Assessment of Dichloroacetate Effect on TCA Cycle Metabolism in Rat Brain In Vivo using MRSI of Hyperpolarized [2-13C]Pyruvate

Jae Mo Park[1,2], Sonal Josan[2,3], Yi-Fen Yen[4], Ralph E Hurd[4], Daniel M Spielman[1,2], and Dirk Mayer[2,3]

[1]*Electrical Engineering, Stanford University, Stanford, CA - California, United States,* [2]*Radiology, Stanford University, Stanford, CA - California, United States,* [3]*Neuroscience Program, SRI International, Menlo Park, CA - California, United States,* [4]*Applied Science Laboratory, GE Healthcare, Menlo Park, CA - California, United States*

Introduction: Dynamic nuclear polarization (DNP) and the recent development of a dissolution process that retains polarization in the liquid state enables a real-time investigation of *in vivo* metabolism with more than a 10,000-fold signal increase over conventional ^{13}C methods [1]. [1-^{13}C]pyruvate (Pyr) has been regularly used to assess metabolism in healthy and diseased state focusing on the metabolic relation between Pyr and lactate (Lac) [2, 3]. Although both [2-^{13}C]Pyr and [1, 2-^{13}C]Pyr have been successfully used as substrates to assess mitochondrial function in cardiac metabolism by measuring products such as [5-^{13}C]glutamate (Glu), which is generated from the tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate [4, 5], the application of [2-^{13}C]Pyr in studies of brain metabolism has been limited as Lac was the only metabolic product that could be detected so far [6]. This was likely due to the relatively low concentration of the substrate (35mM) used in this study, limiting the amount of Pyr crossing the blood brain barrier. The aim of this work was to exploit the unsaturable component of Pyr transport into the brain by using a high [2-^{13}C]Pyr concentration and measure the effects on brain metabolism when the Pyr dehydrogenase (PDH) flux is up-regulated by dichloroacetate (DCA), a Pyr dehydrogenase kinase (PDK) inhibitor [7].

Method: All measurements were performed on a clinical 3-T GE MR scanner (40 mT/m gradient amplitude, 150 T/m/s slew rate). A custom-built ^{13}C surface coil ($Ø_{inner}$ = 28 mm) operating at 32.1 MHz was used for both RF excitation and signal reception. A proton birdcage coil (Ø = 70mm) was used to acquire proton MRI for anatomical reference. Three healthy male Wistar rats (160-396 g weight) were anesthetized with 1-3% isoflurane in oxygen (~1.5L/min), and were injected through the tail vein with 2.5-3.0 mL of a 125-mM solution of [2-^{13}C]Pyr that had been hyperpolarized using HyperSense DNP (20-25% liquid-state polarization). MRSI data were acquired 25 s after the start of Pyr injection using a single time-point phase-encoded free induction decay chemical shift imaging (FID CSI) sequence (variable flip angle leading up to 90^{o}, TR = 75 ms, FOV = 43.5 mm, 16x16 matrix, spectral bandwidth = 10,000 Hz, 512 spectral points, T_{acq} = 19 s). Slice thickness varied from 6 to 8 mm in order to cover the part of the brain between olfactory bulb and cerebellum. Two injections of [2-^{13}C]Pyr per animal were performed before DCA infusion to increase signal-to-noise ratio (SNR). A dose of 150 mg DCA per kg body weight (dissolved in 30 mg/mL of saline) was administered through the tail vein catheter with 0.5 mL of the DCA solution injected as a bolus and the rest was infused at a rate of 0.1 mL per 3 min. The effect of DCA was examined with two additional hyperpolarized [2-^{13}C]Pyr injections at 45 min and at 2 h from the start of DCA infusion, respectively. Spectra were phase-corrected and metabolite maps were calculated by peak integration.

Result: Spectra averaged over region of interest (ROI) in the brain of a representative rat (8-mm slice) are shown in Fig. 1. To increase the SNR, the data from the two baseline injections and the two post-DCA injections were averaged, respectively. The signal intensities were normalized to [2-^{13}C]Pyr to compensate for polarization differences and inhomogeneity for surface coil sensitivity. Besides the resonances of [2-^{13}C]Pyr (207.8 ppm), Pyr hydrate (97 ppm, in equilibrium with Pyr), and Lac doublet (71.7 ppm), the [5-^{13}C]Glu resonace (184.3 ppm) was detected, reflecting TCA cycle metabolism. Fig. 2 shows the corresponding metabolic images of [2-^{13}C]Pyr and [5-^{13}C]Glu pre- and post-DCA. Due to the large dispersion of the chemical shift of the metabolic products from [2-^{13}C]Pyr, the limited bandwidth of the slice-selective pulse (2.3 kHz) led to considerable chemical shift displacement artifacts. For example, the imaged slice for Pyr was shifted in S/I direction by approximately 1/3 of slice thickness relative to the slice for Glu. Two additional peaks with lower intensities were detected at 181.8 ppm and 174.2 ppm (peak A and B in Fig 1), which most likely correspond to resonances of [1-^{13}C]citrate(Cit), a TCA intermediate, and [1-^{13}C]acetyl carnitine, a metabolite produced from acetyl CoA in fatty acid synthesis. Because of the DCA-induced up-regulated PDH activity, more ^{13}C-labeled acetyl CoA (produced from [2-^{13}C]Pyr) entered the TCA cycle, resulting in increased [5-^{13}C]Glu. Relative to the Glu/Pyr ratio at baseline, Glu/Pyr increased by a factor of 1.31±0.1 (mean±SE, n = 3, P = 0.1) 45 min after DCA infusion, and 1.69±0.08 (P = 0.04) 2h post-DCA. Although the Cit peak was also clearly elevated after DCA infusion, its change could not reliably be quantified due to the low SNR.

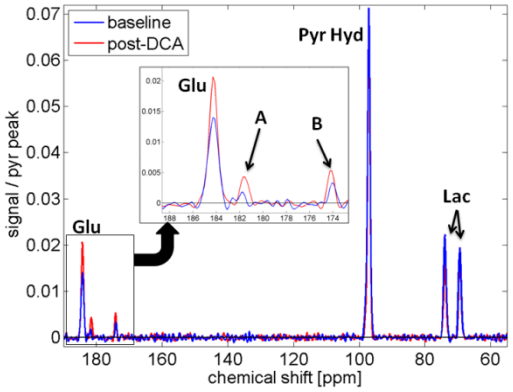

Figure 1: Spectra averaged over brain ROI before (blue line) and after (red) DCA infusion.

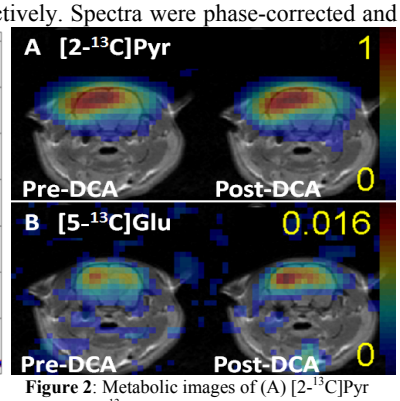

Figure 2: Metabolic images of (A) [2-^{13}C]Pyr and (B) [5-^{13}C]Glu acquired from 8-mm axial slice of a representative rat brain using FID CSI before and after DCA infusion.

Discussion and Conclusion: The presented results demonstrate that hyperpolarized [2-13C]Pyr can be used for the *in vivo* investigation of mitochondrial function and TCA cycle metabolism in brain. Unlike [1-^{13}C]Pyr, where the labeled carbon is eliminated as Bic, [2-^{13}C]Pyr has the advantage that the label is retained when converted to [1-^{13}C]acetyl CoA, which subsequently can enter the TCA cycle. DCA has been reported as a potential drug for cancer treatment, since it promotes suppressed mitochondrial function and lowers Lac in cancers [8, 9]. Therefore, hyperpolarized [2-^{13}C]Pyr is potentially a better substrate to assess the efficacy of DCA as a cancer drug as it can provide a better measure of both Lac dehydrogenase (LDH) activity and TCA cycle metabolism. The chemical shift displacement artifact can be addressed by using 3D CSI acquisition schemes and/or higher bandwidth RF pulses.

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