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MRI and MRS alterations in the preclinical phase of murine prion disease: Association with neuropathological and behavioural changes

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Prion diseases are fatal chronic neurodegenerative diseases. Previous qualitative magnetic resonance imaging (MRI) and spectroscopy (MRS) studies report conflicting results in the symptomatic stages of the disease, but little work has been carried out during the earlier stages of the disease. Here we have used the murine ME7 model of prion disease to quantitatively investigate MRI and MRS changes during the period prior to the onset of overt clinical signs (20+ weeks) and have correlated these with pathological and behavioural abnormalities. Using in vivo MRI, at the later stages of the preclinical period (18 weeks) the diffusion of tissue water was significantly reduced, coinciding with significant microglial activation and behavioural hyperactivity. Using in vivo MRS, we found early (12 weeks) decreases in the ratio of *N*-acetyl aspartate to both choline (NAA/Cho) and creatine (NAA/Cr) in the thalamus and hippocampus, which were associated with early behavioural deficits. Ex vivo MRS of brain extracts confirmed and extended these findings, showing early (8–12 weeks) decreases in both the neuronal metabolites NAA and glutamate, and the metabolic metabolites lactate and glucose. Increases in the glial metabolite *myo*-inositol were observed at later stages when microglial and astrocyte activation is substantial. These changes in MRI and MRS signals, which precede overt clinical signs of disease, could provide insights into the pathogenesis of this disease and may enable early detection of pathology.

Keywords: Magnetic resonance; Diffusion; *N*-acetyl aspartate; Prion; Chronic inflammation; Brain; Neurodegeneration; Thalamus

Introduction

The prion diseases are a group of transmissible spongiform encephalopathies (TSE), which are fatal neurodegenerative disorders that occur in man and animals. In humans, the initial non-specific signs of prion disease are dominated by psychiatric symptoms, which include withdrawal, anxiety, insomnia, depression and a loss of interest in previously rewarding activities (Roos et al., 1973; Spencer et al., 2002). These clinical signs of prion diseases are not apparent until the end stages of the disease and usually herald a rapid deterioration followed by death. The neuropathogenesis of prion disease is strongly associated with the conversion of normal PrP^{c} to the protease resistant PrP^{Sc} (Prusiner, 1982). Late stages of the neuropathology include PrP^{Sc} deposition, vacuolation, neuronal cell death, astrocytosis and microglia activation (Betmouni et al., 1996; Williams et al., 1997). Despite these effects, diagnosis of TSEs, and in particular human new variant Creutzfeldt–Jakob Disease (vCJD), are still very difficult and can only be confirmed neuropathologically at autopsy. For all types of prion disease, a high-throughput, non-invasive test is needed that can diagnose infected individuals at the preclinical stage. In the absence of a current ante-mortem biochemical test for prion disease it is possible that imaging techniques could play a role in diagnosis.

A number of TSE models have been developed in mice. These models vary in the length of the incubation period from the time of infection and in the profile of vacuolation within the brain (Bruce, 2003). In the course of the pathogenesis, some overt clinical signs develop which include impaired grooming, abnormal posture, ataxia and incontinence (McFarland and Hotchin, 1980; Hunter et al., 1986; Cunningham et al., 2005). In C57BL/6J mice intracerebrally injected with ME7 scrapie agent, the incubation period is about 165 days but the first neuropathological

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signs appear as early as 8 weeks post-inoculation (Betmouni et al., 1996; Williams et al., 1997). Formal behavioural testing has revealed signs of neurological dysfunction in this model from 12 weeks after inoculation via the intracerebral route (Betmouni et al., 1999; Deacon et al., 2001), long before the overt clinical signs of the disease become apparent.

To date, only a small number of studies have investigated animal models of prion disease using magnetic resonance imaging (MRI) and spectroscopy (MRS) and these have reported conflicting results. All of these studies have been qualitative and have been carried out during the clinical stage of the disease. In the hamster model, it has been shown that focal blood–brain barrier (BBB) disruption occurs, as detected with gadolinium enhanced imaging (Chung et al., 1995), and increases in signal intensity on T_2-weighted images have been found at the sites of greatest PrP^{Sc} protein accumulation: the septum, hippocampus, thalamus and the cortex (Sadowski et al., 2003). These findings in animal models agree with those of clinical studies of prion disease, in which increases in signal intensity have been observed on T_2-weighted images. However, there have been no investigations in animal models of changes in tissue water diffusion, despite the fact that in human prion disease diffusion-weighted imaging has identified more, and earlier, abnormalities than any other imaging modality (Collie et al., 2001).

In clinical MRS studies of the human Creutzfeldt–Jakob disease (CJD), a decrease in the neuronal metabolite *N*-acetyl aspartate (NAA) is the primary finding at the time of clinical signs (Bruhn et al., 1991; Graham et al., 1993; Pandya et al., 2003; Lim et al., 2004; Oppenheim et al., 2004). Reduced levels of NAA have also been found in the brains of terminal scrapie mice (Bell et al., 1991; Chung et al., 2003), and in hamsters infected with Creutzfeldt–Jakob disease (Behar et al., 1998). In the latter study, alterations in other metabolites such as *myo*-inositol were also found at the clinical stage of the disease.

In the current study, we have used a range of *quantitative* MRI techniques (T_1, T_2 and diffusion mapping) and MRS to investigate changes that occur prior to the onset of late overt clinical symptoms in the ME7 murine model of prion disease. The observed changes are correlated with well described histopathological features that underpin early behavioural changes. The primary aim of this study was to identify early markers of prion disease that can be detected non-invasively by MR.

Methods and materials

Animals

C57BL/6J mice (Harlan, UK) were 8 weeks old on arrival and were housed in a temperature controlled room (21 °C, ±2 °C) on a 12:12 h light:dark schedule. Food (standard laboratory chow, RM1, SDS, UK) and water were readily available, except during the glucose test, when a 5% glucose solution was substituted for water. All procedures described were approved by the United Kingdom Home Office.

Surgery

Mice that were used for the MRI experiments were anaesthetised with 2% isoflurane in 70% N_2O:30% O_2 and mounted on a stereotaxic frame. Animals were injected unilaterally into the dorsal hippocampus with either 10% w/v ME7 brain homogenate, derived from the brain of a C57BL/6J mouse with terminal scrapie disease (referred to as ME7 animals), or 10% normal brain homogenate derived from a normal C57BL/6J mouse (referred to as NBH animals) in a total volume of 1 μl, via a glass microcapillary inserted stereotaxically at 2 mm posterior to Bregma, 1.5 mm lateral (left), and a depth of −1.5 mm. The microcapillary was left in place for 3 min to prevent any reflux of the injected homogenates.

Mice used for behavioural testing were anaesthetised with Avertin (2, 2, 2–tribromoethanol based solution) (0.1 ml per 5 g body weight) and mounted on a stereotaxic frame. Animals were injected bilaterally with either 10% w/v ME7 brain homogenate ($n=44$) or 10% normal brain homogenate ($n=45$) in a total volume of 1 μl at the same co-ordinates as for the MRI study: −2 mm Bregma, ±1.5 mm lateral, −1.5 mm deep. Following surgery, the mice were then placed in a heated recovery chamber (30 °C) and finally re-housed in groups of 5.

Unilateral injections, as previously described (Betmouni et al., 1999), were performed for the MRI experiments to assess the progression of disease pathology along anatomical pathways, including across the cerebral hemispheres, and to control for possible changes induced by the injection although these are known to be minimal (Betmouni and Perry, 1999). It was later found that there was no difference between the hemispheres at the time points studied by conventional MRI techniques.

In vivo magnetic resonance imaging and spectroscopy

MRI data were acquired using a 9.4 T vertical bore magnet with a Varian Inova spectrometer (Varian, Palo Alto, CA). MRI was performed on ME7 animals at 12 ($n=13$), 14 ($n=11$) and 18 weeks ($n=5$) after intracerebral injection, and on NBH animals at the same time points 12 ($n=11$), 14 ($n=10$) and 18 weeks ($n=8$). Animals were positioned in an Alderman-Grant resonator using a bite-bar to prevent movement of the head during image acquisition. A single 1 mm coronal slice centred at the level of the injection site and a second slice 1 mm caudal to the injection site were selected for the full imaging protocol. Initial T_1-weighted (TR = 0.5 s, TE = 20 ms) and T_2-weighted (TR = 3.0 s, TE = 80 ms) images were acquired in the coronal plane (1 mm slice; FOV = 3.5 × 3.5, 128 × 128 matrix). Quantitative T_1 maps (Inversion Recovery, TIR = 0.025, 0.25, 0.5, 0.75, 2, 5 s, TR = 5 s) and T_2 maps (TE = 0.02, 0.04, 0.06 s, TR = 3 s) were obtained. Subsequently, diffusion-weighted images were acquired using a navigated pulsed-gradient spin-echo sequence (TR 2.0 s; TE 0.41 s), with diffusion weighting b values of 125, 750 and 1500 s mm^{-2}, a diffusion time (Δ) of 41 ms and a diffusion gradient duration (δ) of 35 ms. Diffusion gradients were applied separately along three orthogonal axes, and navigator echoes were used for motion correction (Ordidge et al., 1994).

MRS data were acquired from ME7 animals at 12 ($n=6$), 14 ($n=5$) and 18 weeks ($n=5$) after intracerebral injection and on NBH animals at the same time points 12 ($n=7$), 14 ($n=4$) and 18 weeks ($n=6$). Proton MR spectra were obtained from two separate voxels (each approximately 7 × 1.5 × 4 mm^3) placed across the thalamus and hippocampus, respectively. Data were acquired using a PRESS sequence (TE = 39 ms, TR = 3 s, 256 averages, total acquisition time = 15 min) with CHESS water suppression. During MRI and MRS data collection, anaesthesia was maintained with 0.5–1.2% isoflurane in 70% N_2O:30% O_2, ECG was monitored and body temperature was maintained at ~37 °C.