In Vivo Detection of Membrane-Bound Radicals using Molecular MRI and Immuno-Spin-Trapping in a Mouse Model for ALS

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Purpose: Reactive oxygen (and nitrogen) species (ROS/RNS) generated from oxidative stress play a crucial role in many diseases, either as modulators of signal transduction or as a cause of tissue injury. Understanding the extent and timing of events triggered by free radicals *in vivo* is important, since these are major determinants of disease evolution and prognosis. Monitoring *in vivo* radicals in a transgenic mouse model for amyo-trophic lateral sclerosis (ALS) by combining molecular magnetic resonance imaging (mMRI) and immuno-spin trapping (IST) technologies for the first time is possible.

Methods: *ALS Transgenic Mouse Model:* As a model for ALS, a human-G93A-SOD1 Tg mouse model was used, compared to a wild-type control (B6SJLF2). DMPO (5,5-dimethyl-pyrroline-*N*-oxide) treatment commenced at 115 days to maximize the spin trapping of protein radicals prior to detection of DMPO-protein/lipid nitrone adducts at 120 days. For controls, G93A-SOD1 mice (n=5) were administered saline instead of DMPO, while wild-type mice (n=5) received DMPO. *Synthesis of DMPO-specific MRI Agent:* The contrast agent, biotin-BSA (bovine serum albumin)-Gd-DTPA, was prepared as previously described[1]. Each animal was injected with 200µg anti-DMPO and 100µg biotin-BSA-Gd-DTPA. Non-specific mouse-IgG conjugated to biotin-BSA-Gd-DTPA was synthesized by the same protocol. *MRI and mMRI:* MRI experiments were performed on a Bruker Biospec 7.0 Tesla/30 cm horizontal-bore magnet small animal system. Morphological imaging was taken by using double echo sequence. T_1-weighted image was acquired with a repetition time (TR) of 2000 ms and an echo time (TE) of 17.5 ms and the T_2-weighted image with TR of 2000 ms and TE of 58.2 ms. mMRI was performed with a variable-TR RARE sequence (rapid acquisition with refocused echoes, with TE of 15 ms and two transverse slices). Signal intensities were calculated in defined regions-of-interest (ROIs) in T_1-weighed images.

Results: MRI was used to detect the presence of the anti-DMPO adducts by either a significant sustained increase ($p<0.05$) in MR signal intensity (Fig. 1) or a significant decrease ($p<0.05$) in T_1 relaxation. The biotin moiety of the anti-DMPO probe was targeted with streptavidin-fluorescently labeled to locate the anti-DMPO probe in excised tissues. As negative controls, either Tg ALS mice were initially administered saline rather than DMPO followed by the anti-DMPO probe, or wild-type mice were initially administered DMPO and then the anti-DMPO probe. DMPO adducts were also confirmed in disease/non-disease tissues from animals administered DMPO.

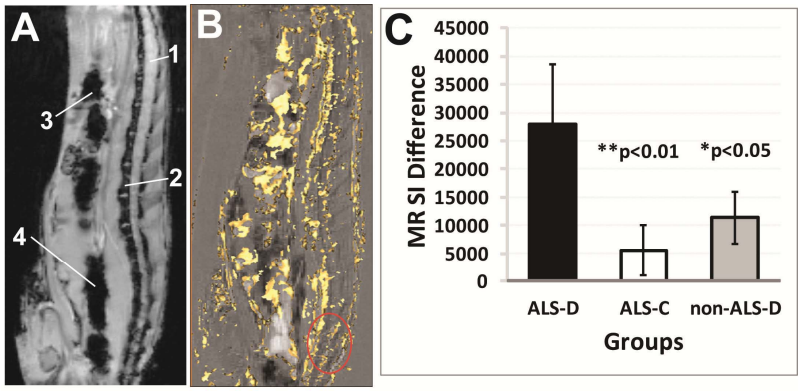

Figure 1: Molecular MRI (mMRI) detection of membrane-bound radical adducts (as detected by the anti-DMPO probe) in a ALS Tg mouse model. (A) Representative T_1-weighted MR image of an ALS transgenic mouse spinal cord. (B) The difference image (image at 120 min post-administration of anti-DMPO probe minus pre-administration image) shows an enhanced region in the lumbar region of the spinal cord (red oval region). (C) Histogram of MRI signal intensity (SI) differences in ALS mice initially administered DMPO and then the anti-DMPO probe (ALS-D), ALS mice administered saline and the anti-DMPO probe (ALS-C) (DMPO control), and wild-type mice administered DMPO and the anti-DMPO probe (non-ALS-D) (disease control). Data is represented as mean ± S.D.. There was a significant increase in the MR image signal intensity difference for ALS mice administered both DMPO and the anti-DMPO probe, compared to the ALS mice administered saline (no DMPO) and the anti-DMPO probe ($p<0.01$) or wild-type controls administered DMPO and the anti-DMPO probe ($p<0.05$).

Discussion: After DMPO is administered, it binds to radicals via a process called spin trapping to form radical adducts. It is anticipated that only radical adducts that are membrane-bound (e.g. protein and/or lipid radical adducts) will be targeted by the Gd-based anti-DMPO probe and detected by MRI. As the anti-DMPO probe mainly targets the cell surface, it is thought that trapped radicals could include oxidized cell membrane-bound protein or lipid radical DMPO adducts that remained in the cell membrane. Uptake of the anti-DMPO probe into spinal cord tissue is thought to be due to a disrupted blood-spinal cord barrier (BSCB) and blood-brain barrier (BBB).

Conclusion: Here we used a combination of mMRI and IST to show for the first time non-invasive *in vivo* detection of spin-trapped membrane-bound radicals in a mouse model for ALS. Using both mMRI and IST provides the advantage of *in vivo* image resolution and spatial differentiation of regional events in heterogeneous tissues or organs and the regional targeting of free radical mediated oxidation of cellular membrane components. This method can be applied towards any radical-associated neuro-pathological condition for the *in vivo* assessment of membrane-bound protein and/or lipid radical levels.

References:

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