Specialty area: Hyperpolarized C-13 Imaging

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Highlights

- Hyperpolarized ^{13}C-labelled cell substrates can be used to image tissue metabolism.
- This metabolic imaging technique has the potential to translate to the clinic where it could be used to detect disease and response to treatment.

TALK TITLE *Imaging metabolism with hyperpolarized ^{13}C-labelled cell substrates*

This talk will be of interest to basic scientists with an interest in metabolism, and to radiologists with an interest in metabolic imaging.

Objectives

An understanding of how hyperpolarized ^{13}C-labeled substrates can be used to image some aspects of tissue metabolism and the likely applications of this technology in the clinic.

Introduction

Cell metabolites are present at ~10,000x lower concentration than tissue water protons and therefore it is not possible to image them with MR at clinical magnetic field strengths, except at relatively low spatial (1 cm^3) and temporal resolution (5 - 10 minutes) (1). Moreover, single ^{1}H spectra or ^{1}H spectroscopic images of tissue metabolites lack dynamic information about metabolic fluxes. In general if you want to know how something works then you need to see it move. One way to do this is to introduce an isotopically labeled substrate and monitor the kinetics of isotope label migration amongst cell metabolites. This approach was pioneered by Bob Shulman's lab in Yale, beginning in the 1970s, where they used ^{13}C MRS to monitor the uptake and utilization of various ^{13}C-labelled cell substrates (2,3). The technique can be used to follow not only which metabolites become labeled, but also which positions in these molecules are labeled. The technique was further enhanced by introducing more than one ^{13}C-labelled cell substrate and then monitoring multiple labeling of individual cell metabolites through spin-spin coupling of adjacent ^{13}C labels. This technique of isotopomer analysis, which could yield detailed information about relative fluxes through various metabolic pathways, was developed by Dean Sherry and Craig Malloy in Dallas in the 1990s (4). Although the sensitivity of ^{13}C label detection can be enhanced via indirect detection through spin-coupled protons (5) this is still not sufficient to enable imaging of these ^{13}C-labeled cell metabolites. The breakthrough for the field came with the demonstration that dissolution dynamic nuclear polarization (dDNP) of ^{13}C-labelled cell substrates could increase their sensitivity to detection in the solution state MR experiment by more than 10,000x (6). This has made it possible not only to image the location of a hyperpolarized ^{13}C-labelled cell metabolite in the body, following intravenous injection of a hyperpolarized ^{13}C-labelled cell substrate, but more importantly, the kinetics of its conversion into other cell metabolites, with spatial resolutions of 2 – 5 mm and temporal resolutions in the sub second range (7). The Achilles heel of the technique, however, is the relatively short half – life of the polarization *in vivo*, which is typically 20 – 30 s. This limits the number of reactions that can be monitored *in vivo* to those which show fast reaction kinetics, which will usually be catabolic rather than anabolic enzyme catalyzed reactions. The limited lifetime also restricts the number of sequential enzyme catalyzed steps through which a ^{13}C label can be followed, although flux of hyperpolarized ^{13}C label from glucose through all 10 steps of the glycolytic pathway to lactate has been detected (8-11) and imaged (12). In coupled spin systems it is possible to access more long-lived singlet states (13), and the generation

and detection of such a singlet state has been demonstrated for [1,2-^{13}C]pyruvate *in vivo* (14). However, in the case of pyruvate the efficiency of generating the singlet state was low and it did not have a longer lifetime than the polarization at high field. Although this approach has yet to deliver radically extended polarization lifetimes in molecules that would be of immediate interest for metabolic imaging (however see (15)), it remains an important area for research.

For the majority of ^{13}C-labelled substrates that have been used for metabolic imaging hyperpolarization has been achieved using the dDNP technique, which can be used to polarize a wide range of molecules and nuclei (16,17). An alternative method is parahydrogen-induced polarization (PHIP), however, with the exception of succinate (18) and phospho-lactate (19), it has not yet been possible to use this technique to hyperpolarize the commonly used ^{13}C-labelled cell substrates. A potential break through in this regard is the demonstration by Aime and co-workers that the carboxyl carbons of pyruvate and acetate can be polarized by PHIP by using a precursor that contains a hydrogenable functionality (20). Polarization is then transferred to the carboxyl carbons and the moiety cleaved to generate free pyruvate or acetate. In principle this technique could be extended to other carboxylic acids, further extending the molecules that can be polarized using this method.

Metabolic imaging with hyperpolarized ^{13}C-labelled cell substrates has the potential to provide unprecedented insights into tissue metabolism *in vivo*, which could also have important clinical applications. In this short review of the field I do not intend to discuss all the metabolites that have been polarized (there are recent reviews that do that (16,17)) but rather instead to focus on those substrates, which in my opinion, have the potential to provide unique insights in basic science studies and those metabolites with the greatest potential to be used in the clinic.

Pyruvate

[1-^{13}C]pyruvate, which was the first hyperpolarized substrate to be used for metabolic imaging, has a number of important attributes that make it an ideal substrate for dDNP. It readily forms a glass and is soluble at high concentration, the latter being an important attribute due to the dilution that takes place during the dissolution process. The molecule is rapidly transported into cells on the monocarboxylate transporters, where it exchanges label with the endogenous alanine and lactate pools in the near-equilibrium reactions catalyzed by alanine aminotransferase and lactate dehydrogenase respectively. The C-1 label can also be liberated as CO_2 in the irreversible reaction catalyzed by the mitochondrial pyruvate dehydrogenase complex (PDH). Exchange of hyperpolarized ^{13}C label between injected pyruvate and endogenous lactate has been used to grade prostate tumors, more aggressive tumors showed more lactate labeling (21), and to detect early responses to drug treatment (22); in general lactate labeling is decreased in responding tumors (17). The kinetics of label exchange are dependent on pyruvate delivery to the tumor, cell uptake and subsequent exchange in the LDH-catalyzed reaction. Increases in lactate concentration increase the exchange rate by raising the near-equilibrium NADH concentration, which is limiting for LDH activity (22,23). Several kinetic models have been used to analyze the exchange data. The first used fitting of the pyruvate and lactate signal intensities to the modified Bloch equations for simple two-site exchange and ignored the effects of pyruvate delivery (22). In a recent comparison of this simple model with more complex models it was concluded that a simple two-site exchange model is sufficient to determine the kinetics of exchange and even simpler unidirectional models are adequate to determine the kinetics of lactate labeling (24). A simpler approach is to take the ratio of the areas under the lactate and pyruvate signal intensity curves, which was shown to be proportional to the first order rate constant (k_{PL}) describing label flux between the injected pyruvate and the endogenous lactate pool (25). An added advantage of this analysis was that it was found to be independent of the pyruvate

arterial input function (AIF), although direct measurements of the pyruvate AIF have shown that it has little influence on k_{PL} (26). However, while estimates of k_{PL} appear to be robust, the key problem for this technique is that we lack an estimate of the pyruvate concentration in the tissue and therefore a true and meaningful estimate of isotope flux. While measuring a first order rate constant and changes in this rate constant with various interventions may be adequate in preclinical animal studies, where the tissues are effectively identical and the pyruvate concentration delivered to the tissue may be very reproducible, this may be more of a problem in the clinic. Although absolute measurements of flux in the clinic will be difficult it may be sufficient simply to measure changes in the rate constant before and after an intervention, for example after tumor treatment. Hyperpolarized [1-^{13}C]pyruvate has now translated to the clinic with a first clinical trial in prostate cancer, where disease that was not detectable in T_2- or diffusion weighted images was shown to be detectable through increased lactate labeling in the metabolic image (27). This was a remarkable study, not only because it was the first time the experiment had been done in man, demonstrating that there was sufficient signal, but because it was accomplished using an adapted preclinical polarizer that was placed in a clean room adjacent to the clinical scanner. A device that does not require a clean room for operation and which could be used clinically is now available (28). In oncology the most likely applications for this experiment would seem to be in grading tumors and detecting early evidence of treatment response. In this respect it will be competing with PET measurements of the uptake of the glucose analog, ^{18}Fluorodeoxyglucose (FDG), which is already widely used in the clinic. The advantages of the hyperpolarized [1-^{13}C]pyruvate experiment, which appears to be comparably sensitive to the FDG-PET experiment for detecting treatment response (29), is that there is no use of ionizing radiation, and thus it is feasible to use multiple imaging exams to guide treatment, in an image – treat – image – treat paradigm. The technique also works in tumors that are not FDG avid, such as prostate and glioma.

In heart muscle [1-^{13}C]pyruvate can be used to assess flux in the PDH complex through the production of hyperpolarized $^{13}CO_2$ and $H^{13}CO_3^-$, with which the $^{13}CO_2$ is in rapid exchange. In systems where there is sufficient carbonic anhydrase activity and the $^{13}CO_2$ and $H^{13}CO_3^-$ pools reach isotopic equilibrium the $^{13}CO_2/H^{13}CO_3^-$ signal ratio can be used to estimate pH (30,31). PDH flux was shown to be modulated in perfused hearts by the availability of fatty acids (32) and *in vivo* by fasting and the induction of diabetes (33). In pig heart *in vivo* temporary coronary occlusion was shown to lead to a loss of $H^{13}CO_3^-$ production, indicating a decrease in PDH activity, despite restoration of perfusion, as indicated by contrast agent enhanced MRI and by the delivery of pyruvate and subsequent labeling of alanine and lactate (34). Further studies in the pig strongly suggest that this experiment with [1-^{13}C]pyruvate will translate to the clinic (35). Studies with hyperpolarized [2-^{13}C]pyruvate in the perfused heart resulted in detectable labeling of [1-^{13}C]-acetylcarnitine, [5-^{13}C]citrate and [5-^{13}C]glutamate, which was decreased following ischemia and reperfusion (36). However, the low signal intensities observed make it unlikely that this labeled form of pyruvate will translate to the clinic. In the perfused liver the primary fate of hyperpolarized [1-^{13}C]pyruvate was shown to be carboxylation, rather than oxidation by PDH, which resulted in labeling of malate and aspartate. The $H^{13}CO_3^-$ that was observed was produced via the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK) (37). However, *in vivo* production of $H^{13}CO_3^-$ in the liver seems to be predominantly via PDH (17).

Fumarate

Hyperpolarized [1-^{13}C]pyruvate has the potential to show whether a drug has hit its target in a tumor; hyperpolarized [1,4-^{13}C]fumarate can show whether there is any cell death as a result of this. Fumarate crosses the cell membrane only slowly on the timescale of the polarization. However, if the cell is necrotic, such that fumarate can get into the cell rapidly or the enzyme fumarase can leak out, then all that is required is water and fumarase will catalyze the rapid hydration of fumarate to produce malate. Detection of malate, following

injection of hyperpolarized [1,4-^{13}C]fumarate, appears to be a sensitive indicator of necrotic cell death (38) and can detect relatively low levels of diffuse cell death (39). This substrate is also likely to translate to the clinic, where it could be used to detect cell death in tumors post treatment, and potentially also in other tissues where disease is present (40).

Glucose

The FDG-PET experiment measures just three steps; delivery via the circulation, cell uptake on the glucose transporters and phosphorylation and trapping in the reaction catalyzed by hexokinase. Both the glucose transporters and hexokinase activity are often up-regulated in tumors. The polarized [1-^{13}C]pyruvate experiment also measures just three steps, delivery via the circulation, cell uptake on the monocarboxylate transporters and exchange in the reaction catalyzed by LDH. In tumors expression of LDH and the monocarboxylate transporters is also often increased. Combining these two experiments using a PET/MR machine has the potential to examine flux in the entire glycolytic pathway. This can also be achieved by using hyperpolarized [U-^{13}C, U-^{2}H] glucose. Deuteration increases the T_1s of the glucose carbons from <1 s to ~10 s and has allowed measurements of label flux from glucose through to lactate. This experiment, which was first done in *E. coli* (41), yeast (9) and tumor cells *in vitro* (11,42), has also been performed in a tumor model *in vivo* (12), where flux was decreased following treatment. As well as observing lactate, signals from glycolytic intermediates and a pentose phosphate pathway intermediate, 6-phosphogluconate, were also observed. The latter offers the possibility of real time flux measurements in a pathway that is responsible, through the generation of NADPH, for resistance to oxidative stress and which is associated with tumor aggressiveness and resistance to treatment. This substrate can provide unique information about real time pathway fluxes in preclinical experiments. However the relatively short half-life of the polarization will make it challenging to translate to the clinic, unless ways can be found to deliver the labeled glucose more rapidly to the tissue of interest.

Ascorbate and Dehydroascorbate

Ascorbate (AA), which is abundant in tissues, is an endogenous buffer of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, reducing them and in the process being oxidized to dehydroascorbic acid (DHA). Extracellular DHA is transported into cells on the glucose transporters where it is reduced to AA. The C-1 labeled molecules have a long T_1 and polarize well (43,44). Despite tumors having a high ROS load, injection of hyperpolarized [1-^{13}C]AA into tumor bearing animals resulted in no detectable oxidation to DHA in the tumor. However, when [1-^{13}C]DHA was injected into these animals there was rapid reduction of DHA in the tumor to AA, which may partly explain why very little oxidation of AA was observed. The rate of DHA reduction may report on the capacity of the tumor cell to resist oxidative stress. However, DHA results in transient respiratory suppression and therefore it is unlikely that this substrate will translate to the clinic.

Future prospects

While hyperpolarized ^{13}C-labelled cell substrates can be used to make unique and informative measurements in preclinical studies, arguably the true power of this technique lies in its capability to make these measurements in the clinic. With the successful completion of the first clinical trial in prostate cancer there can be little doubt now that the technique will “work” in the sense that it will give detectable and interpretable signals in patients. However, the real question is will it find an important clinical application, which affects clinical decision-making and changes the way we treat patients? This is the challenge for the field over the next 5 – 10 years. With first the two substrates about to enter the clinic ([1-^{13}C]pyruvate is already there and [1,4-^{13}C]fumarate may be next), it is perhaps worth speculating in general terms what these applications might be. Many tissue

pathologies are characterized by ischemia, hypoxia and inflammation, with attendant increases in tissue lactate concentration and LDH activities. Therefore, in principle, the presence of many diseases, and their responses to treatment, could be detected using hyperpolarized [1-^{13}C]pyruvate. The presence of disease is also often associated with cell death, for example tumors frequently host high levels of dead cells. Therefore disease and response to treatment should also be detectable with hyperpolarized [1,4-^{13}C]fumarate. FDG has been a commercial and clinical success, because unlike many PET tracers, it can be used in many different disease settings. Perhaps pyruvate and fumarate will be the "FDG tracers" of the DNP world, applicable for detection and response monitoring in many different diseases.

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