Quantitative Measures of T_1 and T_2 Relaxation Time 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 quantitatively investigated T_1 and T_2 relaxation times 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_2$0:30%O_2. 1µl ME7 brain homogenate was injected stereotaxically into the dorsal hippocampus of the left hemisphere. Control animals received an intracerebral injection of normal brain homogenate. Animals were imaged 14 weeks after intracerebral injection of brain homogenate. MR measurements were performed on a vertical bore 9.4T magnet with a Varian Inova spectrometer. Animals were placed in an Alderman-Grant resonator, and anaesthesia was maintained with 0.5–1.5% isoflurane in 70%$N_2$0:30%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). Subsequently, quantitative T_1 maps (Inversion Recovery, TIR=0.025, 0.25, 0.5, 0.75, 2, 5 sec, TR=5s) and T_2 maps (TE=0.02, 0.04, 0.06 sec, TR=3s) were obtained.

Results

Representative T_1 and T_2 maps obtained from infected animals are shown in Figure 1a and b. At 14 weeks post-intracerebral injection, an increase in the T_1 relaxation time was observed in the hippocampus of both hemispheres in all of the mice infected with ME7. A significant difference was found in the left (injected) hemisphere between ME7 injected and control animals, (unpaired t test, $p<0.05$; Figure 2a) but the difference in the right (non-injected) hemisphere did not quite reach significance (unpaired t test, $p=0.055$). In addition, an increase in the T_2 relaxation time of the hippocampus was observed in the ME7 injected animals, which was significant in the left (injected) hemisphere when compared to control animals (unpaired t test, $p<0.02$; Figure 2b). Once again, the difference in the right (contralateral) hemispheres did not quite reach significance (unpaired t test, $p=0.078$). Previously published immunohistochemistry (4) revealed extensive microglial activation and loss of synaptophysin staining in the hippocampus of ME7 infected mice at 14 weeks (Figure 1 c and d).

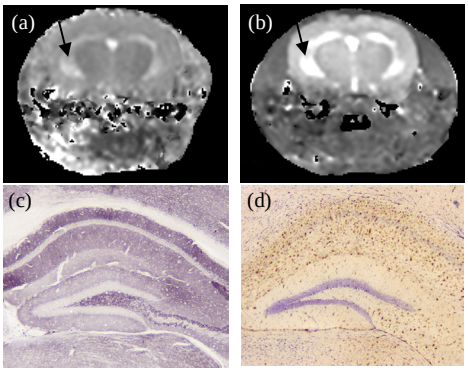

Fig. 1. Prion infection at 14 weeks. T_1 (a) and T_2 (b) maps showing increased relaxation times in the hippocampus (arrows). (c) Reduction in synaptophysin staining indicating synaptic loss (4). (d) Activation of microglia (stained brown) (4).

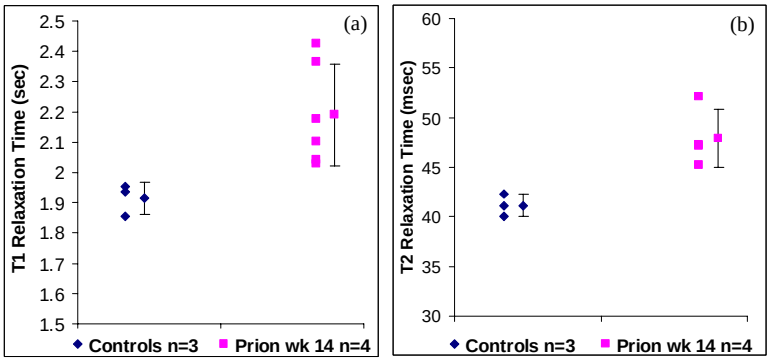

Figure 2. Increased T_1 (a) and T_2 (b) relaxation times in the injected hemisphere of prion infected animals. Unpaired t test, $P<0.05$ and $P<0.02$, respectively. Individual data points and Mean ± S.D. shown.

Discussion

These studies demonstrate, using clinically relevant MRI techniques, that injection of ME7 brain homogenate results in quantitative changes in the T_1 and T_2 relaxation time of the hippocampus. These changes are likely to reflect changes in the cell population in the hippocampus as a result of microglial activation and proliferation (3), or changes in the structure of the hippocampus, such as loss of synapses (4). In this study a unilateral injection of ME7 brain homogenate was used, but increases in T_1 and T_2 relaxation were observed bilaterally indicating that pathophysiology has begun to spread to the contralateral hemisphere by 14 weeks. These increases in relaxation time may provide an early non-invasive clinical marker for Prion disease.

References

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