Investigation of Hepatic Metabolism of DNP Hyperpolarized 1,4-$^{13}C_2$ Succinate

J. W. Gordon[1], K. Wang[1], S. B. Fain[1,2], and I. J. Rowland[2]
[1]Medical Physics, University of Wisconsin-Madison, Madison, WI, United States, [2]Radiology, University of Wisconsin-Madison, Madison, WI, United States

Introduction: Succinic acid, containing two equivalent carboxylic acid carbons, is a prime molecule for use in hyperpolarization studies. Hyperpolarized succinate has many potential uses as it has been implicated as a paracrine signal for liver damage [1] and succinate dehydrogenase (SDH) has been shown to be a tumor suppressor [2]. Mutations in the SDH enzyme lead to accumulation of intracellular succinate, which inhibit hydroxylases that degrade hypoxia inducible factors (HIF) [3]. HIF is a family of transcription factors that promote angiogenesis and up-regulate glycolysis in tumor cells. A rapid and non-invasive technique of determining the activity of the SDH enzyme could potentially provide a means of investigating tumor malignancy.
Hyperpolarized succinate has been shown to be successfully produced via the PHIP method [4]. With the increasing availability of DNP polarizers, we have investigated ^{13}C labeled succinate polarization using the DNP method. At room temperature, succinic acid is a solid and the pure acid exhibits low solubility in water (0.74M) [5]. Whilst these disadvantages can be obviated by using the sodium salt of succinic acid, additional sodium ions could influence achievable polarization levels. We have explored the optimal succinic acid concentrations able to achieve sufficient concentration and polarization for in-vivo investigations of hepatic metabolism. Additionally, we have investigated succinate metabolism *in-vivo* in rats and in mouse organ homogenates.

Materials & Methods: Sodium bicarbonate and 1,4-$^{13}C_2$ labeled succinic acid (Sigma-Aldrich, St Louis, MO, USA) were dissolved in 80:20 D_2O:DMSO by volume to yield a 3.0M solution of monosodium succinate. A trityl radical ('OX63', (tris(8-carboxy-2,2,6,6,-tetra(methoxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithiole-4-yl)methyl sodium salt, GE Healthcare) was subsequently added to yield a radical concentration of 15mM. Immediately prior to polarization, a 100uL aliquot was drawn and combined with 15uL of 1mM gadobutrol (Gadovist®, Schering AG) to enhance the polarization. This mixture was then inserted into an Oxford Instruments DNP polarizer (HyperSense®, Tubney Woods, Abingdon, Oxfordshire, UK), cooled to a temperature of 1.4K, and irradiated with 100mW, 94.092GHz microwaves. A mixture of 1mL H_2O/3mL perfluorcarbon (FC-3282, 3M, St Paul, MN, USA) was loaded into the polarizer to create a 265mM solution upon dissolution. Sodium hydroxide and tris-buffer were added to the resulting dissolution to neutralize the pH before injection. *In-vitro*, *in-vivo* and *ex-vivo* ^{13}C spectra were acquired using a pulse-acquire sequence with a dual tuned $^{1}H/^{13}C$ surface coil on a Varian 4.7T small animal scanner. A varying TR (0.5-2.0s) was employed to distinguish the effects of T_1 and RF decay on the succinate signal. *In-vivo* imaging was accomplished with a radial sequence (eight images, 16 projections per image, constant transmit power, average 5° flip angle, TR of 86ms, ΔTE of 1.3ms, 64 echoes, 10cm by 10cm FOV, slice thickness of 12cm); projections were acquired with a bit-reversal ordering scheme to minimize artifacts. Hepatic localization was achieved by ensuring that the surface coil was positioned under the liver (confirmed by ^{1}H MRI). Organ homogenates were prepared using either glass or electronic homogenizers.

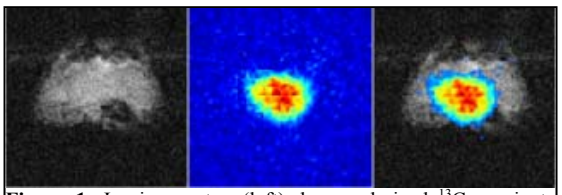

Figure 1: In-vivo proton (left), hyperpolarized ^{13}C succinate (center), and fused (right) images in the liver of a Sprague-Dawley rat. The succinate appears homogenously distributed, while no metabolites are observed. Note that the sensitive volume of carbon is less than that of proton, which is the reason for the smaller FOV of the succinate signal.

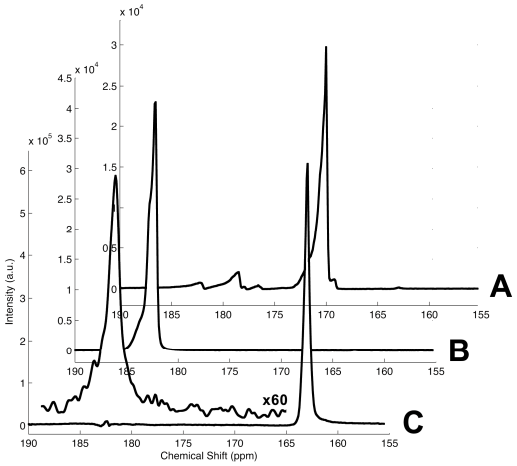

Figure 2: Liver homogenate studied with (A) hyperpolarized pyruvate, (B) hyperpolarized succinate, (C) thermally polarized succinate. The strong signal in C arises from an external urea syringe used for reference. Figure 2A clearly shows strong metabolite signals, while none are observed in either the hyperpolarized succinate or thermal spectrum acquired thereafter.

Results: Solid-state buildup time constants for succinate were 716 ± 61s, allowing for full polarization buildup within 60 minutes. The T_1 relaxation time was measured to be 26s for a 265mM solution in water. *In-vivo* studies revealed that the T_1 of succinate was reduced to ~21s. **Figure 1** is an *in-vivo* image of the biodistribution of succinate in the liver following injection via tail vein cannulation of 0.8 mL of 265mM succinate. No hyperpolarized metabolite signals were observed. Succinate signal distribution within the liver appears to reflect the sensitivity profile of the surface coil and may be interpreted as homogenously distributed. Organ homogenates prepared from heart, kidney or liver also failed to show hyperpolarized or thermally polarized metabolite derived from succinate (**Figure 2b, c**), whereas liver homogenate studies using hyperpolarized 1-^{13}C labeled pyruvate revealed signals attributable to alanine and lactate (**Figure 2a**).

Discussion and Conclusion: We have shown that it is possible to reproducibly generate a concentrated succinate solution that, based on solid state build-up, polarizes to levels similar to that of pyruvate. *In-vivo* and homogenate studies provided succinate resonances with high SNRs. In common with previous studies [4], we did not observe hyperpolarized metabolites within the time-frame of the experiment. Thermal spectra acquired from the homogenate experiments also did not provide evidence for metabolism. However, homogenate experiments utilizing pyruvate clearly showed evidence of enzymatic activity (**Figure 2a**). This may be attributed to the fact that lactate dehydrogenase (LDH) and alanine transaminase (ALT) are non-bound, cytoplasmic enzymes, whereas SDH is bound to the mitochondrial membrane. The process of homogenization may have rendered the SDH enzyme unable to metabolize succinate. While homogenization may have prevented SDH metabolism *ex-vivo*, other factors contributed to the lack of metabolism *in-vivo*, including, for example, slow mitochondrial uptake. Furthermore, it is known that the active site of the SDH enzyme consists of a paramagnetic iron-sulfur cluster [6], potentially able to enhance depolarization. In conclusion, we have demonstrated succinate can be reproducibly polarized in a sufficient concentration for *in-vivo* studies. We have also shown that it is possible to observe pyruvate metabolism in organ homogenates. Further studies are required to determine the value/utility of hyperpolarized ^{13}C succinate.

References: [1] Correa, P. et al., J Hepatol, 2007. **47**(2): p. 262–69. **[2]** King, A., et al., Oncogene, 2006. **25**(34): p. 4675-82. **[3]** Thompson C.B., NEJM, 2009. **360**(8): p. 813-15. **[4]** Bhattacharya, P., et al., JMR, 2007. **186**(1): p. 150–55. **[5]** Rozaini et al., Water Air Soil Pollut, 2009. **198**(1-4): p. 65–75. **[6]** Ohnishi, T., et al., Journal of Biological Chemistry, 1976. **251**(7): p. 2105-09.

Acknowledgements: GE Healthcare, NIH/NIDDK R01 and the Coulter Foundation.