Reductions in NAA Precede Reductions in Water Diffusion in Prion Disease

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Introduction

Transmissible spongiform encephalopathies (TSE's) or prion diseases represent a rare group of infectious, sporadic, and genetic diseases affecting both humans, in the form of Creutzfeld-Jakob disease (CJD) and animals, in the form of scrapie. Prion diseases are chronic neurodegenerative diseases and are characterized by pathological changes in the CNS: degeneration, loss and vacuolation of neurons and the neuropil. Astrocytosis and hypertrophy of microglia occurs in association with accumulation of a protease resistant form of a normal cell membrane protein PrP (prion protein) in plaques. Previous MRI studies in both human and experimental studies have shown contrasting results in the symptomatic stages of the disease (1,2). However, little work has focussed on time points prior to the onset of clinical signs. In this study we have used a well characterised mouse model of prion disease (3), in which pathology characteristically occurs by 12 weeks and the disease is terminal by 24 weeks. We have performed *in vivo* spectroscopy, in association with quantitative Apparent Diffusion Coefficient (ADC) measurements early in the progression of disease pathogenesis with the aim of identifying early markers of disease activity, and to correlate changes with ongoing histopathology.

Methods

Male C57Bl/6J mice (6 weeks old) were anaesthetised with 2% isoflurane in 70%N_20:30%O_2. 1µl ME7 brain homogenate was injected stereotaxically into the dorsal hippocampus of the left hemisphere. Control animals received an intracerebral injection of normal brain homogenate. Animals were imaged 12 (Prion n=7, Control n=6), 14 (Prion n=11, Control n=10), and 18 weeks (Prion n=5, Control n=5) after intracerebral injection of brain homogenate. MR measurements were performed on a vertical bore 9.4T magnet with a Varian Inova spectrometer. Animals were placed in an Alderman-Grant resonator, and anaesthesia was maintained with 0.5–1.5% isoflurane in 70%N_20:30%O_2. ECG and temperature were monitored throughout the MRI examination. Initial T_1-weighted (TR=0.5sec, TE=20ms) and T_2-weighted (TR=3.0sec, TE=80ms) images were acquired in the coronal plane (1mm slice; FOV=3.5x3.5, 128x128 matrix). Proton MR spectra were obtained from a single voxel (approx $7 \times 1.5 \times 4$ mm^3) placed across the thalamus. Data were acquired using a PRESS acquisition sequence with a TE of 39 msec, a TR of 3 sec and 256 averages. Water suppression was achieved using a chemical shift selective sequence. Subsequently, ADC maps were acquired from diffusion weighted images (TR=2.0 sec TE=0.041 msec) using b values of 125, 750, and 1500 s.mm-2, (Δ=41 ms and δ=35 msec), in 3 orthogonal directions. After MRI, the animals were transcardially perfusion-fixed and the brains removed for histological analysis. Microglia activation was investigated using Tomato-Lectin staining. The proton spectra were analysed off-line using the 1D Win-NMR software package (Bruker-Franzen Analytik, Bremen, Germany). Data were zero-filled, Lorentz–Gaussian- and Fourier-transformed, then phase- and baseline-corrected. The spectral peaks from NAA, Cr and Cho were fitted to Gaussian line shapes and integrated. Results are expressed in the following metabolite ratios: NAA/Cr, Cho/Cr and NAA/Cho.

Results

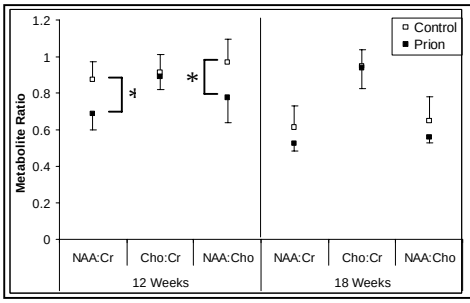

*Figure 1. Reduced Metabolite Ratios (Naa:Cr and Naa:Cho) in prion animals at 12 weeks and 18 weeks post-injection. (*p<0.05 unpaired t test when compared to controls at the same time-point). In addition, the metabolite ratios at 18 weeks were significantly lower than at 12 weeks in both prion (unpaired t tests p<0.02) and in control animals (unpaired t tests p<0.005).*

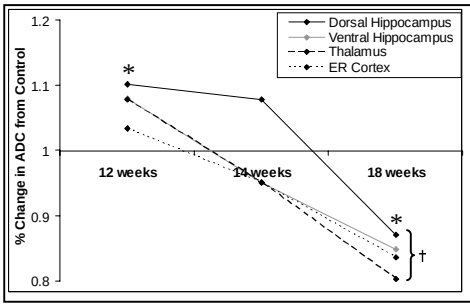

*Figure. 2. Graph to show percent ADC change from controls at each time-point. The ADC at 12 weeks in the dorsal hippocampus was significantly higher (*unpaired t test p<0.05) than controls at the same time-point. In contrast, the ADC at 18 weeks in the dorsal hippocampus was significantly lower than controls at the same time-point. In addition, the ADC at 18 weeks was significantly lower in all regions than at 12 weeks post-injection (†unpaired t tests p<0.01).*

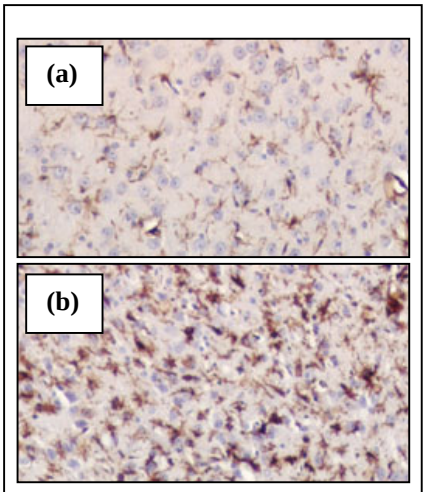

Figure. 3. Microglial activation (stained brown) in the prion thalamus at (a) 12 weeks, and (b) 18 weeks.

Discussion

These studies demonstrate, using clinically relevant MRI and MRS techniques, that injection of ME7 brain homogenate results in early reductions in NAA (N-acetyl aspartate) concentration prior to clinical symptoms, which occur around 23 weeks post-injection (3). In addition, we have observed concomitant increases in ADC, which subsequently decrease by 18 weeks. NAA concentrations are reduced at 12 weeks post-injection in prion animals, and the trend persists until at least 18 weeks, when a reduction is also observed in control animals. NAA is typically a marker of neuronal viability, and neuronal loss has been shown to occur in the later stages of prion disease (4,5). Reductions in NAA have been observed in aging rats (6), so reductions observed in control animals may reflect the ongoing aging process. In prion animals however, the early reductions in NAA suggest that changes in the neuronal structure occur far earlier than the onset of clinical symptoms, and may reflect alterations which contribute to the later reductions in ADC. The early increase in ADC may be brought about by changes in the structure of the hippocampus, such as loss of synapses (7). Decreases in ADC may also reflect changes in the cell population in the thalamus and also in the hippocampus as a result of microglial activation and proliferation (3). These reductions in brain metabolites, specifically NAA, and reductions in ADC observed *in vivo* may provide early non-invasive clinical markers for prion disease.

References

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