Combined Molecular MRI and Immuno-Spin-Trapping for in vivo Detection of Membrane-Bound Radicals in Mouse GL261 Gliomas

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Purpose: Reactive oxygen (and nitrogen) species (ROS/RNS) generated from oxidative stress play a crucial role in cancer, either as modulators of signal transduction or as causal agents of tissue injury. Understanding the extent and timing of *in vivo* events triggered by free radicals is important to consider, as these are major determinants of disease evolution and progression. By combining molecular magnetic resonance imaging (mMRI) and immuno-spin trapping (IST) technologies it is possible for the first time to monitor levels of *in vivo* radicals in rodent glioma models.

Methods: *Mouse Brain Tumor Model:* A GL261 mouse glioma model was used (n=3). Mouse glioma cells (GL261; $2x10^4$ GL261 cells; in 10 µL of PBS) were implanted intracerebrally in C57BL6/J mice. The anti-DMPO probe was administered at 19 days following cell implantation. Mice were treated with DMPO (5,5-dimethyl-pyrroline-*N*-oxide) for 3 days starting at day 16 prior to the administration of the anti-DMPO probe. For a control, GL261 glioma-bearing mice (n=3) were treated with DMPO, but were administered a non-specific mouse IgG-albumin-Gd-DTPA-biotin contrast agent. DMPO (25 µL in 100 µL saline) was administered i.p. 3 x daily (every 6 hours) for 5 days (i.e. 0.42 µL DMPO/µl saline/day). DMPO administration started at day 16 following intracerebral implantation of GL261 glioma cells.

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:* MR experiments were carried out under general anesthesia (1-2% Isoflurane, 0.8-1.0 L/min O_2). MR equipment that was used included a Bruker Biospec 7.0 Tesla/30 cm horizontal-bore imaging spectrometer. Anesthetised (2% Isoflurane) restrained mice were placed in an MR-compatible cradle and inserted in a MR probe, and their brains were localised by MRI. Images were obtained using a Bruker S116 gradient coil (2.0 mT/m/A) and a 72 mm quadrature multi-rung RF coil. Mouse brains were imaged at various intervals (at 7, 10 and 14 days following cell implantation prior to administration of the anti-DMPO probe at day 21). 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.

Results: MRI was used to detect the presence of the anti-DMPO adducts by either a significant sustained increase ($p<0.001$) in MR signal intensity or a significant decrease ($p<0.001$) in T_1 relaxation, measured as $\%T_1$ change. *In vitro* assessment of the anti-DMPO probe indicated a significant decrease ($p<0.0001$) in T_1 relaxation in GL261 cells that were oxidatively stressed with hydrogen peroxide, compared to control samples. The biotin moiety of the anti-DMPO probe was targeted with fluorescently-labeled streptavidin to locate the anti-DMPO probe in excised brain tissues, indicating elevated fluorescence only in tumors from mice administered the anti-DMPO probe. As a negative control a non-specific IgG antibody covalently bound to the albumin-Gd-DTPA-biotin construct was used. DMPO adducts were also confirmed in tumor tissue from animals administered DMPO, compared to non-tumor brain tissue.

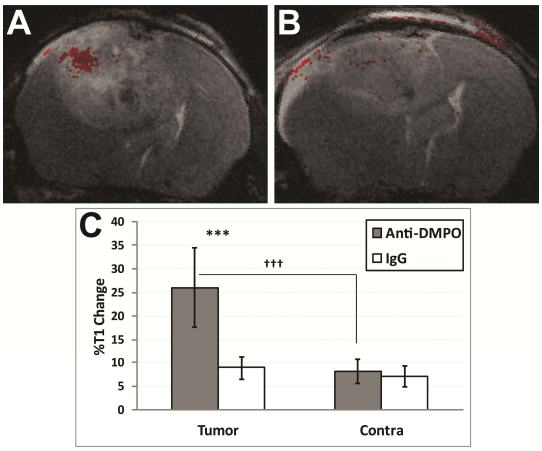

Figure 1: mMRI detection of membrane-bound radical adducts in a GL261 mouse glioma model. (A) T_2-weighted MR image with an overlaid difference threshold image (120 min post- and pre-administration of anti-DMPO probe) following administration of the anti-DMPO probe, taken at 30 days after intracerebral implantation of GL261 cells in mice. Note increased uptake of the anti-DMPO probe in the tumor region. (B) T_2-weighted MR image with an overlaid difference threshold image (120 min post- and pre-administration of the IgG contrast agent) following administration of the IgG contrast agent, taken at 27 days after intracerebral implantation of GL261 cells in mice. Note no specific uptake of the IgG contrast agent in the tumor region. (C) Histogram of percent T_1 change in tumor and contralateral (Contra) regions of GL261 glioma-bearing mice administered either the anti-DMPO probe (Anti-DMPO) or a non-specific IgG contrast agent (IgG).

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 gliomas. 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: 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 mouse gliomas. 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 cancer involving a radical-associated pathological condition for the *in vivo* assessment of membrane-bound protein and/or lipid radical levels.

References:

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