Evaluating Tumor Perfusion with Hyperpolarized HP001 and Comparison with Dynamic Susceptibility Contrast MR Imaging and Pathology Using Orthotopic Human GBM Xenografts

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Introduction: Glioblastoma multiforme (GBM) are characterized by extreme heterogeneity, abnormal neovasculature, and active angiogenesis. Measures of blood vessel volume and permeability are beneficial for evaluating heterogeneous tumor vasculature and predicting response to therapy [1]. The use of hyperpolarized HP001 (*bis*-1,1-(hydroxymethyl)-[1-^{13}C]cyclopropane-d8) may be advantageous over standard gadolinium-based perfusion methods because the signal is directly proportional to concentration and the high polarization and long T1 of HP001 can produce perfusion data with high sensitivity. The purpose of this study was threefold: (i) to characterize tumor perfusion with dynamic ^{13}C HP001 imaging using an orthotopic human GBM xenograft model, (ii) to correlate HP001 data with conventional Gd-based dynamic susceptibility contrast (DSC) imaging data, and (iii) to compare the result from HP001 imaging with immunohistochemistry.

Methods: Ten athymic rats were intracranially implanted with human glioblastoma cells (G55**)** to create an orthotopic brain cancer model [2]. A 50 µL HP001 sample (diluted to 2.78:1 by weight), the trityl radical OX063 (GE Healthcare, Oslo, Norway) and 1.5 mM Dotarem were polarized for ~1 hr using a Hypersense® DNP polarizer (Oxford Instruments, Abingdon, UK), and then rapidly dissolved in ~5.6 mL heated buffer solution (1X phosphate buffered saline). 2.7 ml of the resulting 100 mM HP001 solution was injected into the tail vein of the rat over a 12s duration. Using a GE 3T scanner with a custom-designed ^{1}H/^{13}C RF coil, dynamic imaging data was acquired every second for 90s using a custom single-slice balanced steady state free precession (bSSFP) pulse sequence (TE/TR=6.5/13ms, flip angle=15°, matrix=32x24, resolution=2x2mm, slice thickness=5.4mm) [3]. T1-weighted axial spin-echo images were obtained following the injection of 0.2 mmol/kg Gd-DTPA in order to define the extent of tumor. DSC imaging was performed every second for 150s during a second injection of 0.2 mmol/kg Gd-DTPA using a gradient echo, echo planar imaging sequence (EPI; TE/TR=28.2/500 ms, flip angle 35°, 8x8cm^2 in plane FOV and 40x40 matrix). The in-plane origin, resolution and slice thickness (5.4 mm) were matched between HP001 and DSC imaging, to allow voxel-by-voxel comparisons. Dynamic HP001 images (Fig 1a) were obtained by 2D Fourier-transform of the raw HP001 data and regional magnitude HP001 signals were plotted over time (Fig 1b). Maximum peak (MP) and area under the curve (AU) were normalized by their respective values from arteries and compared between voxels containing tumor and normal tissue using a Wilcoxon sign rank test. The time from MP_{tumor} and MP_{normal} to MP_{artery} (Δt_{max}) was compared between tumor and normal tissues. DSC data were processed using a previously described method [4]. Normalized peak height (nPH) values obtained from the ΔR2* curve in the voxels containing tumor (Fig 1c) were compared with normalized MP from the HP001 data. Anti-α smooth muscle actin (α-SMA) antibody was used to stain for vascular smooth muscle cells.

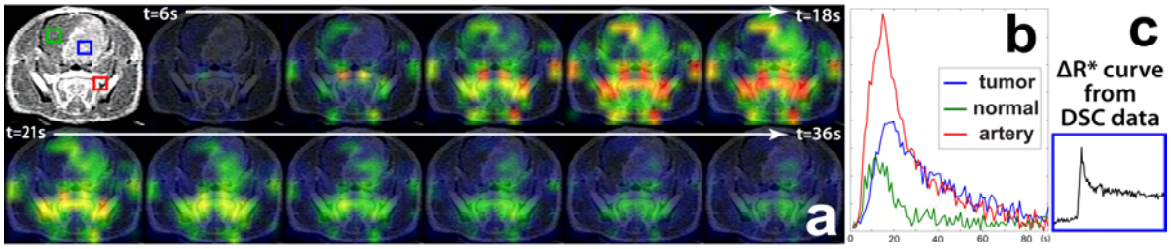

Figure 1. (a) A post-Gd image showing tumor (blue), normal tissue (green), and artery (red) voxels along with dynamic HP001 data displayed every 3s. (b) HP001 signal curves from the corresponding regions. (c) ΔR* curve for the corresponding tumor voxel.

Results: Dynamic ^{13}C imaging allowed for the detection of HP001 signal in tumor, normal tissue, and arteries with heightened temporal (1s) and spatial (2x2x5.4mm) resolution (Fig 1a). Arteries outside of the brain consistently displayed high HP001 signal (Fig 1b). Tumor exhibited significantly higher mean normalized maximum peak (nMP=0.30±0.11) and mean normalized area under the curve (nAU=0.36±0.16) than normal tissue (mean nMP=0.24±0.06, mean nAU=0.24±0.06) (Fig 2). HP001 signal in normal tissue reached maximum 2s (±2s) prior to maximum signal in the artery and HP001 signal in tumor peaked at 2s (±2s) from the maximum peak in the artery (Fig 2). The tumor perfusion data obtained from hyperpolarized HP001 imaging was in excellent agreement with the conventional DSC imaging data. nMP values in 15 tumor voxels from 7 rats were strongly correlated with nPH values from DSC imaging (Fig 3). Figure 4 is α-SMA stained slices from two rats. The rat with a large amount of positive α-SMA in tumor vascular smooth muscle cells exhibited large HP001 parameters (mean nMP=0.5, mean nAU=0.6), while the rat with a very small amount of staining had relatively smaller HP001 parameters (mean nMP=0.2, mean nAU=0.3).

Conclusions: We have demonstrated the feasibility of using hyperpolarized HP001 for investigating tumor perfusion in an orthotopic human GBM model. Distinct HP001 characteristics were found between tumor and normal tissues. HP001 data were strongly correlated with the data from the conventional Gd-based DSC imaging and consistent with the findings from immunohistochemical analysis. The results of this study suggest that this technique may provide an alternate way to evaluate tumor perfusion for brain tumors, which could be applied in patient studies.

References: [1] Essock-Burn et al., *Neuro Oncol*, 2011 [2] Park et al., *J Magn Reson Imaging*. 2011 [3] Von Morze et al., *J Magn Reson Imaging*. 2011 [4] Lupo et al., *AJNR*, 2005

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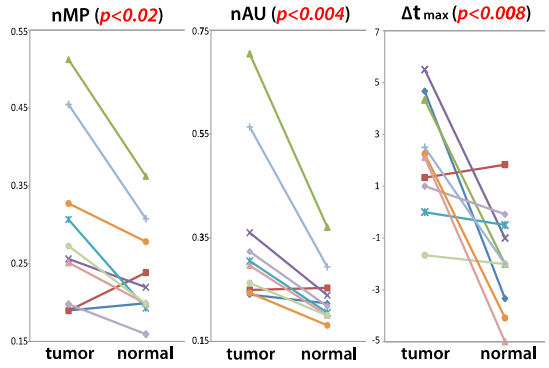

Figure 2. Comparison of HP001 characteristics between tumor and normal tissue. Normalized maximum peak (nMP), normalized area under the curve (nAU) and time from maximum peak to maximum artery peak (Δt_{max}) were significantly different between the two tissue types.

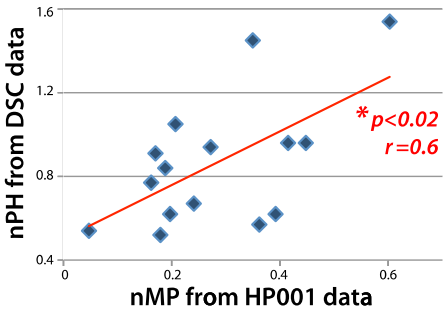

Figure 3. Correlation of normalized maximum peak (nMP) from HP001 data and normalized peak height (nPH) from DSC data.

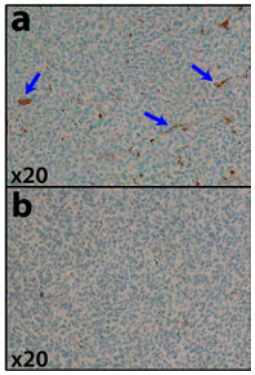

Figure 4. α-smooth muscle actin slides. The rat with a large amount of positive staining (a) had higher nMP and nAU values than the one with little positive staining (b).