REVIEW

Promising Application of Dynamic Nuclear Polarization for in Vivo $^{13}$C MR Imaging

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Use of hyperpolarized $^{13}$C in magnetic resonance (MR) imaging is a new technique that enhances signal tens of thousands-fold. Recent in vivo animal studies of metabolic imaging that used hyperpolarized $^{13}$C demonstrated its potential in many applications for disease indication, metabolic profiling, and treatment monitoring. We review the basic physics for dynamic nuclear polarization (DNP) and in vivo studies reported in prostate cancer research, hepatocellular carcinoma research, diabetes and cardiac applications, brain metabolism, and treatment response as well as investigations of various DNP $^{13}$C substrates.

Keywords: carbon 13 ($^{13}$C), dynamic nuclear hyperpolarization, metabolic imaging, MRI

Introduction

$^{13}$C nuclear magnetic resonance (NMR) has long been applied in the field of in vitro studies and been a must-have tool for determining chemical structure since the mid-1970s. Recent progress in hyperpolarization and dissolution techniques opened a new opportunity to use $^{13}$C MR spectroscopic imaging for in vivo studies. A $^{13}$C NMR probe is desirable because carbon locates in the center of the molecular bone structure to deliver information about surrounding molecules, and the wide chemical shift of $^{13}$C, over 10-fold that of proton NMR, delivers a better split between resonance peaks. However, the NMR receptivity of $^{13}$C is many orders of magnitude lower than that of $^{1}$H because of the low $^{13}$C natural abundance (1.1%) and small $^{13}$C gyromagnetic ratio (1/4 of protons).

Polarization techniques enable the application of $^{13}$C NMR for in vivo measurements and enhance signal 10,000 fold or more.¹ Their use also overcomes the issue of background signal that accompanies use of nonpolarized substrates. Parahydrogen-induced polarization²,³ and dynamic nuclear polarization (DNP)⁴ methods have been developed for in vivo $^{13}$C imaging. We review the DNP method and its in vivo applications for hyperpolarized $^{13}$C magnetic resonance spectroscopic imaging (MRSI). This promising technology is moving toward human clinical trial in the near future.

Technique

Basic physics of DNP

DNP is a polarization process that combines brute force and microwave pumping methods to transfer a high degree of electron polarization to nuclei. Spin energy level splits (Zeeman effect) when polarized material is placed in a high field and at low temperature (brute force method), and the spin population at different energy Eigen states is described by Boltzman distribution. For spin-1/2 particles, the degree of polarization is calculated as the ratio of the population difference between spin-up and spin-down states to the population sum. At 3 tesla and 1 K, the large difference in polarization of electrons (95%) and $^{13}$C (0.08%) reflects the large difference in their gyromagnetic ratios. At 3T and body temperature (at which hyperpolarized substrates can be safely injected into animals), $^{13}$C polarization is even smaller (0.00025%). However, with the help of microwave pumping and fast dissolution technique, $^{13}$C liquid state polarization of 20 to 30% has been achieved routinely at body temperature. We describe how this can be done following.

DNP involves 3 physical phenomena, Overhaus-
er effect, solid effect, and thermal mixing. The Overhauser effect describes the transition between 2 energy states populated by nuclei of the same spin polarity and electrons of the opposite polarity. With microwave pumping at the frequency that matches the energy difference between the 2 states, energy absorption flips the electron spins but does not change the nuclear spins. This does not help enhance nuclear polarization. The solid effect involves the transitions between the states of opposite electron spins and opposite nuclear spins. Microwave irradiation at the transition frequency changes both the electron and nuclear spins. Because the electron $T_1$ relaxation time is shorter than that of the nuclei, the electron spin relaxes back to the ground state and couples with another nuclear spin, and the solid effect continues to build up the nuclear spin population at the higher energy state. If the density of unpaired electrons is sufficient, the electrons couple with each other (dipolar coupling) as well as nearby nuclei. The electron dipolar coupling splits the energy levels to produce continuous energy bands for the electrons. If the electron splitting energy is greater than the nuclear Zeeman splitting, then microwave irradiation can induce a thermal mixing effect, in which 2 electron spin flips drive one nuclear spin flip. In general, the 3 effects can occur simultaneously during the DNP process. A microwave frequency sweep can reveal the relative contribution of these effects to the hyperpolarization of the substrate.

**DNP for $^{13}$C Imaging**

For $^{13}$C imaging, the polarization mixture typically contains the $^{13}$C-enriched substrate, free radical, gadolinium (Gd), and glassing material. The free radical (Trityl) provides unpaired electrons; an aqueous amount of Gd shortens the electron solid-state $T_1$ relaxation time and, hence, improves the efficiency of nuclear polarization; and glassing material, such as water, glycerol, or dimercaptosuccinate (DMS), ensures that the mixture forms glass at freezing to optimize the efficiency of microwave energy transfer. The mixture is placed inside the polarizer, immersed in liquid helium at a high magnetic field (3 to 5T for most existing $^{13}$C DNP polarizers), and cooled to about 1 K by continuous pumping to reduce helium vapor pressure. Microwave irradiation is applied at the optimal frequency (according to the microwave frequency sweep curve) to transfer the electron polarization to the nuclei. A built-in NMR coil surrounds the polarized sample to monitor the build-up of polarization. Solid-state polarization is typically measured by pulse-and-acquire method with a constant 5° flip angle every 5 min. The length of time for polarization to build up varies depending on the polarized substrate. For example, it typically takes [1-$^{13}$C] pyruvate one hour to reach approximately 98% of the maximum level of solid-state polarization.

**Fast Dissolution**

Several factors need to be considered with regard to dissolution. The pH of the dissolved solution needs to be buffered within an appropriate physiological range before the solution is injected into the animal. In the case of [1-$^{13}$C]pyruvate, the polarized material is [1-$^{13}$C]pyruvic acid, and the solvent of choice is NaOH. Tris is also added to buffer the pH at 7.6, and NaCl may be added to ensure that the dissolved solution is iso-osmotic with blood. At dissolution, the dissolution medium is heated and mixed with the frozen polarized mixture, and the dissolved solution is pushed out of the polarizer and collected into a syringe ready for injection. The dissolved $^{13}$C solution should be near body temperature; increased thermal motion somewhat compromises $^{13}$C polarization in dissolution. However, the very fast dissolution process (about one to 2 s) permits the dissolved $^{13}$C solution to maintain high polarization (about 20 to 30% for [1-$^{13}$C]pyruvate).

**Substrates**

The DNP technique has been used to polarize many $^{13}$C substrates, among them, $^{13}$C-urea, $^{13}$C-acetate, $^{13}$C-lactate, $^{13}$C-bicarbonate, $^{13}$C-ketoisocaproyl, $^{13}$C-fuctose, and $^{13}$C-fumarate. The signal-to-noise ratio (SNR) of the $^{13}$C substrate in vivo depends on the concentration, liquid state polarization, and $T_1$ relaxation time. For metabolic imaging applications, the SNR of the metabolic product is of interest and depends on the product’s $T_1$ relaxation time, size of the metabolite pool, and associated enzymatic activities in vivo. Therefore, some $^{13}$C substrates are more applicable than others for metabolic imaging. We will discuss examples of in vivo applications of several different $^{13}$C substrates following.

**In Vivo Applications**

**Early Development**

Early works regarding DNP $^{13}$C MR were reported in the area of angiography. $^{1,5-9}$ $^{13}$C angiography was obtained using hyperpolarized endogenous substances, such as $^{13}$C-urea or bis-1,1-(hydroxymethyl)-1-(13)C-cyclopropane-D, with SNR ranging from 75 in the carotid arteries to 500 in the cardiac region and subsecond temporal resolutions.
DNP $^{13}$C MR was also applied to assess cerebral perfusion$^{10,11}$ to produce maps of cerebral blood flow, cerebral blood volume, and mean transit time. These hyperpolarized endogenous substances acted as tracers and involved no metabolic conversions.

In 2006, Golman and associates$^{12}$ reported the realization of real-time metabolic MR imaging using hyperpolarized [1-$^{13}$C]pyruvate. Maps of $^{13}$C-pyruvate and its metabolic products, $^{13}$C-lactate and $^{13}$C-alanine, obtained in animals using a 1.5T clinical system demonstrated the technique’s utility for in vivo observation of the reduction and transamination pathways of pyruvate. Subsequently, hyperpolarized $^{13}$C-pyruvate metabolic imaging was demonstrated in vivo using a 3T clinical system.$^{13}$ In addition to lactate and alanine images (Fig. 1), this work obtained $^{13}$C-bicarbonate images that confirmed the technique’s utility for observing oxidative decarboxylation into the tricarboxylic acid (TCA) cycle in a clinical system. For disease indications, findings of elevated $^{13}$C-lactate signal in tumors compared to normal tissue$^{14}$ were consistent with the “Warburg effect.”$^{15}$ In 2006, Mansson’s team described future applications of hyperpolarized $^{13}$C MR in metabolic imaging, angiography, perfusion, and catheter tracking and suggested its powerful potential as a new diagnostic platform.$^{16}$

**Prostate cancer**

Chen’s group$^{17}$ described their initial experience studying hyperpolarized $^{13}$C metabolic imaging of mice with transgenic adenocarcinoma of the prostate (TRAMP) using hyperpolarized $^{13}$C-pyruvate and found highly elevated lactate signal in late-stage prostate tumors (Fig. 2). A double spin-echo flyback echo-planar spectroscopic imaging (EPSI) sequence$^{18}$ was used to obtain voxel resolution of 0.135 cc and 3-dimensional (3D) field of vision (FOV) to cover the prostate, kidney, and liver of a mouse in 10 s. This sequence design decreased acquisition time 16-fold compared to conventional phase-encoding chemical shift imaging (CSI) and enabled collection of a large 3D matrix within a short scan time against the $T_1$ relaxation of the hyperpolarized signal.

Applying the above methodology and using a 3D EPSI sequence,$^{18}$ Albers and colleagues$^{19}$ compared hyperpolarized $^{13}$C metabolic imaging of prostate cancer with histology in normal mice and those with TRAMP of various histologic grades. They chose the acquisition time window to coincide with the maximum lactate production determined from dynamic scans and obtained 3D images of $^{13}$C-pyruvate, $^{13}$C-lactate, and $^{13}$C-alanine in 14 s. The level of lactate signal increased with tumor progression and correlated strongly with histologic grade (Fig. 3). Increase in total $^{13}$C signal with tumor grade may have indicated increased substrate uptake with tumor progression. Nelson’s team reviewed applications for prostate cancer.$^{20}$

A Phase I–II clinical trial of hyperpolarized $^{13}$C-pyruvate metabolic imaging in patients with prostate cancer is underway at the University of California in San Francisco. Patients in the trial are on a watch list for surgery and have regular diagnostic follow-up with anatomic and diffusion MR imaging and proton MR spectroscopy. No adverse effect has been reported in the dose-escalation study. The trial’s success will open a new era for clinical research using this novel technique.

**Liver metabolism and hepatocellular carcinoma**

Hu and colleagues$^{21}$ used [1-$^{13}$C]pyruvate to study liver metabolism in fasted rats and found higher lactate-to-alanine signal ratios and lower alanine signal level in the livers of fasted rats than free-fed rats. The low alanine signal is attributed to reduced alanine aminotransferase (ALT) activity in the fasted rat liver during gluconeogenesis. The study suggested that use of a hyperpolarized molecular probe to monitor changes in localized $^{13}$C-alanine distribution can be a more specific assay than a serum ALT test, which can be complicated by high ALT levels that result from non-hepatic causes. Alanine is also a good biomarker for detecting hepatocellular carcinoma (HCC). Using hyperpolarized [1-$^{13}$C]pyruvate, Darpolor and associates$^{22}$ showed elevated alanine and lactate levels that were consistent with enzyme expression analysis on rat HCC tissue extract (Fig. 4). Interestingly, $^{13}$C MRSI showed high alanine signals specifically in HCC tumors but high lactate signals in tumors and vessels. Low $^{13}$C-alanine signals in vessels may be due to the much slower transport of alanine than lactate from cells to blood. Thus, within the one-minute $^{13}$C acquisition window, $^{13}$C-alanine signal was observed minimally in vessels but more extensively in HCC tumors. This is a promising technique for diagnosing liver cancer and monitoring treatment.

Yen’s group$^{23}$ found a large difference in transverse relaxation time ($T_2$) between HCC tumors and normal livers. They injected hyperpolarized [1-$^{13}$C]pyruvate into the liver, selectively excited a single voxel of $1.1 \times 1.2 \times 1.2$ cm in the liver, and acquired the transverse hyperpolarized signal using Carr-Purcell-Meibloom-Gill sequence. The $^{13}$C-alanine $T_2$ of HCC tumor (1.2 ±0.1 s) was longer than
Fig. 1. Hyperpolarized $^{13}$C metabolic spectroscopic imaging of a rat with 5-mm spatial resolution, 20-Hz spectral resolution and 17-s scan time. Figure courtesy of the authors in (13).

Fig. 2. Hyperpolarized $^{13}$C spectroscopic imaging of a 10-s acquisition from a mouse with transgenic adenocarcinoma of the prostate (TRAMP). The prostate tumor has elevated $^{13}$C-lactate signal. Figure provided courtesy of authors in (17).

Fig. 3. Elevated $^{13}$C-lactate signals in different stages of transgenic adenocarcinomas of the prostate in mice (TRAMP) correlate well with histology. Figure provided courtesy of authors in (19).

Fig. 4. Hyperpolarized $^{13}$C magnetic resonance (MR) imaging of pyruvate metabolism in hepatocellular carcinoma (HCC). Elevated $^{13}$C-alanine and -lactate signals in tumors are consistent with the upregulated alanine aminotransferase (ALT) and lactate dehydrogenase-A (LDH-A) enzyme expression in tumors. Figure courtesy of authors in (22).

that of normal liver ($0.38 \pm 0.05$ s), with $P < 3 \times 10^{-5}$, and the $^{13}$C-lactate $T_2$ of HCC tumor ($0.9 \pm 0.2$ s) was longer than that of normal liver ($0.52 \pm 0.03$ s), with $P < 2 \times 10^{-3}$. This may be related to tumor cell morphology, change in iron content, and/or leaky vessels in the fast growing tumors. The large difference in $T_2$ provides an opportunity to develop novel sequence strategies for enhancing image contrast and improving HCC detection.

Diabetes and cardiac applications

Hyperpolarized $^{13}$C-pyruvate has been used to characterize in vivo cardiac metabolism in rats$^{24}$ and pigs.$^{25,26}$ To assess flux through the pyruvate dehydrogenase (PDH) enzyme complex in the heart, Schroeder's group$^{24}$ compared $^{13}$C-bicarbonate production in control rats to that in fasted and diabetic rats following injection of hyperpolarized $^{13}$C-pyruvate and observed reduced $^{13}$C-bicarbonate in the hearts of fasted and diabetic rats, with bicarbonate production negatively correlated with severity of diabetes.

Golman and associates$^{25}$ performed spectroscopic imaging during ischemic episodes to monitor pyruvate metabolism in the heart. They tested 2 ischemic pig models—one group with the artery occluded for 15 min and the other with the artery occluded for 45 min followed by reperfusion in both groups and observed a small reduction of $^{13}$C-bicarbonate in the affected myocardium after 15-min occlusion and its almost complete disappearance in the infarcted myocardium after 45-min occlusion. A single-slice $^{13}$C chemical shift image of the left
ventricle was acquired in this study, with in-plane resolution, 7.5 mm; slice thickness, 20 mm; and total scan time, 13.4 s.

Volume coverage and speed of cardiac imaging have greatly improved recently. Lau’s team used spectral-spatial excitations and cardiac-gated spiral acquisitions to obtain $^{13}$C-pyruvate, $^{13}$C-lactate, and $^{13}$C-bicarbonate images of the whole heart in a single breath-hold. They acquired 6 10-mm slices with in-plane resolution of 8.8 mm in 6 s and repeated the scan to collect dynamic data. With advanced sequence designs, hyperpolarized $^{13}$C MR is rapidly progressing into real-time dynamic metabolic profile mapping, a promising tool to study cardiac and diabetic metabolism.

**Brain metabolism and glioma**

Park and associates assessed the potential use of hyperpolarized $^{13}$C-pyruvate for glioma prognosis in rat models. The signal levels of $^{13}$C-pyruvate and its metabolic product, $^{13}$C-lactate, as well as their relative signal ratios were significantly higher in tumors than normal brain. The $^{13}$C-lactate signal elevation correlated to proliferation marker for tumors. The different $^{13}$C metabolic profiles between the 2 models in the study were consistent with their immunohistochemical data. For normal brain, the blood-brain barrier (BBB) restricts the transport of pyruvate into the brain cells. However, large $^{13}$C-pyruvate uptake was observed in gliomas as a result of disruption of the BBB. For potential neurological applications, the blood-brain transport of pyruvate may be a limiting factor.

In a range-finding study, Hurd and colleagues demonstrated an alternative approach using [1-$^{13}$C]ethyl pyruvate (EP), a lipophilic analog of pyruvate expected to have faster transport than pyruvate across the BBB. They found significantly higher total carbon delivered to the brain for EP than pyruvate, but the lactate level was comparable to that with pyruvate injections. This may be due to the saturation in lactate production, with a much higher concentration with injected pyruvate than in the natural physiological condition. Besides fast transport across the BBB, $^{13}$C-EP has the advantage of higher polarization than $^{13}$C-pyruvate. However, the drawbacks of $^{13}$C-EP include the limitation of injection rate due to cardiac side effects and its relatively short T$_1$ relaxation time (45 s in vitro) compared to that of $^{13}$C-pyruvate (60 s in vitro).

In another study, Hurd’s group characterized the injection bolus, BBB transport, and metabolic effects in anesthetized normal rat brain. They developed a kinetic model to separate the metabolites in the cerebral blood volume (CBV) from those in the brain tissue using a hyperpolarized [1-$^{13}$C]pyruvate time-resolved metabolic imaging technique. The study showed the apparent metabolic rate constants, including intracellular transport of pyruvate and lactate dehydrogenase activity, to be 30- to 100-fold higher than the rate of BBB transport. This implies that any pyruvate transported into the brain is observed as lactate and nearly all pyruvate observed is in the CBV. Hyperpolarized $^{13}$C MR has the potential to measure cerebral dynamic and metabolic changes for both focal and diffuse neurological diseases.

**Therapeutic response**

Using hyperpolarized $^{13}$C MR to study response to cancer treatment, Day’s team reported promising findings suggesting the technique’s potential clinical applications in monitoring early response to chemotherapy. They treated lymphoma tumors with etoposide and showed decreased flux between pyruvate and lactate, which they attributed to NAD(H) loss, decreased lactate dehydrogenase (LDH) activity, and reduced lactate concentration in the treated tumors. The decrease in LDH activity was observed only after a long period of etoposide treatment during which the necrotic fraction increased significantly. This study is the benchmark to demonstrate the feasibility of using hyperpolarized $^{13}$C MR to monitor early treatment effect.

Chen and associates reported similar findings using hyperpolarized $^{13}$C-pyruvate in a study of treatment response by TRAMP tumors. The ratio of $^{13}$C-lactate to -pyruvate was reduced in the TRAMP mice that responded to androgen deprivation therapy and unchanged in those that did not respond to therapy.

**Other substrates for cancer detection**

Karlsson and associates used hyperpolarized $^{13}$C-ketoisocaproate (KIC) to study the role of branched chain amino acid metabolism in 2 different tumor models. KIC is metabolized to leucine by the branched chain amino acid transferase (BCAT) enzyme, a biomarker for metastasis in some tumors and a target of proto-oncogene c-myc. Signals of [1-$^{13}$C] leucine were more than 7-fold higher in murine lymphoma than healthy tissue, a finding consistent with analysis of ex vivo BCAT expression. However, in rat mammary adenocarcinoma, BCAT metabolism was not enhanced relative to healthy tissue, and no [1-$^{13}$C] leucine was observed. This demonstrates the possibility of using hyperpolarized $^{13}$C MR for metabolic profiling at the level of a single gene.
Gallagher’s group reported MR imaging of pH in vivo using hyperpolarized \(^{13}\)C-bicarbonate.\(^{33}\) Extracellular pH is known as a biomarker of interstitial fluid volume in conjunction with lactic acid production.\(^{34}\) Nevertheless, though \(^{31}\)P MRS has been used to measure pH,\(^{35}\) the lower sensitivity of the modality limits its application for human studies with appropriate spatial resolution and imaging time window. With the 5-digit signal enhancement afforded by the DNP technique, Gallagher’s group mapped the pH value of murine lymphoma tumor by applying \(^{13}\)C MRSI following an injection of hyperpolarized \(^{13}\)C-bicarbonate. They used the Henderson-Hasselbalch equation to calculate the pH value in each voxel by the relative concentrations of \(^{13}\)C-bicarbonate and its metabolic product, \(^{13}\)CO\(_2\). Slice thickness was 6 mm; FOV, 32 × 32 mm; phase encoding matrix, 16 × 16; and total acquisition time, 5 s. pH was lower in tumor than surrounding healthy tissue.

Schroeder and colleagues\(^{36}\) compared \(^{13}\)C-bicarbonate-based pH measurements with \(^{31}\)P MRS pH measurements in reperfused hearts and hearts of healthy living rats. Depending on carbonic anhydrase (CA) activity, they obtained good agreement between the 2 methods when CA was at normal levels. However, the \(^{13}\)C-based method underestimated the \(^{31}\)P-measured pH in acidosis when CA was inhibited.

Gallagher’s group\(^{37}\) also reported the use of the substrate hyperpolarized \([1,4-^{13}\)C\(_2\)]fumarate in characterizing cancer and monitoring treatment and found the production of \([1,4-^{13}\)C\(_2\)]malate from the labeled fumarate to be a sensitive marker of cellular necrosis. The conversion was 2.4-fold higher in lymphomas treated with etoposide, which had significant levels of tumor cell necrosis, than in untreated tumors. This technique has clinical potential for monitoring early therapeutic response.

Conclusions

Hyperpolarized \(^{13}\)C MR imaging is a novel and promising technique. Its potential in metabolic imaging has been demonstrated in preclinical animal studies for cancer characterization and treatment monitoring as well as cardiac, diabetic, and neurological applications. The technique offers rich research topics in multi-disciplinary areas, such as development of robust MRSI sequences, coil design, efficient acquisition strategies, new polarized substrates, polarization technique for long-lasting \(T1\), and new applications. Success of the on-going clinical trial will mark a major milestone in translating the use of hyperpolarized \(^{13}\)C MR from bench to bedside.

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