First Hyperpolarized [2-^{13}C]Pyruvate NMR Studies of Human Brain Metabolism

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Motivation: Dissolution Dynamic Nuclear Polarization (dDNP) provides over 10,000 fold signal enhancement for carbon-13 NMR, enabling an emerging stable-isotope molecular imaging technique for preclinical and recently clinical research studies[1]. Hyperpolarized (HP) ^{13}C-pyruvate NMR metabolic imaging is presently applied to identify tumor metabolism, assess aggressiveness, evaluate treatment response and probe organ function[2,3]. The investigation of HP [1-^{13}C]pyruvate conversion to [1-^{13}C]lactate catalyzed by lactate dehydrogenase (LDH) demonstrated clinical potential detecting the hallmark Warburg Effect in tumors with several-fold upregulated LDH activity in cancer patient Phase I trials[4,5]. In approaching the tricarboxylic acid (TCA) cycle however, [1-^{13}C]pyruvate is enzymatically metabolized by pyruvate dehydrogenase (PDH) yielding HP $^{13}CO_2$ and preventing direct detection of downstream metabolites in the TCA cycle. Prior animal studies using HP pyruvate labeled in the 2-position ([2-^{13}C]pyruvate) have successfully shown direct detection as the HP ^{13}C atoms are metabolized into Acetyl-CoA and then onto the TCA cycle and/or acetyl-carnitine and other molecules[2,3]. Therefore, HP [2-^{13}C]pyruvate provides new metabolic information from its unique position atop anapleurotic and catapleurotic metabolic cascades in the TCA cycle with known fast conversions. The goal of this study was to develop methods for the hyperpolarization and preparation of sterile [2-^{13}C]pyruvate with FDA-IND and IRB approval for first-ever human studies. We sought to investigate HP [2-^{13}C]pyruvate conversion to [2-^{13}C]lactate and [5-^{13}C]glutamate in the healthy brain in four human volunteers, demonstrating a significant advance for HP metabolic *in vivo* NMR to diagnose and detect early stage neurological disorders.

Methods: [2-^{13}C]pyruvate was produced and supplied by MilliporeSigma Isotec Stable Isotopes (Miamisburg, OH) following Good Manufacturing Practices (GMP) for first-ever use in human HP NMR studies. A 5 Tesla GE SPINlab polarizer was used to hyperpolarize [2-^{13}C]pyruvate prior to injection. A 400 µsec hard pulse excitation provided an approximately 2.5 kHz bandwidth with a nominal flip angle of 40° at a center frequency of about 141 ppm, calibrated using a built-in urea phantom on a 32-channel head array receiver. The [2-^{13}C]pyruvate, [5-^{13}C]glutamate and [2-^{13}C]lactate doublet resonances saw 7°, 30°, 5° and 2.1° flip angles respectively. The acquisition used temporal and spectral resolutions of 2 seconds and 2.4 Hz. The 32-channel data was combined using a phase-sensitive summation followed by line broadening of 5 Hz.

Results: HP [2-^{13}C]pyruvate, [2-^{13}C]lactate, [5-^{13}C]glutamate and other metabolites were quantitatively measured in the brain for the first time in four normal volunteers. T1 values for [2-^{13}C]pyruvate known to be shorter than [1-^{13}C]pyruvate were determined from solid-state buildup measurements. Polarization levels were back-calculated from the time of dissolution given exponential T1 decay. In humans, dynamic spectroscopic data was collected and summed yielding kinetic rates and curves. Quantitative post-processing analysis was performed across MestReNova (Santiago de Compostela, Spain) and MATLAB (Natick, MA). Peak identifications were assigned following those by Park et al. from their study of HP [2-^{13}C]pyruvate in the murine brain[6].
Dynamic conversion of HP [2-^{13}C]pyruvate to [2-^{13}C]lactate and [5-^{13}C]glutamate from each volunteer were represented with kinetic traces. Area-under-curve (AUC) metabolite ratios and [2-^{13}C]pyruvate to [2-^{13}C]lactate conversion rates (k_{PL}) from nonlinear models were calculated and found in accordance with prior [1-^{13}C]pyruvate data.

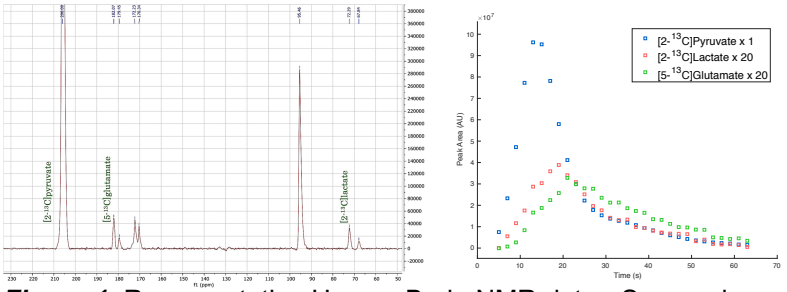

Figure 1. Representative Human Brain NMR data. Summed spectra and dynamic kinetics of [2-^{13}C]pyruvate conversion to [2-^{13}C]lactate and [5-^{13}C]glutamate with temporal resolution = 2 seconds.

Conclusion: We developed methods for the hyperpolarization and preparation of sterile [2-^{13}C]pyruvate with FDA-IND & IRB approval for first-ever human studies. Using a 32-channel ^{13}C-headcoil, NMR was acquired following the injection of HP [2-^{13}C]pyruvate in four human volunteers. We were able to detect the dynamic conversion of HP [2-^{13}C]pyruvate to [2-^{13}C]lactate, [5-^{13}C]glutamate and other compounds in the normal brain, demonstrating a significant advance for HP metabolic imaging to diagnose and detect early stage neurodisorders.

References: [1] Ardenkjaer-Larsen JH., *Proc Natl Acad Sci USA*. (2003); [2] Schroeder MA., *Circ Cardiovasc Imaging*. (2012); [3] Schroeder MA., *FASEB J.* (2009); [4] Albers MJ., *Cancer Res*. (2008); [5] Brindle KM., *Magn Reson Med.* (2011); [6] Park JM., *NMR in Biomedicine* (2013);