PD, and multiple sclerosis, which has been proposed to also play a role in other neurological disorders including TBI.^{21,61–63} This suggests that downstream iron brain regulation is further complicated by enzymatic factors.

Iron-Based Pathology in mTBI: Neurodegenerative

Mechanism of injury

Research points to abnormal iron as a potential source of secondary injury following mTBI. The ability of free iron to generate ROS is well established. In what is known as the Haber–Weiss reactions, Fe^{3+} is reduced by a superoxide radical and then reacts with hydrogen peroxide to produce a hydroxyl radical, an ROS of particularly high reactivity.⁶⁴ When the production of ROS exceeds the capacity of cellular protective mechanisms, a cell is said to be in a state of oxidative stress,⁶⁴ in which ROS are free to react with and damage proteins, lipids, and DNA, leading to cellular dysfunction and death.⁶⁵ Iron-mediated oxidative stress is implicated in the pathology of a wide array of diseases, including acute and chronic neurological disorders.⁶⁵

Recent studies have established oxidative stress as a source of dysfunction in mTBI. In both weight-drop and fluid percussion rat models of mTBI, protein oxidation and lipid peroxidation resulting from oxidative stress were observed following injury.^{66–68} Oxidative stress was also linked to decreased synaptic plasticity with corresponding behavioral deficits.^{67,68} It is important to note, however, that in addition to Haber–Weiss reactions, there are several other potential contributions to ROS formation following TBI, such as calcium-mediated activation of phospholipases, nitric oxide synthase, and xanthine oxidase.⁶⁹

Free iron may also further exacerbate damage caused by ROS that are generated by any mechanism, and prolong the effects of oxidative stress. In cultured rat cortical neurons, free iron was found to facilitate ROS-related damage to neuronal DNA.⁷⁰ As such, further research is necessary to quantify and determine the specific long-term contribution of iron-generated ROS to oxidative stress following mTBI, and the synergistic effects this may have on oxidative stress arising from other mechanisms.

There is evidence that iron plays a role in histopathological changes observed in CTE. Studies using transgenic mice and neuron-like cell lines, suggest that HO-1 overexpression promotes NFT formation through accumulation of free iron, which in turn induces tau phosphorylation.⁷¹ Furthermore, *in vitro* studies examining tau in the form of paired helical filaments found that iron-mediated oxidative stress and iron-induced tau aggregation may be linked.⁷² Beta amyloid deposition is also seen in a subset of CTE patients,² and iron has also been found to increase the neurotoxicity of beta amyloid. Rats given injections of beta amyloid and iron into the basal ganglia sustained significantly more neuronal loss and DNA damage than did rats injected only with beta amyloid, iron, or saline.⁷³

Treatment

Secondary injury mechanisms including such things as iron-mediated oxidative stress provide potential novel therapeutic targets. Recent research has focused on several different points in the injury cascade.

Free iron has been directly targeted as a therapeutic site. In a controlled cortical injury model of TBI, rats treated with deferoxamine, an iron-chelating agent, every 12 h for 28 days starting 2 h after injury were found to have significantly less neuronal loss and lower levels of brain iron than untreated rats. The untreated rats also demonstrated deficits in a Morris water maze task, which were attenuated in the treated rats.¹³ These findings suggest that decrease in neuronal loss and cognitive ability resulted from the removal of pathological iron deposited as a result of TBI.

ROS have also been investigated as therapeutic targets. In a fluid percussion rat model of mTBI, injection with hydrogen-rich saline – an antioxidant – for 7 days after injury, attenuated the decrease in synaptic plasticity, cognitive deficits, and lipid peroxidation compared with rats injected with regular saline.⁶⁸ In a similar rat model, administration of dietary vitamin E for 4 weeks before injury was also found to protect synaptic plasticity and improve performance on a Morris water maze compared with rats not given vitamin E.⁶⁷

Conclusion

In summary, in a broad range of pre-clinical and clinical studies, abnormal accumulation of brain iron has shown to be detectable following mTBI. In addition to focal deposition of heme-iron in the white matter as a result of microhemorrhage, there is separate evidence of abnormal iron in the deep gray matter, which we believe occurs on a cellular level. Recent advances in MRI technology enable *in vivo* detection of iron-mediated field inhomogeneities, and may help characterize distribution of iron. In particular, combined measurements of the MR signal's phase (e.g., using SWI) and magnitude via the transverse relaxation (e.g., T2* or MFC), allows one to identify and quantify iron accumulation at the cellular level. New studies on the deleterious effects of free iron on the brain and the injury cascade resulting from mTBI have helped elucidate mechanisms that may potentially contribute to secondary injury and long-term changes in CTE. However, the study of the role of iron in the pathology of mTBI remains in its infancy. It has become clear in recent years that the disease burden of mTBI to society is significant, and, as a possible source of secondary injury, the presence of abnormal iron warrants further investigation. More studies on iron in both acute and chronic mTBI are necessary to address our current gaps in knowledge, including what the sources of increased iron may be, and what role iron plays in ROS generation and neurotoxicity.

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Author Disclosure Statement

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