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Measuring iron in the brain using quantitative susceptibility mapping and X-ray fluorescence imaging

Weili Zheng [a], Helen Nichol [b], Saifeng Liu [c], Yu-Chung N. Cheng [a], E. Mark Haacke [a,c,*]

[a] HUH-MR Research/Radiology, Wayne State University, Detroit, MI, USA
[b] Department of Anatomy & Cell Biology, University of Saskatchewan, Saskatoon, SK, Canada
[c] School of Biomedical Engineering, McMaster University, Hamilton, Ontario, Canada

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ABSTRACT

Measuring iron content in the brain has important implications for a number of neurodegenerative diseases. Quantitative susceptibility mapping (QSM), derived from magnetic resonance images, has been used to measure total iron content in vivo and in post mortem brain. In this paper, we show how magnetic susceptibility from QSM correlates with total iron content measured by X-ray fluorescence (XRF) imaging and by inductively coupled plasma mass spectrometry (ICPMS). The relationship between susceptibility and ferritin iron was estimated at 1.10 ± 0.08 ppb susceptibility per μg iron/g wet tissue, similar to that of iron in fixed (frozen/thawed) cadaveric brain and previously published data from unfixed brains. We conclude that magnetic susceptibility can provide a direct and reliable quantitative measurement of iron content and that it can be used clinically at least in regions with high iron content.

Introduction

Iron is an important endogenous biomarker for many neurological diseases and normal aging (Haacke et al., 2005; Schenck and Zimmerman, 2004). Previous histological work has shown that focally elevated iron deposition is associated with various neurological and psychiatric disorders, including multiple sclerosis (MS) (LeVine, 1997), Alzheimer's disease (Bouras et al., 1997; Hallgren and Sourander, 1960; LeVine, 1997), Huntington's disease (Chen et al., 1993; Dexter et al., 1991) and Parkinson's disease (Chen et al., 1993; Dexter et al., 1991). Increased iron accumulation has been detected in chronic hemorrhage, MS lesions, cerebral infarction, anemia, thalassemia, hemochromatosis, and NBIA (neurodegeneration with brain iron accumulation) (Haacke et al., 2005). An in vivo non-invasive and quantitative estimation of non-heme iron deposition (predominantly ferritin) is essential to understand the cause of iron accumulation and its distribution patterns as well as its physiological role in any given disease (Bartzokis et al., 2007; Gerlach et al., 1994; Ke and Qian, 2003).

A variety of methods have been used in the past to quantify iron using magnetic resonance imaging (MRI) (Haacke et al., 2005). The standard workhorses in this area are T2 (House et al., 2007; Jensen et al., 2010; Mitsumori et al., 2012) and T2* (or R2* = 1/T2*) imaging methods that create T2* or R2* maps derived from multi-echo gradient (recalled) echo magnitude images. The latter are particularly useful since gradient echo sequences are very sensitive to the local susceptibility induced magnetic field inhomogeneity due to iron (Bartzokis et al., 1993; Haacke et al., 1989, 2005; Ordidge et al., 1994; Peters et al., 2007; Reichenbach et al., 1997). Further, T2* or R2* maps provide an important contrast mechanism to investigate brain tissue microstructure and to detect abnormal levels of brain iron (Bartzokis et al., 2007; Bouras et al., 1997; Chen et al., 1993; Dexter et al., 1991; Haacke et al., 2005, 2009; Hallgren and Sourander, 1960; LeVine, 1997; Wallis et al., 2008).

In this paper, we focus on susceptibility measurements from phase images. Phase has been used as a means to measure iron content (Haacke et al., 2007). However, phase is dependent on the geometry of the object and so it can be misinterpreted. The solution lies in using a susceptibility map reconstructed from the phase information. In theory, this approach is independent of field strength, echo time, the object's relative orientation to the main field and the object's shape (Cheng et al., 2009b; de Rochefort et al., 2010; Haacke et al., 2010; Kressler et al., 2010; Li et al., 2011; Liu et al., 2009; Marques and Bowtell, 2005; Schweser et al., 2011; Shmueli et al., 2009; Wharton and Bowtell, 2010; Yao et al., 2009). Recent work has suggested that susceptibility changes in the basal ganglia, thalamus and other deep gray matter nuclei have better correlation with iron concentration than phase information (Bilgic et al., 2012; Fukunaga et al., 2010; Langkammer et al., 2012b; Schweser et al., 2011, 2012; Shmueli et al., 2009; Wharton and Bowtell, 2010; Yao et al., 2009) and, therefore, quantitative susceptibility mapping (QSM) may provide a good means to study tissue iron content.

Currently, the neuroscience community relies upon the 50 year old data on iron in cadaveric brains published by Hallgren and Sourander (Hallgren and Sourander, 1958). Total iron in cadaveric brain has been measured using synchrotron X-ray fluorescence (XRF) iron mapping

* Corresponding author at: HUH-MR Research/Radiology, Wayne State University, 3990 John R Street, Detroit, MI 48201, USA. Fax: +1 313 745 9182.
E-mail address: nmrimaging@aol.com (E.M. Haacke).

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Table 1
Methodology and data processing.

	ICPMS	XRF	SWI Background phase removal	QSM
Ferritin samples	√	√	Quadratic fitting	Forward fitting
Cadaveric brain		√	SHARP	Truncated k-space division (Haacke et al., 2010)

(Hopp et al., 2010; Zheng et al., 2012), proton-induced X-ray emission mapping (Butz et al., 2000), inductively coupled plasma mass spectrometry (ICPMS) measurements (Langkammer et al., 2010, 2012a) and atomic absorption spectrometry measurements (House et al., 2007). Among these, the first two techniques can provide a voxel by voxel quantification of iron content which can then be compared with MR iron quantification.

In this paper, our goal is to develop an absolute quantification scale by separating the iron induced susceptibility change from other potential sources by comparing ferritin-gelatin phantoms with quantified XRF iron maps of basal ganglia from cadaver brains and ICPMS iron values.

Materials and methods

Preparation of ferritin phantoms

Horse spleen ferritin (Ref. F4503, Sigma-Aldrich, USA) was used to prepare ferritin-gelatin phantoms. The iron concentration as determined by the supplier using ICPMS was 7.13 ± 0.15 mg/ml. The ferritin solution was first diluted by adding 4 ml of original solution with 16ml warm 7% gelatin resulting in a stock solution with iron concentration of about 1426 ± 30 μg/ml. This stock solution was serially diluted six times in warm gelatin by a factor of 2 each time. The ferritin-doped gelatin solutions as well as pure gelatin were loaded into straws and then embedded in a pure gelatin matrix. Total iron was measured in aliquots of the ferritin-doped gelatin by XRF and ICPMS. See the detailed scheme of the experiment in Table 1.

Rapid scanning X-ray fluorescence (RS-XRF)

All XRF measurements were conducted at the Stanford Synchrotron Radiation Lightsource (SSRL). RS-XRF images of ferritin phantoms and cadaveric brain were acquired at wiggler beam line 10–2 at SSRL. The samples were mounted onto a set of motorized stages oriented at 45° to the incident beam. The incident beam (12 keV) passing through a tantalum aperture produced a 100 μm × 100 μm spot on the sample which was raster-scanned in the beam using a dwell time of 15 ms/point. Fluorescent energy windows were centered for Fe (6.21–6.70 keV) as well as all other biologically interesting elements, scatter and total incoming counts. Elements were quantified in μg iron/g wet tissue by comparison of signal strength with XRF calibration standards (±5% uncertainty) (Micromatter, Vancouver, BC, Canada) according to Hopp and colleagues (Hopp et al., 2010) using Sam's Microanalysis kit (Webb, 2010). An area of the ferritin-doped gelatin block was mapped and average counts were compared with XRF calibration standards.

Inductively coupled plasma mass spectrometry

To confirm the total iron content of the ferritin phantoms, 5 ml samples were taken from the straws after MR imaging and the iron content was determined by ICPMS using an ELAN 9000 system (PerkinElmer, Waltham, MA, USA) (American Environmental Testing Laboratory Inc., California). The samples were diluted to the range acceptable for ICPMS via serial dilutions.

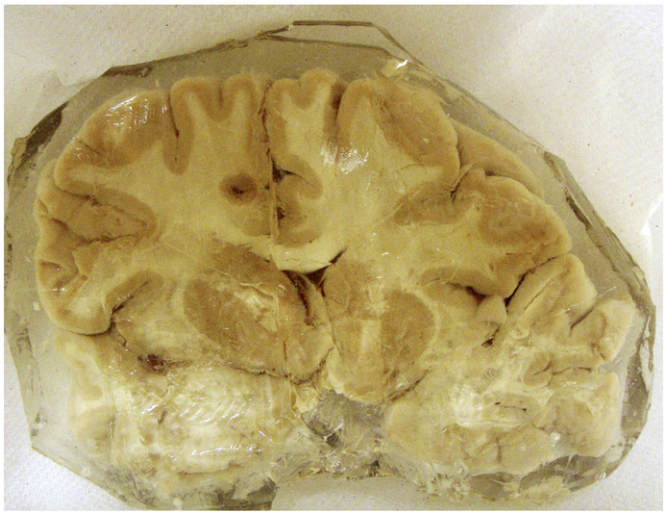

Fig. 1. Photograph of the cadaveric brain sample in gelatin.

Preparation of the cadaveric brain sample

One frozen coronal section (96 mm long × 132 mm wide × 5 mm thick) of human cadaveric multiple sclerosis (MS) brain (MS 3852) (see Fig. 1) was obtained from the Human Brain and Spinal Fluid Resource Center, Los Angeles, CA, under the University of Saskatchewan ethics approval BioREB 06-250. Coronal sections showed extensive irregular demyelination throughout the brainstem. There were also a few small scattered demyelinating periventricular foci (bilateral). The surface of the sample (a 5 mm thick section) showed patchy areas of slight rarefaction without significant axonal loss or change in oligodendrocyte density. There were varying degrees of associated gliosis. The areas of rarefaction were associated with extensive demyelination. To reduce storage artifacts such as leaching of metals, fresh autopsy brain was flash frozen and the slices were shipped on dry ice and stored frozen until they were thawed by immersion in buffered formalin. After 6 h of fixation, the brain slice was drained and sealed in plastic prior to initial synchrotron imaging of the surface of the slice. To resolve regions of interest, the slices were embedded in gelatin for MR imaging. The brain hemispheres were sectioned to expose the region of interest and then the slice was sealed in metal-free thin polypropylene film. RS-XRF images were acquired and quantified at SSRL (see the detailed scheme of the experiment in Table 1).

MR imaging and image processing

Imaging and phase processing of ferritin samples

MR data of ferritin samples were collected on a 3 T Siemens Verio system using a multi-echo susceptibility weighted imaging (SWI) sequence with 11 echoes (TR = 40 ms, FA = 15°). The resolution was 1 mm × 1 mm × 1 mm with a matrix of 256 × 256 × 128. The shortest echo time was 5 ms with a 2.39 ms increment for the other 10 echoes. Magnitude and phase images were reconstructed from the raw data for each individual and combined channel. The geometry of the ferritin samples was segmented from multi-echo spin echo images (TR = 2000 ms, resolution 0.22 mm × 0.22 mm × 3 mm).

In order to reconstruct a susceptibility map, a pristine phase map was required. That is, the phase was unwrapped and all spurious phase information was removed. Phase images (TE = 21.73 ms) were unwrapped using Prelude in FSL (Jenkinson, 2003). To remove