Quantitative Perfusion with Hyperpolarized 13C-tert-butanol: Correlation against ASL and Quantitative Immunohistopathology

Leo L Tsai[1], Xiaoen Wang[1], Gopal Varma[1], Benjamin Edelstein[1], Rupal Bhatt[2], David C Alsop[1], and Aaron K Grant[1]

[1]*Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA, United States,* [2]*Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, United States*

Purpose: Hyperpolarized ^{13}C-labeled tert-butanol (IUPAC name 2-methylpropan-2-ol) is freely diffusible in tissue, allowing perfusion imaging with high SNR[1]. Studies on renal cell carcinoma mouse models have demonstrated the high sensitivity of this method in characterizing early response and resistance to antiangiogenics, but correlation has not previously been made to histology or other quantitative imaging methods. Here we report comparisons between ^{13}C-tert-butanol, Arterial Spin Labeling (ASL), and CD34-labeled pathologic samples in an RCC xenograft mouse model treated with sunitnib.

Methods: 12 mice were implanted with A498 RCC tumors. Five were treated with sunitinib, five controls were administered phosphate-buffered saline (PBS), and two were untreated. Sunitinib-treated mice were imaged pretreatment, 5-7 days after treatment initiation, and at resistance. Control mice were imaged pre-PBS, 5-7 days with PBS, and at a tumor limit of 20 mm. Tumors were harvested after final images for immunohistological analysis. Mouse imaging was performed on a 4.7 T horizontal-bore animal system (Bruker Biospec) using methods approved by our Institutional Animal Care and Use Committee. Tert-butanol was polarized to ~5% by DNP as described previously[1]. ^{1}H images were obtained using rapid acquisition with refocused echoes (RARE) for anatomical localization (TR/TE 1000/40 ms, echotrain of 8, 4 NEX, 4.8 x 4.8 cm FOV, 2.3 mm slice, 125^2 µm resolution). Approximately 10 s after injection of 200 µL of 230 mM ^{13}C-tert-butanol solution via a tail vein, 100 successive images were acquired at the level of the tumor via a balanced steady-state free precession (bSSFP) sequence (60° flip angle, TR/TE 4/2 ms, 8.5 cm FOV, 3.3 mm slice, 1^2 mm resolution, 512ms/frame). Arterial-spin-label (ASL) perfusion mapping was performed with a background-suppressed flow-sensitive inversion-recovery scheme (3.3 mm slice thickness, TR/TE 10000/5.1 ms, 3 NEX, IR 30–2300 ms, 3.5 cm FOV, 270^2 µm resolution). All tumors were processed for histopathologic correlation with CD34, isolectin, and Hoechst immunolabeling on 4 µm slices across the center of the tumor, matching slice orientation to the MR images. Vascular counting was performed using confocal microscopy and optical imaging software (Volocity). ^{13}C-tert-butanol perfusion maps were normalized to total integrated flow across the great vessels so that comparisons could be made across different tumors.

Results: ^{13}C-tert-butanol was more sensitive than ASL for low-perfusion areas (Figure panels b and c); 18 % of ROIs demonstrated perfusion with ^{13}C-tert-butanol but not ASL. This was particularly true in treatment-resistant tumors with minimal flow, which causes poor correlation between the two MR methods for this subgroup (R^2 0.02). ASL and ^{13}C-tert-butanol demonstrated improved correlation in greater-perfused control tumors (R^2 0.5, blue graph). The lower limit of perfusion detection with ^{13}C-tert-butanol corresponds to a vascular density of ~20 per low-field (~1^2 mm) view (red graph).

Discussion: Quantitative MR-based perfusion imaging is valuable for *in-vivo* assessment of tumor dynamics and treatment response. ^{13}C-tert-butanol is attractive because it is freely diffusible, provides high SNR, and possesses a low toxicity profile. Here we show quantitative *in vivo* perfusion measurements with ^{13}C-tert-butanol correlating to ASL and vascular density measurements, with relatively greater sensitivity in lower-flow, early-resistant tumors. This, alongside metabolic markers such as ^{13}C-pyruvate, can provide more accurate detection of treatment response and resistance.

Conclusion: ^{13}C-tert-butanol allows high-sensitivity quantitative *in-vivo* perfusion maps, even in poor-flow states.

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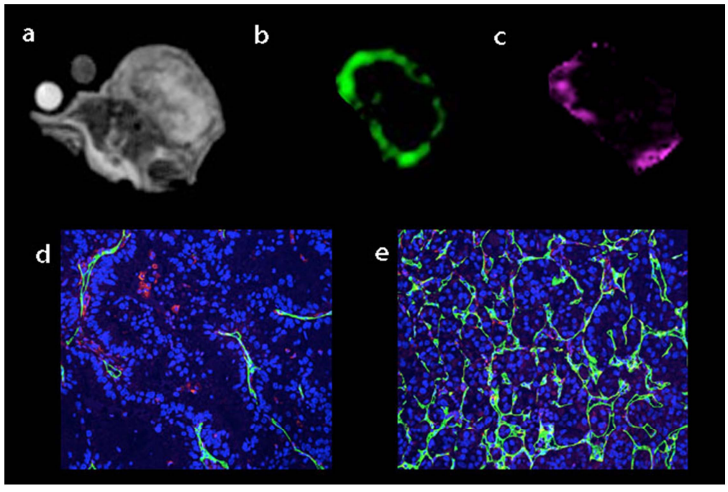
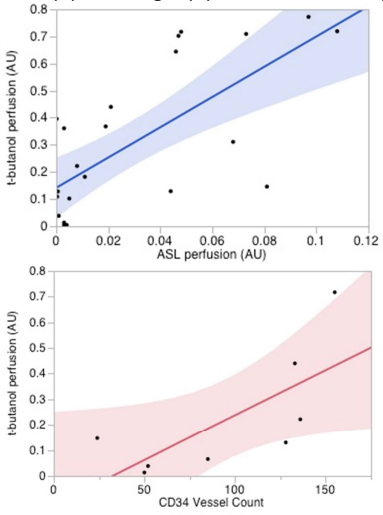
Figure: Proton image (a) of an RCC tumor resistant to sunitinib. Perfusion demonstrated by ^{13}C-tert-butanol (b) and ASL (c). Sample confocal microscopic images from the harvested tumor demonstrate low (d) and high (e) vascular density, denoted by green CD34 labeling, matching areas of low and high perfusion as seen on imaging. The blue graph depicts scatter plot and fit between ASL and t-butanol measurements. The red graph shows t-butanol perfusion measurements against vascular counts in a control tumor.





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