Investigating tumor perfusion and metabolism using multiple hyperpolarized ^{13}C compounds: HP001, urea, and pyruvate

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Introduction: Preliminary studies of the metabolically inactive hyperpolarized ^{13}C compounds HP001 (*bis*-1,1-(hydroxymethyl)-[1-^{13}C]cyclopropane-d_8) and [^{13}C]urea have demonstrated feasibility for perfusion imaging (1,2). The addition of perfusion information to the metabolic information available from spectroscopic imaging of [1-^{13}C]pyruvate would be of great value in exploring perfusion and metabolism anomalies in cancer (3). This study describes our initial data from dynamic perfusion imaging of HP001 (a specialized HP perfusion agent with very long T_1 of 95 sec *ex vivo* and good polarization) in normal and cancerous murine models, interpreted in combination with metabolic data from spectroscopic imaging of pyruvate, with or without co-polarized urea (4) for simultaneous assessment of perfusion, in the same animals.

Methods: *Sample preparation:* Samples were initially prepared as follows: HP001- mixed with water in ratio of 2.78:1 by weight, pyruvate- neat, and urea- dissolved in glycerol to 6.4M. Each sample was then mixed with the trityl radical OX063 (GE Healthcare, Oslo, Norway) and 1.0-1.5 mM Dotarem. For each experiment, a prepared sample was loaded into the 3.35T magnet of the HyperSense polarizer (Oxford Instruments Biotools, Oxford, UK), where it was cooled to 1.3 K and irradiated for ~1 hr, and then rapidly dissolved in ~4.5 mL heated buffer solution (HP001- 1X phosphate buffered saline, samples with pyruvate- NaOH/Tris buffer), resulting in a 80-115 mM solution (HP001- 100 mM, pyruvate- 80 mM, urea- 115 mM) of pH ~7.5. For the co-polarized studies, urea and pyruvate were loaded into the sample cup separately in frozen layers to avoid mixing, by quickly immersing the cup in a liquid nitrogen bath after dispensing each layer. Animals were injected with 350 μL (mice) / 2.4 mL (rats) of the hyperpolarized solution over 12 sec. *Animal experiments:* Four rats and seven mice were imaged in a clinical GE 3T scanner, with custom dual-tuned ^{1}H / ^{13}C volume RF coils. Six of the mice were from transgenic cancer models: three from the transgenic adenocarcinoma of mouse prostate (TRAMP) model (5), and three from a liver tumor model (6). The rest of the animals were normal. Dynamic imaging of HP001 was performed for all animals, using a custom multi-slice pulse sequence employing balanced steady state free precession (bSSFP) with flip angle ramped over time to match T1 and T2 decay, at 10 timepoints every 6 sec. Sequence parameters: matrix= 32x32, slices= 8, spatial resolution= 2.5 mm x 2.5 mm x 6 mm (0.038 cm^3), TE / TR = 6ms / 12ms. Metabolic imaging of [1-^{13}C]pyruvate was also performed in the mice, using 3D echo planar spectroscopic imaging (EPSI) with adiabatic double spin echo preparation and compressed sensing for fourfold resolution enhancement (7). Sequence parameters: matrix= 16 x 16 x 16, spatial resolution= 2.5mm x 2.5mm x 5.4mm (0.034 cm^3), TE / TR = 140 ms / 215 ms, spectral bandwidth= 581 Hz, covering lactate to pyruvate at 3T, scan duration= 16 sec, acquisition timing= 30 sec post-injection in liver tumors, 35 sec in others. In three of the mice, a multi-compound study was performed where [^{13}C]urea was co-polarized and imaged using MRSI along with pyruvate and its metabolic products, folding into the spectral window between lactate and alanine. *Data analysis:* For the dynamic HP001 data, blood flow maps were computed through deconvolution of the dynamic HP001 signal curves (with SVD thresholding at 10%), with arterial input functions (AIF's) defined in the descending aorta. Peak signal maps were also created. For the spectroscopic data, images of pyruvate, lactate, and urea were generated from the spectral peak areas. All data was interpolated to 256x256 in-plane. Regions of interest (ROI's) corresponding to the kidneys, liver, prostate, and any tumors were manually drawn on anatomic T2-weighted FSE images, co-registered to the ^{13}C imaging and spectroscopy data, and regional signals were tabulated. Raw signals were normalized by the percent polarization measured from an aliquot of the dissolved sample in a separate low-field spectrometer.

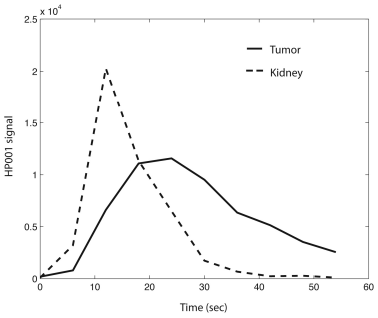

Fig 1. HP001 signal curves for mouse kidney and liver tumor

Results: Among the rats, consistent mean peak HP001 signals (normalized by polarizations 17-31%) and mean blood flows were measured in regions of the kidneys (pk: 138±7, BF: 156±29 in a.u./sec) and liver (pk: 83±22, BF: 47±11). The most prominent feature of the perfusion data from tumor tissues in comparison to normal tissues was greatly increased spatial heterogeneity. For example, the spatial variability for peak HP001 signals in liver tumor tissues was 2.9x greater than normal liver tissue (in terms of the coefficient of variation). Similar to previous studies, high mean lactate-to-pyruvate ratios were detected in the tumors (liver tumors- 3.5, TRAMP's- 0.65). Furthermore, lactate-to-pyruvate ratios were inversely correlated with the peak HP001 signals (correlation coefficient R= -0.67). In the animals with co-polarized urea data, urea was seen to have a very similar distribution to HP001 (especially when comparing the closest timepoint in the dynamic data), and correlation was detected between the urea and peak HP001 data (R= 0.64).

Discussion: The spatial heterogeneity of tumor perfusion is attributable to abnormal neovascularization, and formation of sub-regions of viable tumor, necrosis, and edema within the tumor region. The long relaxation time and high polarization of HP001 make it an ideal perfusion agent, but the use of urea is attractive because it is endogenous with a known safety profile and can be easily imaged simultaneously with pyruvate using existing fast MRSI methods (unlike HP001, which is spectrally separated from pyruvate by ~5 kHz). Correlation between the MRSI urea signals with separate dynamic HP001 imaging shows the value of co-polarizing urea with pyruvate for simultaneous assessment of perfusion and metabolism.

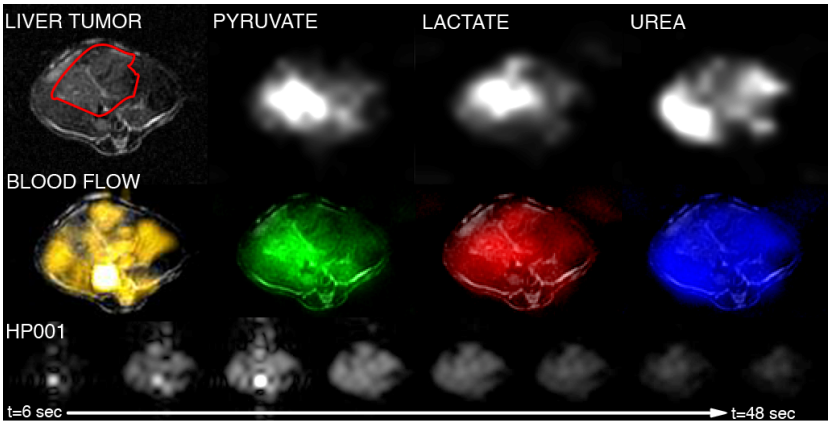

Figure 2. Images of liver tumor perfusion / metabolism with multiple hyperpolarized ^{13}C compounds. Top row: Anatomic ^{1}H image and ^{13}C MRSI images from co-polarized pyruvate / urea study. Middle row: Overlays for ^{13}C MRSI data and blood flow image derived from dynamic HP001 data. Bottom row: dynamic HP001 data.

References- 1. Golman et al. *PNAS.* 2003. 2. Johansson et al. *MRM.* 2004. 3. Mankoff et al. *J Nucl Med*. 2002. 4. Wilson et al. *JMR*. 2010. 5. Greenberg et al. *PNAS.* 2005. 6. Schachaf et al. *Nature*. 2004. 7. Hu et al. *MRM*. 2010.