Metabolic Response of Glioma to Dichloroacetate Measured by Hyperpolarized 13C MRSI

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Introduction: Glioblastoma multiforme (GBM) is one of the most aggressive cancers with less than 1.5 years of median survival even being optimally treated with radiation therapy and concomitant chemotherapy [1]. Unlike normal tissues, which derive the bulk of energy needs via oxidative phosphorylation (OXPHOS) of multiple energy substrates, solid tumors, including glioma, have altered metabolism (known as Warburg effect) that derives disproportionate energy via glycolysis (GLY), even under normal oxygenation [2]. Hyperpolarized ^{13}C magnetic resonance spectroscopy (MRS), using Dynamic Nuclear Polarization (DNP) in combination with a rapid dissolution process to retain high levels of nuclear spin polarization in the liquid state, enables the real-time investigation of *in vivo* metabolism [3, 4]. The shift of metabolism towards GLY in tumors leads to elevated lactate (Lac) labeling in metabolic imaging using hyperpolarized [1-^{13}C]pyruvate (Pyr), a substrate occupying a key nodal point in the glucose metabolic pathway. The PDH-mediated flux from Pyr to acetyl CoA, the first step towards entering the tricarboxylic acid (TCA) cycle, can be indirectly measured through the detection of ^{13}C-bicarbonate (Bic). Whereas the flow of ^{13}C label from Pyr to Bic is readily observed in the heart due to its highly active TCA cycle [5], it has proven difficult to visualize ^{13}C-labeled Bic in other organs well enough for quantitative analysis, in particular in for brain, especially for tumor models [6]. Dichloroacetate (DCA) [7], which promotes mitochondrial function by inhibiting Pyr dehydrogenase kinase (PDK), a PDH inhibitor, increasing the ratio of OXPHOS to GLY, has been shown to reduce tumor growth both *in vitro* and *in vivo* [8, 9], and is currently in clinical trials for GBM, non-small-cell lung cancer, and breast cancer (see clinicaltrials.gov). Grant et al. recently demonstrated that the reduction of Lac in TRAMP after DCA administration could be consistently observed, but Bic signal was only detected post-DCA. In this work, we optimized the acquisition protocol for spiral chemical shift imaging (CSI) [10] to measure both Lac and Bic in transplanted C6 glioma and quantitatively assess the effect of DCA on Pyr metabolism of tumor and normal brain.

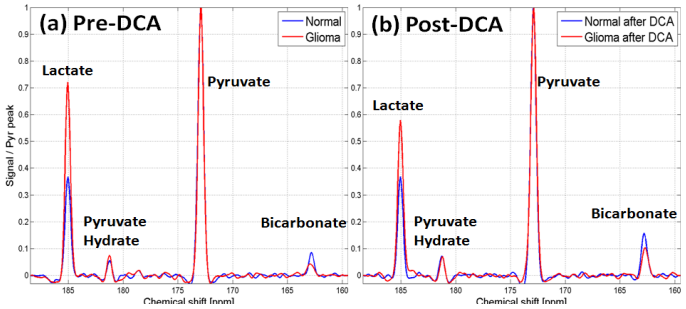

Figure 1: Spectra (a) before and (b) 45 min after DCA infusion from ROIs in glioma and normal brain

Method: All measurements were performed on a clinical 3-T GE MR scanner. A custom-built dual-tuned (^{1}H/^{13}C) quadrature rat coil (Ø=50mm) was used for both RF excitation and signal reception. Approximately 10^6 C6 rat glioma cells, derived from a nitrosourea-induced tumor, were implanted into the brains of adult male Wistar rats (n=5) 10.5 days prior to the ^{13}C experiment. The tumor-bearing rats (204-258 g) and three healthy rats (248-273 g) were anesthetized with 1-3% isoflurane in oxygen (~1.5L/min), and were injected through their tail veins with 2.5-3.0 mL of a 125-mM solution of [1-^{13}C]Pyr polarized using HyperSense DNP (20-25% liquid-state polarization). Each animal received two injections of [1-^{13}C]Pyr; one before and one 45 min after DCA infusion (200 mg/kg body weight dissolved in saline at 30mg/mL). Single time-point MRSI data were acquired following each Pyr injection using spiral CSI with four spatial interleaves (spectral bandwidth = 932.8 Hz, variable flip angle leading up to 90°, FOV=43.5 mm). Slice thickness varied between 6-8 mm depending on the tumor size, and the nominal in-plane resolution was 2.7-mm.

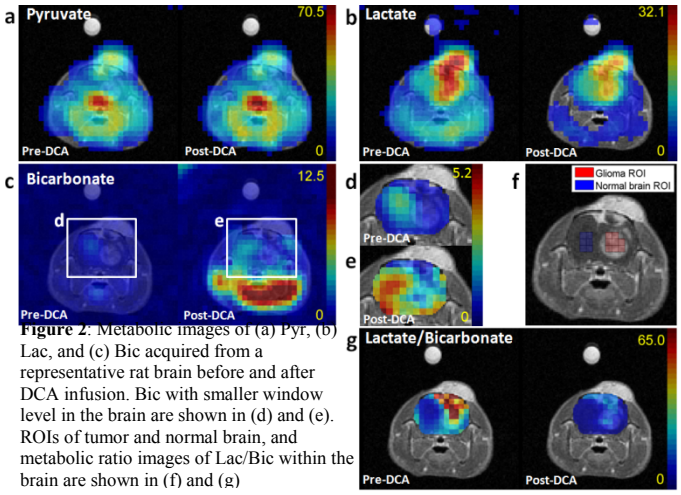

Figure 2: Metabolic images of (a) Pyr, (b) Lac, and (c) Bic acquired from a representative rat brain before and after DCA infusion. Bic with smaller window level in the brain are shown in (d) and (e). ROIs of tumor and normal brain, and metabolic ratio images of Lac/Bic within the brain are shown in (f) and (g)

Results: Fig. 1 shows representative spectra averaged over ROIs in glioma and normal appearing brain, respectively, acquired from an 8-mm brain slice before and after DCA infusion. Metabolic images of Pyr, Lac, and Bic along with Lac/Bic are shown in Fig. 2. The data illustrate the Warburg effect as Lac/Pyr was higher and Bic/Pyr was lower in glioma compared to normal appearing brain. Although the metabolite pattern (Lac/Pyr high, Bic/Pyr low) remained, DCA shifted the tumor metabolism towards normal brain metabolism, indicating a shift from GLY towards OXPHOS. The Lac/Bic was the metric most sensitive in differentiating glioma from normal brain and to assess the DCA effect (Fig. 3).

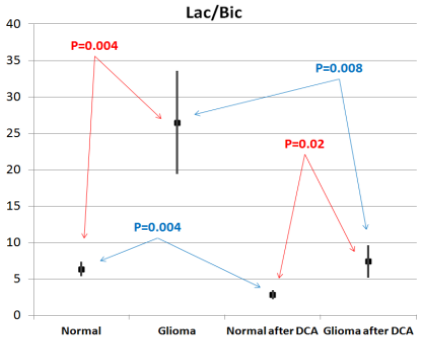

Figure 3: Summary of statistics of Lac/Bic ratios for all glioma rats (n=5)

Discussion & Conclusion: Results clearly demonstrate the feasibility of quantitatively detecting ^{13}C-Bic in tumor-bearing rat brain *in vivo*, permitting the measurement of DCA-modulated changes in PDH flux. The simultaneous detection of both Lac and Bic, representing LDH and PDH activity, respectively, will improve the assessment of DCA as a cancer drug. Lac/Bic appears to be a better metric than Lac/Pyr or Bic/Pyr to differentiate between the metabolism in glioma and normal brain, as it reflects both cytoplasmic and mitochondrial metabolism. Future work will be aimed at increasing the SNR of Bic to a level sufficient for dynamic metabolic imaging, which will enable the calculation of apparent conversion rate constants.

References: [1] Wen PY., et al, *N Engl J Med*. 2008 Aug 21;359(8):877, [2] Gatenby RA., et al, *Nat Rev Cancer*. 2004 Nov;4(11):891-9, [3] Ardenkjær-Larsen, JH., et al, *Proc Natl Acad Sci* 2003;100(18):10158-10163, [4] Golman, K., et al, *Cancer Res*. 2006; 66:10855-10860, [5] Schroeder MA., et al, *Proc Natl Acad Sci* U S A. 2008 Aug 19;105(33):12051-6, [6] Day SE., et al, *Magn Reson Med*. 2011 Feb;65(2):557-63, [7] Stacpoole PW, *Metabolism*. 1989 Nov;38(11):1124-44, [8] Bonnet S. et al, *Cancer Cell* 2007; 11:37, [9] Cao W., et al, *Prostate* 2008; 11:1223, [10] Mayer D, *Magn Reson Med*. 2011 May;65(5):1228-33

Acknowledgements: NIH: RR09784, AA05965, AA018681, AA13521-INIA, and EB009070, DOD: PC100427, Nadia's Gift, The Lucas Foundation, and GE Healthcare