Monitoring changes in tumor perfusion and metabolism following anti-angiogenic therapy using hyperpolarized tracers

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Introduction: The signal enhancements provided by hyperpolarization present new opportunities for imaging of perfusion and metabolism. Recently we demonstrated the use of hyperpolarized tert-butanol as a freely diffusible contrast agent for perfusion-weighted imaging in the rat brain [1]. This agent can be used in combination with pyruvate to study changes in tumor metabolism and perfusion resulting from targeted therapies [2]. In particular, antiangiogenic agents targeted at VEGF can greatly decrease perfusion and vascular permeability [3]. In such therapies, the much lower delivery of pyruvate to the tumor may introduce systematic uncertainties in metabolic studies. Here we describe the use of tert-butanol in conjunction with pyruvate for monitoring changes in perfusion and metabolism resulting from anti-angiogenic treatment in a model of renal cell carcinoma.

Materials and Methods: **Animal Handling:** Xenograft tumors were prepared by subcutaneous implantation of human renal cancer cells (A498 cell line, ATCC, VA) in athymic nude mice (Charles River Labs, MA). After tumors reached sufficient size, animals underwent imaging with hyperpolarized tert-butanol and pyruvate in two sessions separated by three days. Prior to imaging, mice were anesthetized by inhaled isoflurane in O_2 and a catheter connected to a thin extension tube was inserted in the tail vein. During each session, mice received two contrast injections of 250 microliters each containing butanol and pyruvate separated by at least one hour. Prior to the second imaging session, mice received three daily doses of sunitinib (Pfizer Inc), an anti-angiogenic agent, by gavage at a dose of 53.6mg/kg. All methods were approved by our institutional animal care and use committee. **Contrast preparation:** ^{13}C labeled perdeuterated tert-butanol (Sigma-Aldrich, MO) was combined with protonated glycerol (50/50 v/v) and 21mM FINLAND radical (GE Healthcare, London UK). 1-^{13}C labeled pyruvic acid (Cambridge Isotopes, MA) was combined with 15mM OX063 radical. Samples were polarized at 1.4K and 100mW microwave power in a DNP polarizer (Oxford Hypersense, Oxfordshire UK). Pyruvic acid was polarized for 40 minutes or more, while tert-butanol was polarized for 2 hours or more. Samples were dissolved in saline containing 250mg/l EDTA and, for pyruvic acid, 40mM TRIS and NaOH to obtain neutral pH. The final concentration was 19 mg/ml for tert-butanol and 80mM for pyruvate. **Imaging:** All images were acquired using a Bruker Biospec 4.7T scanner (Bruker Inc., MA) equipped with a pair of nested, linearly polarized, inductively decoupled volume coils: an outer 72mm proton coil and an inner 36mm ^{13}C coil. tert-butanol was imaged using 2D balanced SSFP (bSSFP) with 3.3mm slice, 64x192 (frequency x phase) matrix on a 7x21cm FOV (1.1mm in-plane resolution), TR/TE=4/2ms, 27kHz bandwidth, 40º tip angle. Scanning was initiated immediately prior to contrast administration to enable imaging of the input bolus, and 100 consecutive frames were acquired with 0.77s temporal resolution. Examination with hyperpolarized pyruvate consisted of two scans. Ten seconds after the end of the bolus injection, a single FID was acquired with a non-selective 3º RF pulse to provide a measure of the total hyperpolarized carbon signal. Thirty seconds after the end of the bolus, spectroscopic image data were acquired using 2D CSI on a 10mm slice, 3.5cm field of view with 16x16 matrix size and elliptic k-space sampling for a total scan time of 16s. **Analysis:** Perfusion was quantified by determining the vascular concentration of the tracer, $C_V(t)$, using an ROI placed over a large vessel. Local tissue flow F was then determined from the tissue image intensity $C_T(t)$ by means of the relation $C_T(t) = F\int_0^t e^{-R(t-t')}C_V(t')dt'$, where the decay constant *R* includes effects of T_1 and T_2 decay as well as outflow. For pyruvate data, the total carbon signal was obtained from the RMS signal in the non-selective FID. CSI data were apodized with a 15Hz line-broadening filter, zero-filled to 2048 spectral points, and phased; metabolite signals were determined from line integrals. The reduction in tumor blood flow following treatment was determined. Metabolite signals were summed over the tumors to determine the lactate:pyruvate ratios and the average tumor lactate signal normalized to the total carbon signal.

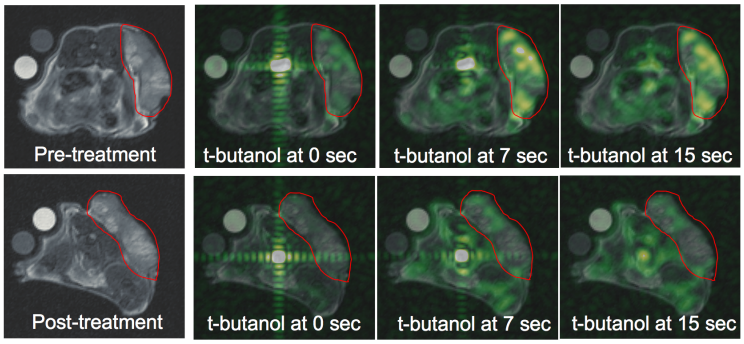

Fig 1: Perfusion-weighted images acquired in tumor A before (top) and after (bottom) treatment. The tumor is outlined in red.

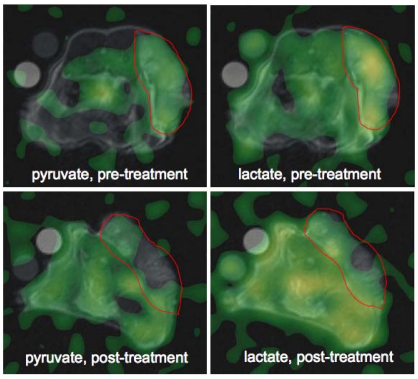

Fig 2: Pyruvate and lactate images acquired pre- (top) and post-treatment (bottom) on a common intensity scale.

Tumor	Change in flow	Change in Lactate:Pyruvate	Change in Lactate:Total Carbon
A	-77%	+22±5 %	-54±2%
B	-64%	-38±15%	+11±15%
C	-66%	+20±24%	-59±4%

Table 1: Changes in flow, lactate:pyruvate ratio, and lactate:total carbon ratio after sunitib treatment. Errors on metabolite signals are statistical errors based on SNR.

Results and Discussion: Figure 1 shows representative perfusion-weighted images of tert-butanol before and after treatment. The prominent intravascular signal rapidly dies away while regions of high flow show persistent high signal. The reduction in tumor blood flow following treatment is readily apparent. Though earlier perfusion studies with ASL previously demonstrated similar decreases in flow, the diffusible and long T1 t-butanol studies help to confirm the flow decrease is not an artifact of transit time or vascular shunts in the short T1 ASL [4]. Figure 2 shows pyruvate and lactate images before and after treatment in the largest tumor. In the post-treatment study the average pyruvate and lactate signals are reduced, presumably because of reduced blood flow. Table 1 summarizes measurements acquired in three tumors. All cases show sizable reductions in flow. However, changes in the lactate:pyruvate ratio show some variability, decreasing by 38% in one case and increasing slightly in the other two cases. The lactate signal normalized by the total carbon signal also varies from case to case, increasing slightly in one case and decreasing by 50-60% in the others. Note that while lactate:total carbon is reduced in tumors A and C, the lactate:pyruvate ratio still increases in these tumors because the pyruvate signal falls by a still larger amount. In summary, these data show that tert-butanol can be used to quantify the effects of anti-angiogenic therapies, while metabolic imaging with pyruvate shows mixed results that may depend upon multiple competing factors including blood flow, tissue oxygenation, and substrate availability.

[1] Grant *et al* MRM 2011 66:746 [2] Day *et al* Nature Med 2007 13:1382 [3] Morgan *et al*, J. Clin. Oncol. 2003 21:3655 [4] Schor-Bardach *et al* Radiololgy 2009 251:731.