A multiparametric assessment of Prion disease

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Abstract: Human prion diseases are fatal neurodegenerative disorders which cause cognitive impairment and neurological deficits. Our previous studies in mice-intracerebrally inoculated with misfolded prion proteins using quantitative magnetic resonance imaging shows great promise for monitoring prion protein misfolding in vivo. However, little work has been carried out using quantitative MRS, MT and quantitative T1 and T2 during the course of the disease. Additional measures of tissue status are necessary for improving the sensitivity and specificity of clinical diagnosis as in many cases clinical forms of prion disease are commonly mistaken for other forms of dementia. Using in vivo MT significant changes were detected in cortex and thalamus of late stage prion infected when compared to control mice. To our surprise a significant increase of MTR was detected in thalamus and cortex between the group of control mice suggestive of developmental changes occurring in healthy brain. Using quantitative T2 maps significantly higher values were measured in thalamus of prion mice at all stages of the disease (T_2=40msec, p=0.001) while $T_1$was found to be significantly higher in cortex (T_1=1.89sec, p= 0.0007) and hippocampus of only late stage prion mice when compared to aged-matched controls (T_1=1.67sec). Using quantitative MRS significant changes were detected in glutamate (Glu) and myo-inositol (Ins) at all stages of prion disease compared with the control group. NAA, Cr, Lactate and Lipids were only found to be significantly different at early and late stages of the disease while Taurine (Tau) was only significantly increased in the asymptomatic stage without any significant change in the early and late stage of the disease. 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.

Introduction

Spongiform encephalopathies, of which Prion disease is a particular form, are fatal neurodegenerative disorders that have prolonged asymptomatic incubation periods. Whilst the most common form of prion disease occurs sporadically (sporadicCreutzfeldt–Jakob disease, sCJD), other forms are caused by prion protein gene mutations, or exposure to prions in the diet or by medical procedures, such us surgeries. The neuropathogenesis of prion disease is strongly associated with the conversion of normal PrPc to the protease resistant PrPSc. However, the mechanism by which prions cause brain damage remains unclear. Therefore, characterization of early pathological aspects would be of benefit for the diagnosis and treatment of this chronic neurodegenerative disorder.

For all types of prion diseases, neuropathological findings apart from prion protein staining include vacuolation, neuronal cell death, astrocytosis and microglia activation. Despite these effects, diagnostic criteria for CJD rely more on clinical and histopathological signs with limited utility for imaging. To date, numerous models of prion disease have been developed in mice mimicking disease symptoms. In these models the length of the incubation period varies while developed clinical signs include ataxia and abnormal posture. Several investigations using preclinical magnetic resonance imaging yielded inconclusive findings. In hamsters, increased signal intensity on T_2-weighted images was observed in the hippocampus at a pre-symptomatic stage (120 days) with the signal alterations being more pronounced with disease severity. In addition, the observed neuropathological changes included accumulation of PrPsc and astrogliosis without any spongiform changes (1). In a second study, sheep were exposed to Prion disease. However, the amount of PrPsc found in the brain of the animals was below the detectable limits of the protein assays. Other MRI findings include diffuse cerebral atrophy in asymptomatic and symptomatic sheep, with the origin of this finding being unclear (2) .

The findings in animal models agree with clinical studies of prion disease suggestive of the use of quantitative imaging measures such as diffusion weighted images (DWI) and T2-weighted scans as potential imaging biomarkers at the early stages of the disease. In the current study, we have used a range of quantitative MRI techniques (T1, T2 and Magnetization transfer) and MRS to investigate changes that occur longitudinally in C57BL/6J mice intracerebrally inoculated with misfolded prion proteins. The observed changes are correlated with well described histopathological features that underpin early metabolic changes. The primary aim of this study was to identify early markers of prion disease that can be detected non-invasively by MR.

Methods

Animal studies

Control and prion-infected mice

Work with mice was performed under licence granted by the UK Home Office and conformed to the University College London institutional guidelines. Two groups of 7-week-old FVB mice were intracerebrally inoculated with 30µl of 1% brain homogenate from Rocky Mountain Laboratory prion-infected mice (n=19) or brain homogenate from uninfected mice as controls (n=11). The prion-infected group was separated into three groups of mice scanned at different stages of prion disease: 80 days post injection (dpi) – asymptomatic-stage (n=6), 130 dpi – early-stage (n=6), and 160 dpi – late-stage (n=7). Control mice were separated into two groups: 80 dpi (n=5) and 160 dpi (n=6). All mice were anaesthetized (1.5-1.8% isoflurane in 1.5% oxygen with balance in air) and scanned on a 9.4 T Agilent system using a 33-mm-diameter transmit/receive coil (Rapid Biomedical).

Anatomical MRI and MRS protocol

Anatomical scans were acquired in an axial slice using a fast spin-echo sequence (data matrix: 256x128, TR=3000ms, TE=20ms, FOV=20x20mm). *In vivo* proton spectra were acquired using a PRESS sequence (TR = 5000msec, TE = 7.5msec and TE = 144 mec, 128 averages, total acquisition time=10 min) with a voxel centred on the thalamus (1.7 x 4.3 x 1.8 mm^3). After first and second order shimming, the typical linewidth of the water resonance was 20-23Hz.

MRS data analysis: Metabolite concentrations were estimated using TARQUIN (3). Experimental data were modelled as a linear combination of modified simulated basis signals. The Cramer-Rao lower bounds were used as a reliability measure of the metabolite concentration estimates. All metabolite concentrations are presented as mean ± standard deviation. Statistical analysis was performed using a two-tailed t-test. Significant changes in metabolite concentrations are indicated by $p<0.05$.

MT protocol

All images were acquired in a single slice (thickness=2mm), centred on thalamus. MT measurements were acquired using a gradient-echo sequence (matrix: 64x64, TR=2.11ms,

TE=1.07ms, FOV=20x20mm²) with a train of off-resonance Gaussian pulses applied at 10 µT (n=30, pulse length=50ms, flip angle=6000°, 99% duty cycle). The presaturation pulses were applied at 10 ppm. In addition, for improving the image SNR and reducing the physiological noise during MRI acquisition the measurements were collected three times and the averaged values have been calculated for each mouse.

MT Data analysis

Regions-of-interest (ROIs) were drawn on the anatomical image of each mouse in the cortex and thalamus for analysis of the corresponding MTR maps. In this study, MTR were calculated as the difference in signal on either side of the water peak at 10 ppm. B_0 correction used the minimum of a fitted spline as indicated in our previous study.

T_1 and T_2 measurements

An inversion recovery EPI sequence was used to quantify the T_1 values. A global adiabatic inversion pulse (fa=180°, duration=2ms) was applied followed by 10 inversion times exponentially spaced from 8.1ms to 7.5s. For the quantification of T_2 values a CPMG sequence was used, consisting of a 90° Sinc-shaped excitation pulse along the x axis (duration=2ms) followed by 15 Sinc-shaped refocusing pulses along the y axis (fa=180°, duration=1.6ms).

Post processing

Data processing was performed using custom-written scripts in MATLAB (Mathworks Waltham, MA). T_1 and T_2 maps were obtained assuming mono-exponential decay for longitudinal and transverse relaxation.

Results

To investigate signal alterations in prion infected mice experimental work was carried out to assess whether changes in metabolites concentration, or alterations in magnetization transfer could be detected between prion infected and control mice. Additional measures of tissue status were evaluated by means of quantitative T_1 and T_2 maps. Finally, T_2-weigthed images in the brain of prion-infected mice were visually inspected for pathological changes throughout the disease course.

Structural changes in Prion disease

Figure 1 shows conventional T_2 weighted images obtained from the brains of prion-infected mice at 80 days post injection (dpi), 130 dpi and 160 dpi which correspond to asymptomatic, early-signs and late-stage Prion disease. Increased grey matter signal intensity has been observed in 80 dpi mice which is more pronounced in cortical and hippocampal regions. As the disease progresses increased signal intensity has been also detected in thalamus and basal ganglia. It is worth noting that the origin of hyperintensities might be related to the reaction of the brain to the injection of brain cells, and might not be directly related to the progression of the disease in this model. At the late-stage of the disease brain atrophy has been detected in cerebellum, brain stem and hippocampal regions.

MRS studies

The neurochemical profile of prion disease in mice at different disease stages was evaluated using high-quality MR spectra obtained in thalamus. Seven metabolites were measured *in vivo* longitudinally. The metabolite concentrations were evaluated for age dependence between the two control groups scanned at 80 dpi and 160 dpi. The only change with age between both control groups was an increase in N-acetylaspartate (NAA) (p=0.009) and creatine (Cr) (p=0.005). Therefore, the control groups were merged into one group when compared with prion-infected mice for all the metabolites except NAA and Cr. Significant changes were detected in glutamate (Glu) and myo-inositol (Ins) at all stages of prion disease (80 dpi, 130 dpi, 160 dpi) when compared with the control group. However, there was no significant change in Choline (Cho). NAA, Cr, Lactate and Lipids were only found to be significantly different at 130 dpi and 160 dpi compared with the control group. Moreover, Taurine (Tau) was only significantly increased at 80 dpi without any significant change at 130 dpi and 160 dpi (see Figure 2). Figure 3 shows representative MRS spectra in thalamus from a control and a prion-infected mouse at 160 dpi.

Magnetization transfer results

Magnetization transfer contrast was evaluated to visualise changes in macromolecular concentration and composition, which might take place through both the developmental course as well as through the direct effect of the disease. Significant changes have been detected in cortex and thalamus of 160 dpi prion-infected mice when compared to 160 dpi control mice (see Figure 4). Moreover, a significant increase of MTR has been detected in thalamus and cortex of 160 dpi versus 80 dpi control mice. The MT levels for the early and late stage prion-infected mice were not significantly different, however, control mice of 130

days old were not scanned therefore it is difficult to conclude if there are any significant MT changes at the early stage of the disease.

T_1 and T_2 results

T_2 values were found to be significantly higher in thalamus of prion mice at all stages of the disease (T_2=40msec, p=0.001) when compared to control mice (T_2=37sec) while the T_1 was found to be significantly higher in cortex (T_1=1.89sec, p= 0.0007) and hippocampus of only late stage prion-infected mice when compared to aged-matched controls (T_1=1.67sec).

Discussion

In this study, we used quantitative MRI measures with a view to increase our understanding of the preclinical development of prion disease. Additionally, we could detect early changes in MRI parameters which would facilitate early diagnosis of prion disease in humans. In the RML mouse model we have used, clear evidence of abnormal brain function has been detected about 80 days post-inoculation, when subtle symptoms become apparent in prion-infected mice. Clinically this corresponds to phase 2 of prion propagation where the disease-related prion protein is well characterised throughout the brain. Despite the evidence that neuropathological symptoms have not been observed, brain function is affected while abnormalities in the brain are detectable by conventional MRI such as quantitative T2 mapping throughout the disease course. Additionally, MRS which reports on neuronal function and metabolism in the brain shows deficiencies throughout the disease course. The concentration of MI and Glu, were statistically different in all prion-infected mice. Furthermore, MT abnormalities reporting on macromolecular concentration and composition were found to de reduced at the end-stage of the disease. These findings are discussed in more details below.

In vivo MRS changes

MRS analysis is promising for identifying relevant and early biological markers of the disease, since it provides biological information occurring at a cellular level. Here, changes in brain metabolite concentrations provide key insights about disease progression. Glu is a major excitatory neurotransmitter and high concentrations can be found in the brain (~ 7.2 mmol/kgww), followed by NAA (~ 6.3 mmol/kgww) and Ins (~ 4.9 mmol/kgww). Alterations in Glu concentration are indicative of imbalance in metabolic activity (Krebs Cycle) and a decrease in glutamate concentration is related to a reduced metabolism (4). The roles of Ins are not well understood; however, it is believed that increased concentrations of Ins in neurodegenerative diseases reflect an increase of glial cells, because it is primarily expressed

in such cells. In prion-infected mice, an increase in Ins might be also related to astrogliosis (5).

NAA represents a marker of neuronal density or neuronal function since it recovers after stroke or multiple sclerosis (6). Reduced NAA in prion-infected mice is indicative of neuronal loss, which is similar to evidence given by human studies (7). Under conditions of anaerobic metabolism or inflammation, lactate levels become elevated and a characteristic peak at 1.3ppm overlaps with the lipid resonance (8). In prion-infected mice, lactate and lipid levels are elevated; this was further confirmed by the collection of MRS spectra at high TE=120 ms. To date, limited MRS data have been obtained from individuals with CJD. Similar with our findings significant reductions in NAA levels were reported (9) while it has been argued that these changes reflect neuronal cell death. Additionally, a decline in NAA levels might be associated with a functional deficit through synaptic loss.

MT changes

The MTR ratio was found to be significantly increased in 160-day-old mice, compared to that in 80-day-old mice. The increase in MT could be attributed to decreased water content as a result of the accumulation of myelin, lipids, proteins, proteolipid-proteins, cholesterol and amino-acids. Furthermore, the findings – including MT changes in healthy mouse brains and reduced MT values in the disease state – are in line with the literature of other neurodegenerative diseases such as Alzheimer's disease or amyotrophic lateral sclerosis (ALS) (10),(11). Taking this into account, MTR might not likely be a useful diagnostic biomarker as abnormalities have been described in other neurodegenerative disorders. For clinical practise, it would be beneficial to compare MTR changes in patients with prion diseases and other neurodegenerative disorders. On the positive side, in our previous studies, we showed that NOE* which reports on conformational changes occurring in $\beta - \mathrm{PrP}$ is significantly decreased throughout the disease course in the prion infected group. Thus, combining both indices a useful predictor of clinical disease onset might be established.

T1 and T2 relaxation changes

In all regions studied, T_1 was found significantly different only at the end-stage of the disease in contrary to T_2 which was significantly higher in thalamus throughout the disease course. These findings support the hypothesis that the early symptoms of the disease are more likely caused by processes which affect normal brain function rather than abnormalities at the structural level. It is known that early stages of the pathology are related with synaptic loss

resulting to the loss of neurons at the late stages of the disease. Additionally, at the end stage extensive vacuolation and brain atrophy alter water distribution which might influence the relaxation times. According to the literature increased T_2 values were obsvered in prion-infected hamsters suggestive of increased tissue water content which might related to the presence of cell death (12). However, these are qualitative observations taken from T2-weighted images, which have a number of inherent confounds. In contrast, our data obtained by quantitative methods and provide evidence for widespread degeneration of the brain integrity during the preclinical stages of the disease in this murine model.

Conclusion

Several MRI modalities were applied in prion infected mice for gathering information regarding the pathogenesis of this fatal neurodegenerative disorder. Early signs of the disease are observed at about 130 dpi, however, brain function is affected from around 80 dpi as shown from our results. Alterations in the brain are detectable by histopathological staining as well as by non-invasive quantitative measurements of MT, T2 and MRS. A major goal of this study was to identify preclinical markers of prion disease for early detection and/or diagnosis. Among all our measurements decreases in Glu and MI concentration as well as an increase in T2 has the potential to warn of impending clinical symptoms. Overall, these results indicate functional down-regulation in advance of neurodegeneration. Further studies are required in earlier timepoints for predicting disease onset by means of MRI.

Acknowledgements

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