

Chemical Exchange Saturation Transfer on a prototype model of neurodegeneration.

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Target audience: Scientists interested in the application of Chemical exchange saturation transfer (CEST) in neurodegenerative diseases.

Introduction: Prion disease is associated with the accumulation of abnormal proteins rich in beta sheets due to misfoldings, leading to neuronal degeneration and ultimately death [1]. Chemical exchange saturation transfer (CEST) is a promising MRI technique for the detection of changes in concentration and possibly conformation of proteins [2]. Here, we apply CEST to examine potential changes related to protein folding and aggregation occurring in a terminal mouse model of prion disease.

Methods: Mice (n=6) were inoculated intraperitoneally with 100 μ l of 1% brain homogenate from Rocky Mountain Laboratory (RML) infected mice (n=3) or Phosphate Buffered Saline (PBS) as controls (n=3). 200 days-old mice, corresponding to the end stage of Prion disease, were anaesthetized and scanned on a 9.4T Agilent scanner using a transmit/receive RF coil with 33mm inner diameter (Rapid Biomedical). Anatomical scans were acquired using a fast spin echo multislice sequence (data matrix 256x128, TR=3000ms, TE=20ms, FOV=20x20mm). CEST measurements were acquired using a gradient echo sequence (TR=2.11ms, TE=1.07ms, FOV=20x20mm², slice thickness=2mm, matrix size=64x64) with a saturation train prior to the readout consisting of 80 Gaussian pulses at three different powers. These power settings were optimized for Nuclear Overhauser Effect (NOE), amide and amine proton exchange respectively. The applied power for NOE was 0.8 μ T, (pulse length=50ms, flip angle=480, 95% duty cycle), for amides 1.2 μ T (pulse length=50ms, flip angle=720, 95% duty cycle) and for amines 10 μ T (pulse length=50ms, flip angle=6000, 95% duty cycle). Saturation was applied at 71 frequency offsets between -5.0 and 5.0ppm.

Data analysis: Two representative regions-of-interest (ROI) one in the cortex and one in the basal ganglia were chosen for data analysis of the three power settings (figure 1). MTR asymmetry was calculated between 3.3 and 3.7ppm for both 0.8 μ T and 1.2 μ T power (figure 3). In addition APT* and NOE* values were calculated within the same range by finding the difference between the Z-spectrum and a linear interpolation [3]. For the 10 μ T data the MTR asymmetry was calculated between 0-5.0ppm (figure 1). Bo correction was calculated from the minimum of the fitted to spline curve of the 0.8 μ T CEST dataset.

Results: CEST data show a decrease (p<0.05) in the MTR asymmetry of 10 μ T in the prion mice compared to the control group in the cortex and in the basal ganglia (figure 1). The MTR asymmetry at 1.2 μ T and 0.8 μ T showed no significant difference (p>0.05) (figure 3). A trend towards increased APT* at 1.2 μ T in the Prion mice was found in the cortex and basal ganglia but was not statistically significant (p> 0.05) when compared with the control group. The NOE* for both groups was the same at either power 0.8 μ T or 1.2 μ T (figure 4).

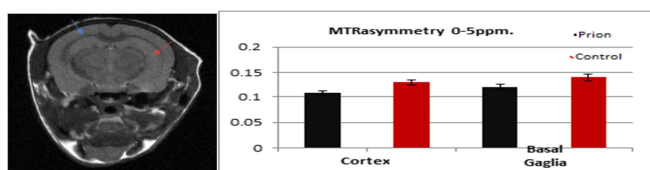


Figure 1 shows the MTR asymmetry at 10 μ T in the cortex and in the basal ganglia of prion (black) and control mice (red). Significant decrease (p<0.05) of MTR was detected in both regions.

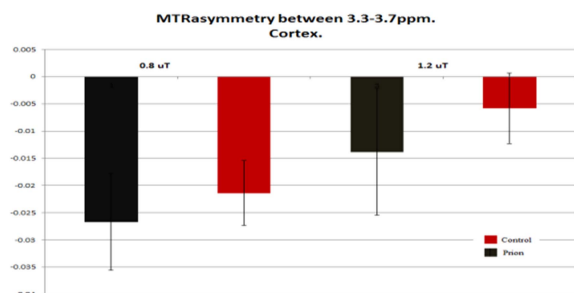


Figure 3 shows the MTR asymmetry in the cortex between 3.3-3.7ppm for prion (black) and control (red) mice.

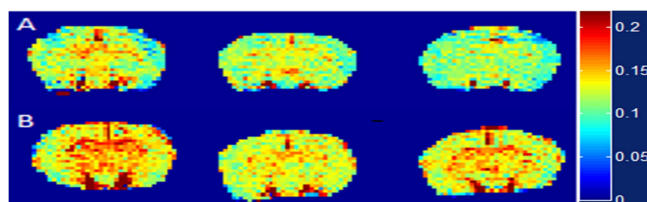


Figure 2 displays the MTR asymmetry maps at 10 μ T between 0-5ppm for prion (A) and control mice (B).

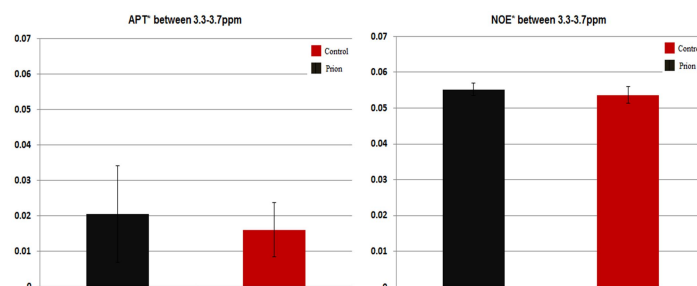


Figure 4 shows the APT* and NOE* at 1.2 μ T in the basal ganglia of prion (black) and control mice (red). No significant difference (p>0.05) found in either APT* or NOE*.

Discussion and Conclusion: In this preliminary study, we applied CEST to detect changes related to Prion protein misfolding present in Prion-infected mice. The results show no significant changes in NOE* indicating a lack of difference in intracellular pH between groups. In addition, APT* only showed a trend towards an increase signal in Prion animals, in line with a potential cytosolic accumulation of misfolded proteins. Additional datasets on more animals would indicate the validity of this finding. However, this was also accompanied by a significant reduction found in the MTR asymmetry at 10 μ T power in both basal ganglia and cortex of the diseased animals, suggestive of changes happening in the local proteasome possibly indicating cell death at this late stage of this disease, in addition to a reduced number of exposed amine groups due to Prion protein misfolding.

Acknowledgements This project was funded by the UCL Grand Challenges Scheme **References** [1] K.M.Pan et al Proc Natl Acad Sci USA. Dec 1, 1993; 90(23): 10962–10966. [2]Moritz Zaiss et al.NMR in biomedicine 2013;26(12):1815-1822; [3] Xu J. et al. NMR Biomed. 2014 Apr;27(4):406-16.