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Diffusion-weighted magnetic resonance imaging detection of basal forebrain cholinergic degeneration in a mouse model

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ABSTRACT

Loss of basal forebrain cholinergic neurons is an early and key feature of Alzheimer's disease, and magnetic resonance imaging (MRI) volumetric measurement of the basal forebrain has recently gained attention as a potential diagnostic tool for this condition. The aim of this study was to determine whether loss of basal forebrain cholinergic neurons underpins changes which can be detected through diffusion MRI using diffusion tensor imaging (DTI) and probabilistic tractography in a mouse model. To cause selective basal forebrain cholinergic degeneration, the toxin saporin conjugated to a p75 neurotrophin receptor antibody (mu-p75-SAP) was used. This resulted in ~25% loss of the basal forebrain cholinergic neurons and significant loss of terminal cholinergic projections in the hippocampus, as determined by histology. To test whether lesion of cholinergic neurons caused basal forebrain, hippocampal, or whole brain atrophy, we performed manual segmentation analysis, which revealed no significant atrophy in lesioned animals compared to controls (Rb-IgG-SAP). However, analysis by DTI of the basal forebrain area revealed a significant increase in fractional anisotropy (FA; +7.7%), mean diffusivity (MD; +6.1%), axial diffusivity (AD; +8.5%) and radial diffusivity (RD; +4.0%) in lesioned mice compared to control animals. These parameters strongly inversely correlated with the number of choline acetyl transferase-positive neurons, with FA showing the greatest association ($r^2 = 0.72$), followed by MD ($r^2 = 0.64$), AD ($r^2 = 0.64$) and RD ($r^2 = 0.61$). Moreover, probabilistic tractography analysis of the septo-hippocampal tracts originating from the basal forebrain revealed an increase in streamline MD (+5.1%) and RD (+4.3%) in lesioned mice. This study illustrates that moderate loss of basal forebrain cholinergic neurons (representing only a minor proportion of all septo-hippocampal axons) can be detected by measuring either DTI parameters of the basal forebrain nuclei or tractography parameters of the basal forebrain tracts. These findings provide increased support for using DTI and probabilistic tractography as non-invasive tools for diagnosing and/or monitoring the progression of conditions affecting the integrity of the basal forebrain cholinergic system in humans, including Alzheimer's disease.

Introduction

The cholinergic basal forebrain is a key modulator of neurotransmission, function and plasticity of the hippocampus and amygdala, as well as the entire cortical mantle (Mesulam et al., 1983), and is strongly implicated in regulating attention, learning and memory (Mufson, 2003). Pathologically, loss of basal forebrain cholinergic neurons is a pathological hallmark of Alzheimer's disease at autopsy (Mufson et al., 2002; Whitehouse et al., 1982). Furthermore, numerous studies have shown that degeneration of the basal forebrain is linked, albeit indirectly, to early signs of cognitive decline in Alzheimer's disease patients, including deficits in spatial navigation and memory (George et al., 2009; Grothe et al., 2010, 2011; Muth et al., 2010). In these studies, magnetic resonance imaging (MRI) methods were used to detect structural changes associated with atrophy of the basal forebrain (Grothe et al., 2011), which has been correlated with cognitive decline in cohorts of patients diagnosed with Alzheimer's disease or its prodromal stage, mild cognitive impairment (MCI) (Grothe et al., 2011). In some cases, basal forebrain atrophy was observed as early as 4.5 years before the onset of overt clinical symptoms (Hall et al., 2008).

By the time atrophy of a structure can be detected as a volumetric reduction with structural MRI, significant cell and tissue loss has

Abbreviations: AD, axial diffusivity; ChAT, Choline acetyl transferase; DePeX, Distrene-80/Plasticizer/Xylene; dMRI, diffusion MRI; DTI, diffusion tensor imaging; FA, fractional anisotropy; i.p., intraperitoneal; MD, mean diffusivity; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; NHS, normal horse serum; $p75^{NTR}$, p75 neurotrophin receptor; PB, phosphate buffer; PBS, phosphate buffered saline; PBT-X, PB containing 0.2% Triton X-100; RD, radial diffusivity; ROI, region of interest; SAP, saporin; s.c., subcutaneously; TBSS, Tract-based spatial statistics.

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already occurred. The drugs currently prescribed for the treatment of Alzheimer's disease, inhibitors of acetyl cholinesterase, enhance basal forebrain cholinergic function by inhibiting the degradation of acetylcholine produced by the surviving neurons. However, as the diagnosis of MCI or Alzheimer's disease currently occurs at a stage at which the cholinergic system has been irreversibly damaged, it is not surprising that these drugs have mild to little effect in prolonging cognitive function (Mancuso et al., 2011). Non-invasive early detection of basal forebrain neurodegeneration prior to overt tissue loss therefore has the potential to improve the window of therapeutic efficacy.

Diffusion MRI (dMRI), a technique used to analyze structural integrity and brain connectivity, has been successfully employed to detect differences between Alzheimer's patients and healthy control subjects (Kiuchi et al., 2009; Pievani et al., 2010; Teipel et al., 2010). In particular, tractography analysis of axonal regions known to degenerate in Alzheimer's disease, such as the fornix and the cingulum, have shown significant changes in diffusion parameters in humans with the condition (Oishi et al., 2011; Pievani et al., 2010). However the pathophysiological reason for these changes is not known. The aim of the current study was to determine, using a mouse model, whether mild to moderate loss of basal forebrain cholinergic nuclei and axons could underpin changes to dMRI measures and tractography prior to the point at which changes correlate with overt atrophy. The most commonly used transgenic mouse models of Alzheimer's disease show only certain pathological features of the human condition (Kokjohn and Roher, 2009), failing to reproduce the loss of basal forebrain cholinergic neurons characteristic of Alzheimer's disease. Consequently, both diffusion and structural imaging studies of transgenic Alzheimer's disease mice have focused on brain regions other than the basal forebrain (Harms et al., 2006; Lau et al., 2008; Maheswaran et al., 2009; Mueggler et al., 2004; Sun et al., 2005; Thiessen et al., 2010). However, one study of a transgenic mouse model using structural MRI has reported a significant interaction between age and genotype in a variety of brain regions including the medial septum, a sub-nucleus of the basal forebrain (Badea et al., 2010). In the current study, we investigated a robust model of selective basal forebrain cholinergic neuron loss, which results in a similar spatial navigation and memory deficit in mice (Berger-Sweeney et al., 2001; Moreau et al., 2008; Perry et al., 2001) to that seen in Alzheimer's disease patients (Hort et al., 2007). In this model, the toxin saporin, conjugated to an antibody to a receptor almost exclusively expressed by basal forebrain cholinergic neurons (p75 neurotrophin receptor; $p75^{NTR}$) is injected into the ventricles (Berger-Sweeney et al., 2001). Therefore, unlike in transgenic mouse models of Alzheimer's disease, the changes in dMRI induced by cholinergic degeneration can be studied with little influence from other pathological features.

Materials and methods

Animals

Experimentally naive male C57Bl/6 J mice were housed in groups of four and maintained on a 12 h light/dark cycle, with food and water provided *ad libitum*. All procedures were approved by the University of Queensland Animal Ethics Committee.

Surgery

Twelve 8- to 10-week-old male C57Bl/6J mice (22–24 g) were anesthetized by intraperitoneal (i.p.) injection with a mixture of ketamine (130 mg/kg) and the muscle relaxant xylazine (6 mg/kg). Each mouse was then placed in a stereotaxic frame (with the incisor bar maintained at −3.3 mm below horizontal to achieve a flat skull position). Bilateral infusions of mu-p75-saporin (Advanced Targeting Systems, San Diego, CA; 5 mice) or control rabbit-IgG-saporin (Advanced Targeting Systems; 7 mice) dissolved in phosphate buffered saline (PBS; 0.2 μg/ventricle) were performed using a 30 G needle attached to a 5 μl Hamilton syringe. The needle was lowered into the lateral ventricle using the following co-ordinates: A–P, −0.3 mm; M-L, ± 1.0 mm; D-V, −2.0 mm from Bregma (Franklin and Paxinos, 2007). Infusions were conducted over 5 min, after which the needle was left in place for 10 min to allow for diffusion. Immediately after surgery, mice were injected subcutaneously (s.c.) with the analgesic torbogesic (1.3 mg/kg), and the antibiotic Baytril® (0.43 mg/kg).

Fourteen days post-surgery mice were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 20 ml of 0.9% saline, containing 1% sodium nitrite and heparin (5000 I.U./ml), followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were removed from the skulls and post-fixed overnight in the same fixative, followed by repeated washing in PBS (pH 7.4) for 4 days prior to MRI acquisition.

After close examination of whole brain MR scans and histological sections in each animal, two mice (one control and one lesioned animal) were excluded from the study prior to analysis due to injuries to the fimbria/fornix caused by the injections.

MRI acquisition

Mouse brains were immersed in Fomblin oil (Y06/6 grade, Solvay, NY) and diffusion weighted magnetic resonance images were acquired according to the protocol of Moldrich et al. (2010). Briefly, a small animal MRI system (16.4 T vertical bore; Bruker Biospin, Rheinstetten, Germany; ParaVision v5.0) with a 15 mm linear SAW coil (M2MImaging, Brisbane, Australia) was used. A 3D diffusion-weighted spin-echo sequence was acquired with TR/TE = 400 ms/22.3 ms, signal average of 1, and 1.4 phase encoding accelerations using partial Fourier acquisition. High angular resolution diffusion images were acquired with two minimally diffusion-weighted ($b = 0$ s/mm^2), and 30 high diffusion-weighted images ($\delta/\Delta = 2.5/14$ ms, $b = 5000$ s/mm^2) with the encoding gradient vectors uniformly distributed using the electrostatic approach (Jones et al., 1999). The image data resolution was 100 μm isotropic (uninterpolated) and total acquisition time was 16 h.

Segmentation and DTI analysis

DTI reconstruction of the diffusion data was performed using MRtrix (v0.2.9, www.nitrc.org/projects/mrtrix). The basal forebrain was drawn manually on 12 consecutive coronal slices using color-coded fractional anisotropy (FA) maps and using the mouse brain atlas of Franklin and Paxinos (2007) as a guide. Volumes of the basal forebrain were created, starting at approximately 1.34 mm anterior to Bregma and finishing at approximately 0.26 mm anterior to Bregma, thereby assuring that the mask predominately encompassed the medial septum and the vertical and horizontal diagonal bands of Broca. The anterior-posterior distance covered was 1200 μm (12 slices of 100 μm). However, given the lack of specific anatomical boundaries observable by MRI contrast, the masks were also likely to contain minor parts of the lateral diagonal band of Broca (also called the magnocellular preoptic nucleus), as well as the basal part of the substantia innominata in the posterior section of the mask (ventral part of the mask in Fig. 2E).

The hippocampal volume, starting at approximately −0.88 mm posterior to Bregma and finishing at approximately −4.04 mm posterior to Bregma, and the whole brain volume, were manually segmented on gray-scale FA maps. The basal forebrain volume and hippocampal volume were normalized to the whole brain volume. The whole brain, hippocampal and basal forebrain volumes were measured by a researcher blind to group status. The basal forebrain mask was used to extract FA, mean diffusivity, axial diffusivity and radial diffusivity values.