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Tissue magnetic susceptibility mapping as a marker of tau pathology in Alzheimer's disease

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A B S T R A C T

Alzheimer's disease is connected to a number of other neurodegenerative conditions, known collectively as 'tauopathies', by the presence of aggregated tau protein in the brain. Neuroinflammation and oxidative stress in AD are associated with tau pathology and both the breakdown of axonal sheaths in white matter tracts and excess iron accumulation grey matter brain regions. Despite the identification of myelin and iron concentration as major sources of contrast in quantitative susceptibility maps of the brain, the sensitivity of this technique to tau pathology has yet to be explored. In this study, we perform Quantitative Susceptibility Mapping (QSM) and T2* mapping in the rTg4510, a mouse model of tauopathy, both *in vivo* and *ex vivo*. Significant correlations were observed between histological measures of myelin content and both mean regional magnetic susceptibility and T2* values. These results suggest that magnetic susceptibility is sensitive to tissue myelin concentrations across different regions of the brain. Differences in magnetic susceptibility were detected in the corpus callosum, striatum, hippocampus and thalamus of the rTg4510 mice relative to wild type controls. The concentration of neurofibrillary tangles was found to be low to intermediate in these brain regions indicating that QSM may be a useful biomarker for early stage detection of tau pathology in neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD) is defined by the presence of amyloid-β plaque and neurofibrillary tangle (NFT) tau pathology found primarily in grey matter regions of the brain. These insoluble plaques and tangles have both been found to contain iron (Lovell et al., 1998; Good et al., 1992). Iron levels need to be tightly regulated in the brain but homeostasis can become disturbed during neuroinflammation which is thought to increase iron levels in neurons and microglia (Urrutia et al., 2013). Oxidative stress is associated with the dysfunction of oligodendrocytes in AD (Cai and Xiao, 2015) and white matter degradation has been detected by histopathological examination in over 50% of patients (Sjöbeck et al., 2005). Numerous white matter changes in AD have been reported in post mortem studies including decreased myelin density (Sjöbeck et al., 2005), decreased myelin basic protein (Wang et al., 2004), loss of oligodendrocytes (Sjöbeck et al., 2006), activation of microglia (Gouw et al., 2008), as well as denudation of the ventricular ependyma, gliosis and the loss of myelinated axons (Scheltens et al., 1995). *In-vivo* biomarkers sensitive to tissue neuroinflammatory processes and the concentration of iron and myelin in brain tissue, may play a key role in tracking the progressive pathology of AD and provide a means by which to measure the efficacy of therapeutics.

Quantitative Susceptibility Mapping (QSM) (Wang and Liu, 2015; Shmueli et al., 2009; Haacke et al., 2015; Liu et al., 2014; Deistung et al., 2016), uses the phase of the MRI signal to calculate maps of the bulk magnetic susceptibility of tissue. Myelin is diamagnetic and has been shown to be a predominant source of susceptibility contrast between white and grey matter (Liu et al., 2011a; Klohs et al., 2013; Lee et al., 2012). Furthermore, magnetic susceptibility measurements in white matter regions using QSM have been shown to be more specifically related to myelin concentration than measures of diffusion using DTI (Argyridis et al., 2014). In addition to its dependence on myelin, magnetic susceptibility has been shown to correlate with iron concentrations in tissue (Langkammer et al., 2012; Bilgic et al., 2012), and, like findings of reduced T2*in AD (Moon et al., 2012; Zhao et al., 2017), these increases have been attributed to increased iron deposition.

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In a recent clinical study, significant increases in magnetic susceptibility were detected in AD patients relative to controls in the putamen, a sub region of the striatum (Acosta-Cabronero et al., 2013). In the ArcAβ, an amyloid mouse model of AD, smaller susceptibility increases over time were observed relative to controls in a longitudinal study using a linear mixed effects modelling analysis that incorporated estimates from multiple brain regions (Klohs et al., 2013). Thus far, no susceptibility mapping studies have been performed in mice exhibiting tau pathology associated with AD.

The rTg4510 mouse model of tauopathy contains the P301L human tau mutation, and accumulates NFTs in a progressive manner along with motor and behavioural deficits that are similar to those in human AD (Lewis et al., 2000). Previous work in this model has identified abnormalities in the white matter of the corpus callosum using DTI and Electron Microscopy (Wells et al., 2015; Sahara et al., 2014). Additionally, reactive microglia and astrocytes, associated with neuroinflammation and iron accumulation, are known to be present in higher quantities in the rTg4510 than in controls (Maphis et al., 2015; Yoshiyama et al., 2007). We hypothesized that QSM might provide a sensitive *in-vivo* method to non-invasively probe these pathological traits of the rTg4510 mouse model.

In this study, we present *in-vivo* QSM and T2* maps in the rTg4510 mouse, supported by higher resolution measurements from *ex-vivo* datasets. A semi-automatic segmentation of the quantitative parameter maps was employed to calculate magnetic susceptibility and T2*values in selected grey matter and white matter regions. The biological factors contributing to magnetic susceptibility and T2* measurements in the tissue were investigated by comparison with histological stains for myelin, iron, and neuroinflammatory markers to aid interpretation of the MRI findings.

2. Materials and methods

2.1. Animals

Female rTg4510 transgenic mice were licensed from the Mayo Clinic (Jacksonville Florida, USA) and bred for Eli Lilly by Taconic (Germantown, USA) (Ramsden et al., 2005a). Mice were imported to the UK for imaging studies at the Centre for Advanced Biomedical Imaging, University College London. All studies were carried out in accordance with the United Kingdom Animals (Scientific Procedures) act of 1986.

2.2. In-vivo data acquisition

In-vivo imaging was conducted on rTg4510 mice (n = 10) and wild-type (WT) controls (n = 10) aged 7.5 months. Data were acquired with a 9.4 T VNMRS horizontal bore scanner (Agilent Inc.). A 72 mm inner diameter volume coil (Rapid Biomedical) was used for RF transmission and signal was received using a two-channel head array (Rapid Biomedical). Mice were anaesthetised under 2% isoflurane in 100% O_2 and were immobilised by securing the head with a bite bar and ear bars. The anaesthesia was subsequently reduced to 1.5% isoflurane and maintained at this level throughout imaging. Core temperature and respiration were monitored using a rectal probe and pressure pad (SA instruments). Mice were maintained at ~37 °C using heated water tubing and a warm air blower with a feedback system (SA instruments). Shimming was performed using an automatic 3D gradient echo shim function (VNMRJ, Agilent Inc.) in a voxel (1 cm^3) centred in the cortex resulting in linewidths of 47 ± 7 Hz. Data for QSM was collected using a 3D single echo spoiled gradient recalled echo (GRE) sequence with first order flow compensation applied in three dimensions. Subsequently, a multi-echo sequence was run without flow compensation to acquire data for T2* mapping. Parameters for MRI pulse sequences are provided in Table 1. Flow compensation can improve phase estimation *in vivo* by reducing errors caused by mislocalisation of signal and accumulation of spins due to motion at a constant velocity (Haacke et al., 2015; Xu et al., 2014; Deistung et al., 2009). Consequently, the multi-echo data, which were not flow compensated, were not used for QSM.

Table 1
Imaging pulse sequences and parameters. For *in-vivo* acquisition, a flow compensated 3D GRE sequence was used to generate data for QSM and a Multi-Echo 3D GRE sequence for T2* mapping data. A Multi-Echo 3D GRE acquisition was used to collect *ex-vivo* QSM and T2* mapping data. Abbreviations: NE: Number of echoes, NSA: Number of signal averages.

Parameter \ Pulse seq.	*In-vivo* (QSM): Flow compensated 3D GRE	*In-vivo* (T2*): 3D multi-echo GRE	*Ex-vivo* (QSM/T2*): 3D multi-echo GRE
TR (ms)	250	250	200
minTE/ ΔTE/ maxTE (ms)	15	2.31/2.46/29.32	3.05/3.92/46.21
FA (^{0})	32	32	36
NE	1	12	12
NSA	1	1	5
Scan time	1hr,2s	1hr,2s	10hr,45min,2s
Spectral width (Hz)	50000	100000	73529
FOV (mm)	18 × 18 × 18	18 × 18 × 18	18 × 17.2 × 14.4
Matrix	120 × 120 × 120	120 × 120 × 120	225 × 215 × 180
Resolution (μm)	150 × 150 × 150	150 × 150 × 150	80 × 80 × 80

2.3. Preparation of ex-vivo samples

Animals were terminally anaesthetised with Euthanal administered via intraperitoneal injection immediately after *in-vivo* imaging. Fixation was then carried out by perfusion through the left ventricle: first with 15–20 mL of saline (0.9%) and heparin; second with 50 mL of buffered formal saline (10% solution, Sigma-Aldrich), at a flow rate of 3 mL per minute. Brains (in-skull) were then removed and stored at 4 °C in buffered formal saline. After 4 weeks, brains were transferred to phosphate buffered saline (50 ml PBS refreshed weekly, Sigma-Aldrich) for rehydration (Zhang et al., 2012; Shepherd et al., 2009; Benveniste and Blackband, 2002) for a further 3 weeks.

2.4. Ex-vivo data acquisition

Ex-vivo imaging was conducted on rTg4510 mice (n = 8) and wild-type controls (n = 8) using a 3D spoiled GRE acquisition with parameters given in Table 1. Four mice from the *in-vivo* cohort were excluded from *ex-vivo* processing due to non-optimal perfuse fixation. Each brain (in-skull) was secured individually in a 20 ml syringe filled with 10 ml proton MR signal-free, non-viscous Fomblin perfluoropolyether (PFS-1, Solvay Solexis SpA., Bollate, Italy) prior to imaging in a 26 mm diameter birdcage coil (Rapid Biomedical GmbH, Germany) at 9.4T. Shimming was conducted manually using a pulse-acquire sequence giving a linewidth of 47 ± 5 Hz.

Following *ex-vivo* imaging, the brains were then transferred to buffered formal saline before being dispatched for histology.

2.5. Quantitative Susceptibility Mapping and T2* mapping

The reconstruction of the *in-vivo* phase data required a pre-processing step to combine the signal from the two receive coils using a global offset correction technique to remove phase shifts between channels (Hammond et al., 2008). *In-vivo* phase data were unwrapped using Laplacian unwrapping (*unwrapLaplacian.m*, MEDI toolbox, Cornell MRI Research Lab, http://weill.cornell.edu/mri/pages/qsm.html (Liu et al., 2011b)) before background field removal using a Variable Sophisticated Harmonic Artifact Reduction for Phase data (VSHARP) (Schweser et al., 2011; Wu et al., 2012) (minimum kernel width = 3 voxels). Thresholded k-space division (TKD) (Shmueli et al., 2009) (threshold t = 5) was then performed on the field map to calculate magnetic susceptibility maps.

Ex-vivo QSMs were generated by path-based unwrapping