Investigation of Hepatic Metabolism of DNP Hyperpolarized 1,4-\(^{13}\)C\(_2\) Succinate

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Introduction:
Succinic acid, containing two equivalent carboxylic acid groups, is a prime molecule for use in hyperpolarization studies. Hyperpolarized succinate has many potential uses including 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 inhibits 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 [6]. We have explored the optimal succinate concentrations able to achieve sufficient concentration and polarization for in-vivo investigations of hepatic metabolism. Additionally, we have investigated succinate metabolism 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\(_2\)O:DMSO by volume to yield a 3.0M solution of monosodium succinate. A tritryl radical (\(1\)′\,\(\text{O}_{63}\))\(_2\), \(\text{tris}(\text{8-carboxy-2, 6, 6-}
\text{tetra(methoxyethyl)benzo[1,2-d:4,5-d'][\text{tris}(1,3\text{dithiole-4-y})\text{methyl sodium salt}, GE Healthcare) was subsequently dissolved 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 signal. 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 100W, 94.092GHz microwaves. A mixture of 1mL H\(_2\)O: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\(^{\circ}\) flip angle, TR of 86ms, ATE of 1.3ms, 64 echoes, 10cm by 10cm FOV, slice thickness of 12cm); projections were acquired with a bit-reversing 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.

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 homogeneously 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.

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References: