In Vivo Detection of Membrane-Bound Radicals in Mouse Brains with Sepsis using Molecular MRI and Immuno-Spin-Trapping

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Purpose: Oxidative stress, particularly involving free radicals, plays a major role in sepsis. Understanding the extent and timing of free radical triggered events in an *in vivo* environment is of importance to our understanding of these major determinants involved in disease evolution and prognosis. With the use molecular magnetic resonance imaging (mMRI) and immuno-spin trapping (IST) technologies combined, it is possible for the first time to monitor *in vivo* radicals in a mouse model for sepsis.

Methods: *Sepsis Model:* As a model for sepsis, cecal ligation puncture (CLP) was used. Eight to 10-week-old male C57BL/6 mice (n=6; n=3 CLP and n=3 sham) were anesthetized with isoflurane (2-3%) under aseptic conditions. The cecum was exposed and ligated with sterile 3-0 silk below the ileocecal junction. The cecum was punctured once with an 18-gauge needle and squeezed to empty its content through the puncture. The cecum was returned to the peritoneal cavity, and the abdominal muscle and skin incisions were closed. Mice in the sham operation control group were subjected to identical procedures, except that ligation and puncture of the cecum were omitted. DMPO (5,5-dimethyl-pyrroline-*N*-oxide) (25 µL in 100 µL saline) was administered (i.p.) every 1.5 h over a period of 6 hours, prior to injection of the anti-DMPO probe. *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. Multiple brain ^{1}H-MR image slices were taken in the transverse plane using a spin echo multislice (repetition time (TR) 0.8 s, echo time (TE) 23 ms, 128x128 matrix, 4 steps per acquisition, 3x4 cm^2 field of view, 1 mm slice thickness). Mouse brains were imaged at 0 (pre-contrast), 20, 40, 60, 120 and 180 min intervals post-contrast agent injection. Mice were injected intravenously with anti-DMPO or non-immune-IgG antibodies tagged with a biotin-Gd-DTPA-albumin-based contrast agent (200 µL/kg; 1 mg antibody/kg; 0.4 mmol Gd^{+3}/kg). T_1-weighted images were obtained using a variable TR (repetition time) spin-echo sequence. Pixel-by-pixel relaxation maps were reconstructed from a series of T_1-weighted images using a nonlinear two-parameter fitting procedure. The T_1 value of a specified region-of-interest (ROI) was computed from all the pixels in the ROI by the following equation (processed by ParaVision 4.0, Bruker): $S(TR) = S_0(1- e^{-TR/T1})$, where TR is the repetition time (units: ms), S_0 is the signal intensity (integer machine units) at TR>>T_1 and TE=0, and T_1 is the constant of the longitudinal relaxation time (units: ms).

Results: *In vitro* assessment of the anti-DMPO in oxidatively-stressed mouse astrocytes indicated a significant decrease ($p<0.0001$) in T_1 relaxation, compared to control samples. MRI was used to detect the presence of anti-DMPO adducts via a substantial decrease in T_1 relaxation, measured as $\%T_1$ change (Fig. 1), within the hippocampus, striatum, occipital and medial cortex brain regions ($p<0.01$) in septic animals compared to sham controls. Fluorescently-labeled streptavidin was used to target the biotin moiety of the anti-DMPO probe to locate the targeting contrast agent in excised tissues, detecting increased signal in the brains, liver and lungs of septic animals, compared to sham controls. *Ex vivo* DMPO adducts were also confirmed in septic brain tissues from animals administered DMPO, compared to sham controls.

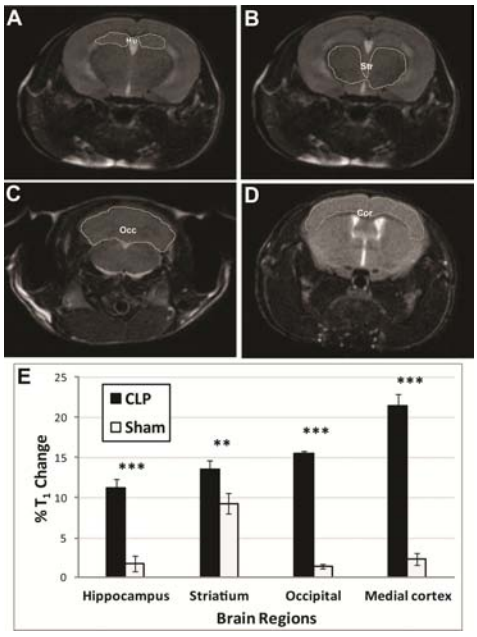

Figure 1: mMRI detection of membrane-bound radical adducts in brain regions of a CLP-induced mouse model for sepsis. Outlined brain regions including the hippocampus (A), the striatum (B), the occipital lobe (C) and the medial cortex (D). (E) Histogram of the percent T_1 difference in the brains of mice with CLP-induced sepsis (CLP) or control shams (Sham). Significantly elevated % T_1 differences were found in the hippocampus (*p<0.05), the striatum (**p<0.01), the occipital lobe (***p<0.001), and the medial cortex (**p<0.01).

Discussion: After DMPO is administered, it binds to radicals 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. Here we used a combination of mMRI and IST to show for the first time non-invasive *in vivo* detection of membrane-bound radicals in neurological effects from sepsis. 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.

Conclusion: This is the first report regarding the detection of *in vivo* levels of MBR from free radical-associated neurological pathology within a sepsis model. This novel non-invasive method can be applied to investigate free radical mechanisms in various sepsis models and different tissues in addition to the brain, toi elucidate the role of free radicals in sepsis-associated pathogenesis.

References:

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