Detection of protein accumulation by Amide Proton Transfer (APT) in the spinal cord of SOD1 mice using exchange-modulated PRESS

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Target audience: Preclinical analysis with APT-CEST on animal models for neurodegenerative diseases

Purpose

Amyotrophic Lateral Sclerosis (ALS), the most common variant of Motor Neurone Diseases (MND), is one of the most devastating neurodegenerative diseases causing death typically within 2-5 years of diagnosis (1). Approximately 10% of cases are familial ('fALS'), inherited in an autosomal dominant mode, with 20% of these cases caused by mutations in the Cu, Zn-superoxide dismutase (SOD1) gene. Cytosolic protein aggregates are a characteristic feature of ALS and are common to both sporadic and familial forms of ALS (2), as well as transgenic mouse models expressing mutant SOD1 protein (3). The formation of SOD1-rich fibrils inclusions in the spinal cord is an early and prominent feature of ALS (both sporadic and familial) in patients and SOD1 mutant models of this disease, suggesting that the conversion of soluble SOD1 into amyloid fibrils may play an important role in its aetiology. In this study we explore the possibility of detecting the formation of SOD1-rich fibrils inclusions in the spinal cord using amide proton transfer (APT), by indirect detection of saturated amide protons through chemical exchange saturation transfer (CEST) effects (4) using the EXPRESS (exchange-modulated PRESS) sequence (5).

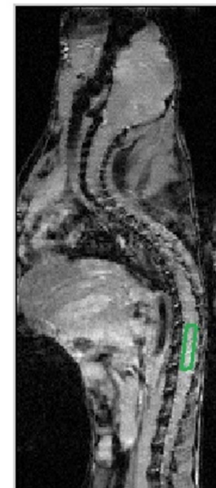

Figure 1: Sagittal image of a mouse indicating the voxel area (in green) at the L3-L4 (Lumbar)

Methods

MRI scanning: The EXPRESS sequence consists of a 6 second saturation train of off-resonance Gaussian pulses (length 50ms, inter-pulse delay= 5ms, BW 54Hz, FA= 500°) and a PRESS readout. To improve SNR, the sequence was run 3 times with 77 offset frequencies over the range of ±6ppm around the water resonance and 2 reference points at 200ppm. The study was conducted under an approved protocol from the Home office. 7 wild type (WT) and 7 SOD1 mice, all male were scanned at 90 days (pre-symptomatically of the disease) and another set with equal numbers at 120 days old (symptomatically and near the disease-end point). Anaesthesia was induced by 3% isoflurane in O_2 and maintained throughout the experiment with isoflurane 1% in O_2. Respiration gating was used to avoid voxel movement. Scanning was performed on a single voxel in the lumbar region (Fig1), deemed to be most susceptible to protein aggregation in SOD1 animals.

Protein assays: Each mouse was euthanized with 0.1ml of Pentobarbitone and cardially perfused immediately after MRI scanning. The spinal cords were extracted, dissected into the Lumbar sections and snap frozen in liquid nitrogen. The samples were then sonicated on ice and ultracentrifuged at 110,000g for 5 minutes. The supernatant was kept as the soluble protein and the fractions were determined using the BCA assay and bovine serum albumin as the standard.

Results and discussion

At 90 days there is a significance difference ($p<0.01$, unpaired T-test) in the APT MRI signal between SOD1 and WT mice. These findings are important as affected mice at 90 days old do not yet display clinical symptoms. There was no difference at 120 days ($p=0.6$). The soluble protein fractions from protein assays follow the same trend as the MRI data (see Fig 2). Mice at 90 days show a trend towards reduced soluble protein levels ($p=0.1$), while 120 days old SOD mice show no significant difference in APT or protein levels to their WT counterparts ($p=0.8$). The higher APT signal at 90 days old could be related to the soluble protein as it shows the same trend as the protein assays. While aggregates form, the level of soluble proteins decreases, and motor neurones die, which potentially explains the APT signal drop at 120 days old.

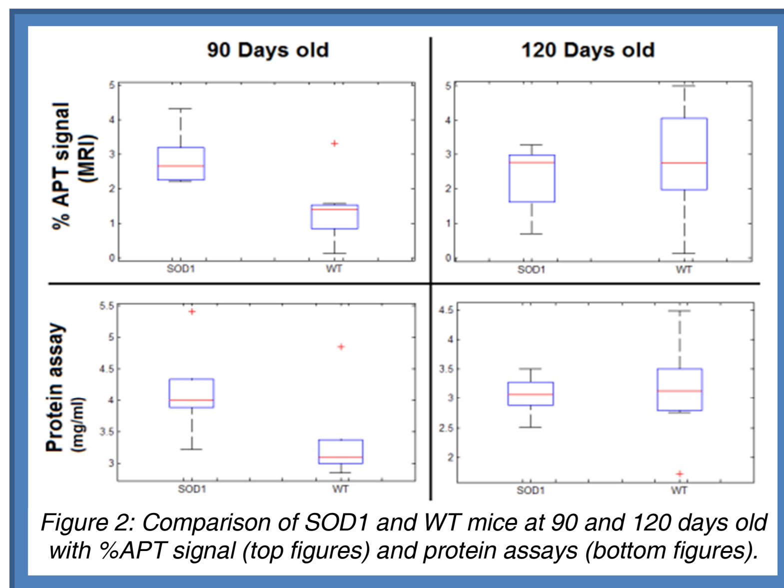

Figure 2: Comparison of SOD1 and WT mice at 90 and 120 days old with %APT signal (top figures) and protein assays (bottom figures).

Conclusion

This study shows that it is possible to obtain Z-spectra from the spinal cord of SOD1-mutant mice, and preliminary results indicate the presence of a detectable significant difference between WT and SOD1 animals. The consequences of these developments and potential translation into the clinic would be of beneficial to patients suffering from ALS, as it would offer the first diagnostic marker for this disease. If confirmed, such methods could also be used as an early marker of response to therapy in addition to potentially providing the first real diagnostic test for this disease. Therefore, we are currently working towards studying SOD1 mice before and after treatment with arimoclomol, a heat shock protein co-inducer known to work on this model by reduction of protein aggregates (6).

References: [1] D. R. Rosen et al., Nature 362, 59 (1993), [2] C. A. Ross, M. A. Poirier, Nat Med 10 Suppl, S10 (2004), [3] L. I. Bruijn et al., Neuron 18, 327 (1997), [4]J.Zhou, et al., Nat Med 9, 1085 (2003), [5] S. Walker-Samuel et al., NMR in Biomedicine, 25(6)(2012). [6] Kieran D, et al., Nat Med 10(4), 402 (2004)