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Quantitative evaluation of MRI and histological characteristics of the 5xFAD Alzheimer mouse brain

N.G. Spencer [a,*], L.R. Bridges [b], K. Elderfield [b], K. Amir [a], B. Austen [a], F.A. Howe [c]

[a] Basic Medical Sciences, St. George's University, London, SW17 0RE, UK
[b] Neuropathology, St George's Hospital, London, SW17 0RE, UK
[c] Clinical Sciences, St. George's University, London, SW17 0RE, UK

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ABSTRACT

Assessment of β-amyloid (Aβ) plaque load in Alzheimer's disease by MRI would provide an important biomarker to monitor disease progression or treatment response. Alterations in tissue structure caused by the presence of Aβ may cause localised changes that can be detected by quantitative T_1 and T_2 relaxation time measurements averaged over larger areas of tissue than that of individual plaques. We constructed depth profiles of the T_1 and T_2 relaxation times of the cerebral cortex with subjacent white matter and hippocampus in six 5xFAD transgenic and six control mice at 11 months of age. We registered these profiles with corresponding profiles of three immunohistochemical markers: β-amyloid; neuron-specific nuclear protein (NeuN), a marker of neuronal cell load; and myelin basic protein (MBP), a marker of myelin load.

We found lower T_1 in the 5xFAD transgenic mice compared to wild type control mice at all depths, with maximum sensitivity for detection at specific layers. T_1 negatively correlated with Aβ staining intensity in the 5xFAD mice which had no changes in NeuN and MBP staining compared to wild type mice. We postulate that these relaxation time changes are due to the presence of β-amyloid in the transgenic mice. It may be clinically feasible to develop a similar layered analysis protocol as a biomarker for Alzheimer's disease in humans.

Introduction

With the advent of new treatment and prevention strategies for Alzheimer's disease (AD), it is increasingly important to have a clinical biomarker of the disease suitable for selecting at-risk individuals for early intervention, as well as providing a means of pre-morbid diagnosis and monitoring of disease progression.

The progression of AD is known to be associated with the accumulation and aggregation of a small (40–42-residue) peptide, termed β-amyloid (Aβ). According to the amyloid hypothesis, Aβ aggregation is thought to be a primary cause of the neuronal dysfunction and memory loss seen in the disease (Selkoe, 2008). It is possible to detect Aβ in the living brain using the radiopharmaceutical compounds such as [11C]-Pittsburgh compound B (Klunk et al., 2005) and [18F]-AV-45 (Choi et al., 2009) and positron emission tomography. However, this imaging modality is only available in a few centres. On the other hand, magnetic resonance imaging (MRI) is widely available but there are not yet full clinically validated MRI imaging protocols for directly assessing amyloid load and its effect on brain in Alzheimer's disease. MR imaging has demonstrated visualisation of individual plaques in transgenic Alzheimer mice using high-field MRI systems with a number of different methodologies applied to *in vivo* and *ex vivo* samples. The sizes of amyloid plaques are at the limit of the MRI resolution available for rodent studies and detection requires careful optimisation of imaging protocols and analysis. Using high magnetic field strengths, high spatial resolution imaging and relatively long acquisition times, individual amyloid plaques can be resolved on T_2 and T_2^* weighted MR imaging without the use of exogenous contrast agents (Benveniste et al., 1999; Jack et al., 2004, 2005; Lee et al., 2004; Poduslo et al., 2002; Vanhoutte et al., 2005; Zhang et al., 2004) and the compartmentalisation of iron within plaques leading to increased paramagnetic susceptibility has been postulated as the major contributing factor to date (Benveniste et al., 1999; Braakman et al., 2006; Falangola et al., 2005a, 2005b; Jack et al., 2004, 2005; Lee et al., 2004; Vanhoutte et al., 2005). An alternative approach uses the administration of exogenous contrast agents to enable the detection of amyloid plaques in the brain by increasing the contrast between the parenchyma and the amyloid deposits (Higuchi et al., 2005; Petiet et al., 2012; Poduslo et al., 2002; Sigurdsson et al., 2008; Wadghiri et al., 2003). However there are major technical and image sensitivity challenges to performing such high spatial resolution MRI for routine clinical MRI studies in man.

Abbreviations: 5xFAD, Five familial Alzheimer's disease mutations; Aβ, β-Amyloid; AD, Alzheimer's disease; FOV, Field of view; H, Hippocampus; LC, Lower cortex; MBP, Myelin basic protein; MRI, Magnetic resonance imaging; NeuN, Neuron-specific nuclear protein; ROI, Region of interest; SI, Signal intensity; TE, Echo time; TR, Repetition time; UC, Upper cortex; WM, White matter.

* Corresponding author.
E-mail address: nspencer@sgul.ac.uk (N.G. Spencer).

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In a more clinically feasible setting where lower magnetic field strengths are used and there is a practical limit to patient imaging time, currently obtainable spatial resolutions cannot resolve individual amyloid plaques. Alternatively, quantitative assessment of mean tissue relaxation times within regions of interest (ROI) may provide indirect means to assess changes in the tissue–water environment associated with amyloid burden. Studies using the low resolution MRI approach in the Tg2576 and APP/PS1 transgenic mouse strains have found T_2 reductions in some brain regions which also correlate with amyloid deposition: in the subiculum (El Tannir El Tayara et al., 2006) hippocampus and cortical regions (Braakman et al., 2006; Helpern et al., 2004; Teipel et al., 2011). The exact causes for the signal changes remains unclear as no changes in T_2 were also seen in other brain regions containing amyloid deposits. Given that brain regions containing intracellular amyloid are associated with gliosis (a marker of neuroinflammation and a hallmark of AD) and neuronal loss (Schmitz et al., 2004) it is likely that changes in T_1 and T_2 measurements may be due to a combination of factors. However, the regional variation of these tissue changes may be too subtle to detect within large ROIs which also average over less affected areas.

The cerebral cortex has a multi-layered architecture of cortical regions each defined by a particular density of cell, myelin and receptors (Zilles, 2004). The use of the profile analysis aids the visualisation of the specific changes in T_1 and T_2 associated with regional changes in tissue architecture of individual layers and may help determine the particular contributions to the T_1 and T_2 changes. MRI of human and mouse brain has demonstrated that the layered structure of the cortex is characterised by variation of T_1 and T_2 relaxation times with depth and these MR relaxation rate changes correlate to the varying levels of myelin and neuronal cell density (Boretius et al., 2009; Eickhoff et al., 2005; Walters et al., 2003). Histological studies also suggest that amyloid deposition is layered (Clinton et al., 1993). Hence, we have performed a detailed correlation of relaxation time profiles with histology as a function of cortical depth in AD transgenic mice to establish whether MR relaxation rate profiles follow layer patterns of Aβ. This approach has a particular advantage over the standard ROI method because it simplifies the visualisation of cortical regions most sensitive for T_1 and T_2 changes.

Using 5xFAD transgenic mice and wild type controls, we co-registered T_1 and T_2 profiles of the cerebral cortex with subjacent white matter and hippocampus with histological profiles of Aβ, neuron-specific nuclear protein (NeuN) and myelin basic protein (MBP) staining in order to compare the MRI with underlying histology. Since it is known that myelin and cell density can affect T_1 and T_2, we used these histological markers as positive controls; to validate our method of mapping T_1 and T_2 changes with the anatomical variations in myelin and cell density and; to confirm that relaxation rate differences between 5xFAD and control mice are associated with Aβ deposition patterns.

We found a spatial pattern of significant reductions in T_1 which broadly corresponded to the pattern of Aβ deposition in the transgenic mice. We postulate that the reductions in T_1 are due to the presence of Aβ or its effects on the structure of brain tissue and as such it may be worth exploring the feasibility of using quantitative profile measures of T_1 and through cortical layers as a biomarker of Alzheimer's disease in humans.

Methods

Transgenic mice

The 5xFAD transgenic mouse developed by Oakley et al. (2006) using three mutations in amyloid precursor protein and two in presenilin-1, achieve massive amyloid burden with gliosis and neuronal loss by 9 months of age with amyloid deposition starting at 2 months. 5xFAD transgenic mice were purchased from Jackson Laboratory [stock number 006554; strain name B6SJL-Tg(APPSwFlLon, PSEN1*M146L*L286V)6799Vas/Mmjax]. A colony of transgenic and wild type controls were produced by crossing hemizygous 5xFAD mice with non-carrying (wild type) mice, B6SJF1/J (stock number 100012). Mice were genotyped using polymerase chain reaction and gel electrophoresis. Six 5xFAD transgenic mice of 11 months of age were used. Six age-matched non-transgenic littermates served as controls. A mixture of males and females were used in both groups.

In vivo MRI

In vivo MR images were acquired on a 4.7 T Varian horizontal bore MRI system with a mouse brain coil setup comprising a volume transmit coil and surface coil receive (RAPID Biomedical GmBH, Würzburg-Rimpar, Germany). Mice were initially anesthetised with isoflurane, consisting of an O_2 mixture (2 l/min), administered in a sealed tank fitted to an isoflurane scavenger system. Subsequently, a 1:2:1 mixture of Hypnorm (0.315 μg/ml fentanyl citrate and 10 mg/ml fluanisone); sterile water and; Hypnovel (5 mg/ml Midazolam) was administered intraperitoneally at 10 mg/kg bodyweight. Body temperature was maintained by a warming bed integrated into the imaging coil apparatus.

A spin echo multi-slice sequence was used for T_2 and T_1 measurements. For T_2 measurements: repetition time (TR) = 2000 ms; echo time (TE) = 15, 25, 40 and 60 ms; field of view (FOV) = 30 × 30 mm; matrix = 128 × 128; nine contiguous slices of 1 mm thickness; total acquisition time = 20 min. For T_1 measurements, TR = 250, 500, 1000, 2000, 4000 ms; TE = 14 ms; FOV = 30 × 30 mm; matrix = 128 × 128; nine contiguous slices of 1 mm thickness; total acquisition time = 17 min. The most anterior of the nine slices was positioned just behind the olfactory bulb and the same slice positioning was used for both T_2 and T_1 measurements. The selection of TE and TR times were chosen to adequately quantify the range of T_2 and T_1 relaxation times in the mouse brain.

ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012) was used to generate T_2 and T_1 quantitative maps. For each dataset, a stack of images was created for each slice with increasing TE (T_2 map) or TR (T_1 map) and Stackreg (Thevenaz et al., 1998) was applied to co-register image slices prior to generating quantitative relaxation time maps. The MRI Analysis Calculator ImageJ plugin by Karl Schmidt was used to transform the image stacks into T_1 and T_2 quantitative maps by fitting signal intensities (SI) to the equations, $SI = M_0\,[1 - \exp(-TR/T_1)]$ and $SI = M_0\,[\exp(-TE/T_2)]$, respectively, on a pixel-by-pixel basis.

Histology

Immediately after MR imaging, mice were culled by decapitation and whole skulls collected and placed into formalin for 7 days. Brains were removed from the skull and sliced into 1 mm thick coronal sections using a matrix to match the imaging slices taken during MRI.

Serial sections taken from the 1 mm mouse brain slices were stained for Aβ (Novocastra monoclonal β-amyloid clone 6F/3D (Leica Microsystems, Newcastle-upon-Tyne, UK)); vertebrate neuron-specific nuclear protein (NeuN, Millipore monoclonal NeuN clone A6, Fisher Scientific UK Ltd, Leics, UK) and; myelin basic protein (MBP, Novocastra monoclonal clone 7H11, Leica Microsystems, Newcastle-upon-Tyne, UK). The Aβ antibody epitope is aa8–17 of mature amyloid A4/Aβ, which is the same as aa660–669 of human amyloid A4/Aβ protein precursor. Staining is transmembrane for APP and extracellular for Aβ. After treatment of sections with epitope retrieval solution pH 9 at 100 °C for 30 min and 90% (v/v) formic acid for 5 min, Aβ antibody was applied for 15 min at a dilution of 1:50. For NeuN and MBP staining, sections were treated first with antigen retrieval solution at pH 6 for 30 min at 100 °C. NeuN reacts with most neuronal cell types throughout the nervous system of mice. NeuN antibody was applied for 15 min at a dilution of 1:100. The MBP antibody is reactive with

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