Current and Potential Applications of Clinical ^{13}C MR Spectroscopy

In this article, the current and potential clinical roles of ^{13}C magnetic resonance spectroscopy (MRS) and ^{13}C magnetic resonance spectroscopic imaging are presented, with a focus on applications to prostate cancer and hyperpolarized ^{13}C spectroscopic imaging. The advantages of ^{13}C MRS have been its chemical specificity and lack of background signal, with the major disadvantage being its inherently low sensitivity and the subsequent inability to acquire data at a high-enough spatial and temporal resolution to be routinely applicable in the clinic. The approaches to improving the sensitivity of ^{13}C spectroscopy have been to perform proton decoupling and to use endogenous ^{13}C-labeled or enhanced metabolic substrates. With these nominal increases in signal-to-noise ratio, ^{13}C MRS using labeled metabolic substrates has shown diagnostic promise in patients and has been approved by the Food and Drug Administration. The development of technology that applies dynamic nuclear polarization to generate hyperpolarized ^{13}C-labeled metabolic substrates, and the development of a process for delivering them into living subjects, have totally changed the clinical potential of MRS of ^{13}C-labeled metabolic substrates. Preliminary preclinical studies in a model of prostate cancer have demonstrated the potential clinical utility of hyperpolarized ^{13}C MRS.

Key Words: molecular imaging; MRI; ^{13}C; magnetic resonance; spectroscopy

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In contrast to anatomic MRI, which detects changes in the relaxivity or density of bulk tissue water, magnetic resonance spectroscopy (MRS) can noninvasively detect multiple small, endogenous molecular-weight metabolites within cells or extracellular spaces associated with several metabolic pathways that have been shown to significantly change in prostate cancer (*1,2*). The clinical use of spectroscopy as an adjunct to MRI has expanded dramatically over the past several years because of recent technical advances in hardware and software that have improved the spatial and time resolution of spectral data and have resulted in the incorporation of this technology on commercial MRI scanners. However, because of sensitivity and MRI scanner hardware issues, most clinical MRI/MRS studies have involved a combination of MRI and proton magnetic resonance spectroscopic imaging (MRSI) (^1H MRSI) (*1,2*). Hyperpolarized ^{13}C-labeled metabolic substrates have the potential to revolutionize the way we use MRSI for assessing prostate cancer. ^{13}C-labeled substrates polarized via dynamic nuclear polarization (DNP) can provide tens of thousandfold enhancement of the ^{13}C NMR signals of the substrate and its subsequent metabolic products. Because of both the unique chemical properties of the ^{13}C nucleus and the dramatic improvement in spectroscopic sensitivity provided by DNP, the use of hyperpolarized ^{13}C-labeled substrates will potentially allow us to assess, in patients, changes in metabolic fluxes through glycolysis, citric acid cycle, and fatty acid synthesis that significantly change in prostate cancer and are not currently obtainable by ^1H MRSI.

CLINICAL ^{13}C MRS USING ^{13}C-LABELED SUBSTRATES

^{13}C nuclear magnetic resonance (NMR) spectroscopy allows observation of the backbone of organic compounds, yielding specific information about the identity and structure of biologically important compounds. Another advantage is the large chemical shift range for carbon (≈250 ppm), compared with that for protons (≈15 ppm), allowing for improved resolution of metabolites that cannot be resolved in proton NMR spectra of tissue. However, clinical ^{13}C spectroscopy has been limited by its low natural abundance of ^{13}C (1.1%) and its low magnetogyric ratio (γ of ^{13}C is one quarter that of ^1H).

One way to improve the sensitivity of ^{13}C spectroscopy is to perform a technique known as proton (^1H) decoupling. Without proton decoupling, NMR signals from ^{13}C nuclei are split by attached ^1H nuclei into (n + 1) peaks, where n is the number of ^1H nuclei attached to the ^{13}C nucleus. The signal-to-noise ratio of ^{13}C resonances can be significantly increased by eliminating these couplings by irradiating the entire ^1H NMR absorption range (called proton decoupling), consequently collapsing ^{13}C resonances to singlets, with an additional enhancement in ^{13}C signal-to-noise ratio being obtained from a phenomenon known as the nuclear Overhauser effect (*3*).

Another way to improve the sensitivity of ^{13}C MRS is to introduce a ^{13}C label into a metabolic substrate. Replacing the ^{12}C (98.9% natural abundance) isotope with the ^{13}C isotope at a specific carbon or carbons in a metabolic substrate does not affect its biochemistry. After administration in cells, animals, or even humans, the uptake of the ^{13}C-labeled substrate and incorporation of the ^{13}C label into metabolites

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can be monitored without interference from background signals. Despite a low inherent signal-to-noise ratio, ^{13}C MRS using labeled substrates has shown diagnostic promise in patients (*4–7*) but has not been approved by the Food and Drug Administration. Specifically, ^{13}C neurochemical data have contributed to the understanding of Alzheimer's disease, Canavan disease, mitochondrial and hepatic encephalopathy, epilepsy, childhood leukodystrophy, schizophrenia, and normal brain development (*4–7*). Some of these clinical studies have further improved the sensitivity of ^{13}C MRS by applying the high sensitivity of ^{1}H MRS using sophisticated data acquisition schemes that detect only ^{1}H attached to ^{13}C labels within molecules (*4*).

Although in vivo ^{13}C MRS has not been used in studies of patients with prostate cancer, preliminary studies have suggested its clinical potential. In a high-resolution, proton-decoupled natural-abundance ^{13}C NMR spectra of unprocessed human pathology specimens of prostate tumors and adjacent nonneoplastic control tissues, metabolic changes were identified that distinguished prostate cancer from benign prostatic hyperplasia. In particular, the tumors contained larger amounts of triacylglycerols, smaller amounts of citrate, and acidic mucins. It was proposed that the transformation-associated deviations from the normally high amounts of citrate and low amounts of lipids in the prostate were consistent with an alteration in either the concentration or the activity of adenosine triphosphate-citrate lyase in the tumors (*8*). Two subsequent studies attempted to translate ^{13}C NMR studies to the clinic (*9,10*). In the first study, proton-coupled natural-abundance ^{13}C NMR spectra were acquired from a $4 \times 4 \times 4$ cm region of normal human prostate using a depth-resolved surface-coil spectroscopy sequence and a dual-tuned, concentric $^{1}H/^{13}C$ 20-cm-diameter transmit and 7.5-cm-diameter receive surface coils in a clinical 1.5-T MRI scanner (*8*). It was concluded that although citrate could be detected in the natural-abundance ^{13}C NMR spectra of the normal human prostate, more sensitive techniques involving proton decoupling and the use of ^{13}C-labeled citrate precursors may be necessary to attain clinically relevant data (*9*). In the second study, proton-enhanced ^{13}C imaging or spectroscopy was performed on phantoms and ex vivo human prostate specimens using polarization transfer techniques in which the sensitivity of the carbon signal was enhanced by transferring the proton spin order to the attached carbon (*10*).

Certainly, another consideration of using ^{13}C-labeled metabolic substrates in clinical studies is the cost of having these labeled compounds synthesized, particularly since physiologic, rather than tracer, doses need to be administered. The cost for the synthesis of a new ^{13}C-labeled metabolic probe is initially high but dramatically reduces with demand for its production. A good example is the pricing for d-glucose-$^{13}C_6$. Around 15 years ago, the market price for this d-glucose-$^{13}C_6$ was about $500/g. Because of increased demand and improvements in the synthesis, the current pricing for d-glucose-$^{13}C_6$ is less than $100/g.

HYPERPOLARIZED ^{13}C SPECTROSCOPIC IMAGING

The development of technology that applies DNP to generate hyperpolarized ^{13}C agents and a dissolution process that prepares them for injection into living subjects has totally changed the clinical potential of MRS of ^{13}C-labeled substrates. The prototype DNP polarizer that was designed in Malmo, Sweden (*11–13*), has been shown to enhance the signal by more than 10,000-fold for detecting ^{13}C probes of endogenous, nontoxic, nonradioactive substances such as pyruvate and to have the potential for monitoring fluxes through multiple key biochemical pathways such as glycolysis (*14,15*), the citric acid cycle (*16*), and fatty acid synthesis (*17,18*). Pyruvate is ideal for these studies: The signal from C-1 carbon relaxes slowly as a result of its long T_1, and C-1 carbon is at the entry point to several important energy and biosynthesis pathways. Preliminary studies that were performed in a whole-body MRI scanner at 1.5 T in rat

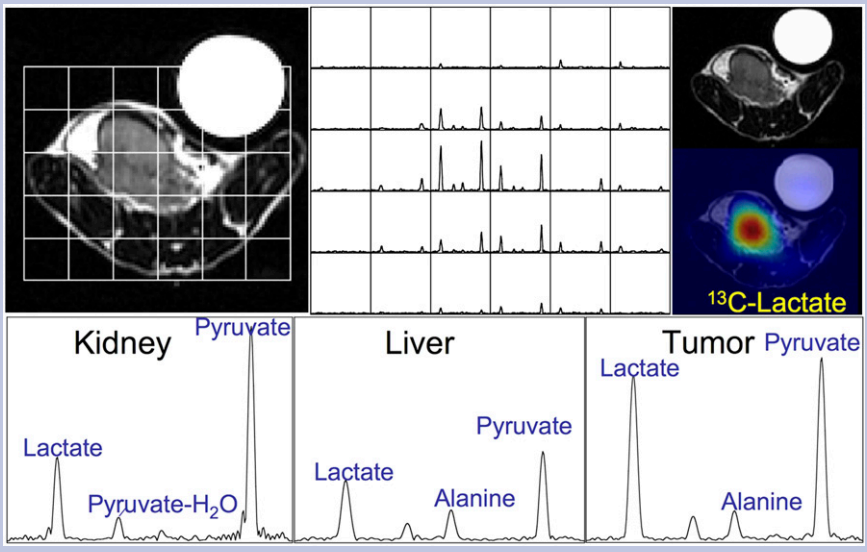

FIGURE 1. Selected spectra from a 3D $8 \times 8 \times 16$ array of ^{13}C MRSI data acquired in 14 s at 3 T demonstrate dramatic increases in ^{13}C lactate signals in a transgenic prostate tumor after injection of ^{13}C pyruvate that was prepolarized in HyperSense system (Oxford Instruments). Lactate SNR was measured to be 59.9 in kidney and 115.9 in tumor.

kidney (*19*) and in tumors (*20,21*) have confirmed that [1-^{13}C]pyruvate is delivered to tissues and converted to alanine, lactate, and bicarbonate with a spatial distribution and time course that varies according to the tissue of interest (Fig. 1).

Although the current commercially available clinical MRI/^{1}H MRSI prostate examination relies on changes in choline, citrate, and polyamine metabolism, lactate and alanine have largely been ignored because of the difficulty of suppressing the large signals from periprostatic lipids, which overlap lactate and alanine. Significantly higher concentrations of lactate and alanine have recently been found in biopsy samples of prostate cancer, compared with biopsy samples of healthy tissue (*22*). High levels of lactate in cancer are also consistent with the findings of prior studies and have been associated with increased glycolysis and cell membrane biosynthesis (*14,23*). ^{18}F-FDG PET studies have shown that rates of glucose uptake in several human cancers are high and that the glucose uptake correlates directly with the aggressiveness of the disease and inversely with the patient's prognosis (*24*). The high glucose uptake leads to increased lactate production in most tumors even though some have sufficient oxygen, a condition known as the Warburg effect (*25*) or aerobic glycolysis (*23*). The increased glycolysis provides the parasitic cancer cells with an energy source, a carbon source for the biosynthesis of cell membranes that begins with lipogenesis (*14*), and an acid source that likely enables the cells to invade neighboring tissue (*23*). The high sensitivity of hyperpolarized ^{13}C MRS and its ability to monitor [1-^{13}C]pyruvate and its metabolic products (lactate and alanine) have the potential to significantly improve the characterization of prostate cancer.

HYPERPOLARIZED ^{13}C MRSI IN A PRECLINICAL MODEL OF PROSTATE CANCER

The first dynamic hyperpolarized ^{13}C spectroscopic imaging studies of prostate cancer were performed at various stages of progression using the transgenic adenocarcinoma of mouse prostate (TRAMP) model (*20*). The ^{13}C metabolic imaging data were acquired using a fast 3-dimensional (3D) MRSI sequence that provided 3D ^{13}C MRSI at a resolution of 0.135 cm^3 in 10 s. Preliminary studies clearly demonstrated the feasibility of obtaining high-spatial-resolution ^{13}C MRSI data with a high signal-to-noise ratio from TRAMP mice by injecting the animals with hyperpolarized [1-^{13}C]pyruvate. Different ^{13}C metabolic characteristics were observed in mouse kidney, liver, and prostate tumor (Fig. 1). Serial ^{13}C 3D MRSI data acquired from the same TRAMP mouse clearly demonstrated that high levels of lactate were produced from hyperpolarized ^{13}C-pyruvate in prostate cancer and that lactate production increased with disease progression (Fig. 2) (*20*). These studies demonstrated the feasibility of the technology to provide noninvasive biomarkers for characterizing prostate cancer tumor aggressiveness at an unprecedented spatial and temporal resolution and the potential for serially monitoring disease progression using hyperpolarized ^{13}C metabolite imaging.

CONCLUSION

It is clear that proton-decoupled ^{13}C MRS using ^{13}C-labeled metabolic substrates has potential in both preclinical and clinical MRS studies on understanding prostate cancer and the diseases of other organs. The advantages of ^{13}C MRS have been its chemical specificity and lack of background signal, with the major disadvantage being its inherent insensitivity and the subsequent inability to acquire data at a high-enough spatial and temporal resolution to be routinely applicable in the clinic. These disadvantages have also meant that MRI scanner manufacturers have not made standard clinical MRI scanners that can perform ^{13}C MRS. The addition of the ability to detect nuclei other than ^{1}H represents an added cost, and the clinical motivation to date has not been adequately demonstrated. The development of technology that applies DNP to generate hyperpolarized ^{13}C agents and a dissolution process that prepares them for injection into living subjects has totally changed the clinical

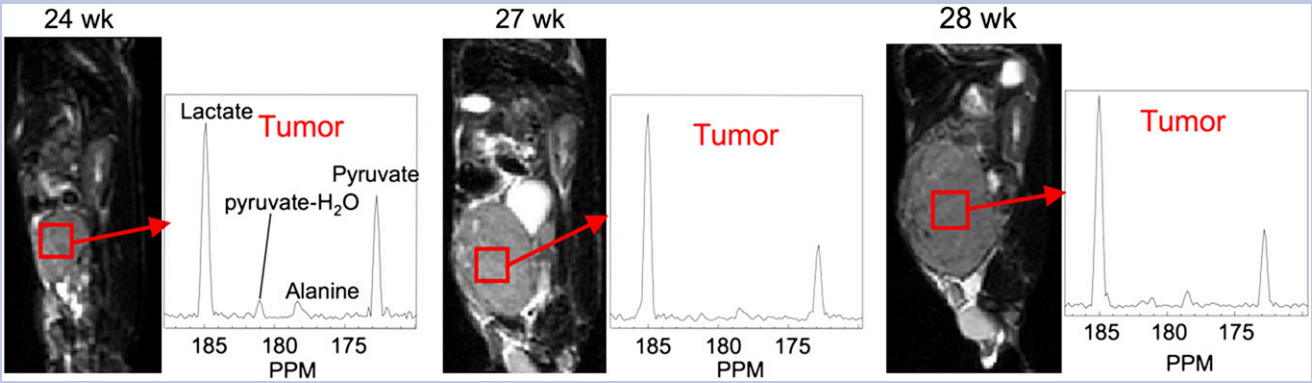

FIGURE 2. Serial studies of same TRAMP mouse demonstrate ability to follow disease progression in this transgenic model of prostate cancer. All ^{13}C MRSI data were acquired in 14 s using 3D double spin-echo flyback echoplanar spectroscopic imaging sequence (*20*) with TR of 215 ms, variable spin echo, and spatial resolution of 0.135 cm^3. This new technique is seen to be feasible for following disease progression and monitoring therapeutic response in preclinical cancer models.

potential of MRS of ^{13}C-labeled metabolic substrates. This excitement has been fueled by the encouraging results of studies involving fast ^{13}C MRSI of prepolarized ^{13}C metabolic substrates in preclinical models of prostate cancer. There clearly remain several technologic hurtles, but the potential is truly amazing.

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