Specialty area: Molecular Imaging

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Highlights

- Imaging metabolism can be used to grade tumors and detect treatment response
- Hyperpolarized ^{13}C-labeled substrates can be used to image unique aspects of tumor cell metabolism

TALK TITLE *How to use hyperpolarized agents to image cancer*

This talk will be of interest to basic scientists with an interest in tumor biology, particularly tumor metabolism, and to radiologists with an interest in tumor therapy and imaging.

Objectives

An understanding of how hyperpolarized ^{13}C-labeled substrates can be used to image tumor cell metabolism and how this can be used to grade tumors and detect treatment response.

Introduction

A better understanding of tumor biology has led to the development of 'targeted therapies', in which a drug is designed to disrupt a specific biochemical pathway important for tumor cell survival or proliferation (1). The problem is that not all patients, with ostensibly the same tumor type, respond equally well to the same drug and therefore the challenge is to match the patient to the drug, selecting those patients that respond and switching the treatment of those that do not. Genome sequencing of tumor biopsies is likely to play an increasingly important role in the selection of the most appropriate drug or combination of drugs for individual patients (2). However, there are some important limitations of this approach. Tumors can be genomically unstable and therefore intrinsically heterogeneous, which could bias the result of any analysis depending on when and where in the tumor the biopsy was taken (3). Moreover, it may not always be possible to biopsy the tumor and its metastases routinely during the course of treatment, when new mutations may arise (2). Therefore, while mutation analysis may be an important first step in patient stratification and therapy selection, imaging is likely to play an important role when treatment is underway; monitoring early regional responses of the primary tumor and its metastases and detecting possible relapse at later time points.

While imaging is likely to play an important role in therapy monitoring in the clinic it is probable that it will be used alongside other non-invasive methods for assessment of treatment response. An important new development in this respect has been the analysis of circulating tumor DNA (ctDNA) in the plasma. This was shown to be capable of detecting response in breast tumors before there were any changes in tumor size, determined by conventional morphological imaging (4). Sequencing of ctDNA has also been used to monitor the evolution of treatment resistance (5). This is a powerful new technology that needs to be evaluated in comparison with more sophisticated imaging measurements of treatment response.

Currently treatment response is still largely assessed by using imaging to monitor reductions in tumor size (6). However this can be a slow method for detecting response, with reductions in tumor size not becoming evident for weeks or even months and in some cases may not occur at all, for example following treatment with anti-angiogenic drugs (7). Imaging tumor metabolism, however, may give a much earlier indication of treatment response. For

example [^{18}F]fluorodeoxyglucose (FDG)-positron emission tomography (PET) measurements of response to Imatinib treatment in patients with gastrointestinal stromal tumors (GIST) showed a marked reduction in glucose uptake following drug treatment, indicating response, despite the fact that the tumors continued to increase in size (8).

Hyperpolarization

Cellular 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) (9). Moreover, single spectra or spectroscopic images of tissue metabolites lack dynamic information about metabolic fluxes. Hyperpolarization of ^{13}C-labelled cell substrates increases their sensitivity to detection in the MR experiment by more than 10,000x (10), making it possible to not only image the location of a hyperpolarized ^{13}C-labelled cell substrate in the body 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 (11). The technique promises unprecedented insights into tissue metabolism *in vivo*, which could have important clinical applications (reviewed in (12-14)).

[1-^{13}C]pyruvate – Has the drug hit its target?

[1-^{13}C]pyruvate has been the most widely used hyperpolarized substrate to date, including for tumor grading (15) and response monitoring (16,17) and was the first to be used in a clinical trial of the technique (18). Given its widespread use it is important to consider what information it can provide about tumor metabolism *in vivo*. Hyperpolarized [1-^{13}C]pyruvate exchanges label with endogenous lactate and alanine, in the reactions catalyzed by lactate dehydrogenase (LDH) and alanine aminotransferase respectively. $^{13}CO_2$ can also be produced in the irreversible oxidative decarboxylation reaction catalyzed by mitochondrial pyruvate dehydrogenase in those tissues with high levels of mitochondrial metabolism, such as the heart (19,20). Low or undetectable levels of $^{13}CO_2$ and $H^{13}CO_3^-$, with which $^{13}CO_2$ is in rapid exchange, have been observed in tumors, see for example (21). The reaction catalyzed by LDH is near-to-equilibrium in the cell and the equilibrium constant is such that when pyruvate enters the cell the reaction will rapidly re-establish chemical equilibrium, with only a very small net conversion of pyruvate into lactate (14,16). What is then observed in ^{13}C MR measurements is predominantly the slower isotopic equilibration of hyperpolarized ^{13}C label between the injected pyruvate and the endogenous lactate pool. Thus hyperpolarized lactate will generally be observed in those tissues that have a large endogenous lactate pool, such as tumors. This explains why the reverse experiment with hyperpolarized [1-^{13}C]lactate has not been so successful (22,23) since endogenous pyruvate is present at much lower concentrations than lactate.

Kinetics of hyperpolarized [1-^{13}C]pyruvate conversion to lactate

The observed rate of hyperpolarized ^{13}C label exchange depends on the rate of pyruvate delivery to the tumor, the rate of pyruvate transport into the cell and the activity of LDH. In lymphoma cells control of the exchange was shown to be shared between the transporter and LDH and this varied according to the lactate and pyruvate concentrations. The observed exchange kinetics for LDH in these lymphoma cells were well described by an ordered ternary complex mechanism, in which the coenzymes NAD+ and NADH (the reduced form of NAD+) bind first (Eqn. 1), and by using rate constants that had been determined previously for the rabbit muscle enzyme using steady state kinetic studies. This analysis showed that the apparent Km of LDH for pyruvate is 13 μM, where Km is the Michaelis constant, defined as the substrate concentration at which the enzyme shows half the maximal velocity (24). The observed Km for pyruvate in cells is higher, depending on the extent to which the transporter limits the exchange (24,25).

$$E + NAD^+ \Leftrightarrow E.NAD^+ + Lactate \Leftrightarrow E.NAD^+.Lactate \Leftrightarrow E.NADH.Pyruvate \Leftrightarrow E.NADH + Pyruvate \Leftrightarrow E + NADH \quad [1]$$

The rate constant describing exchange of hyperpolarized ^{13}C label between pyruvate and lactate (k_P) can be determined by fitting the pyruvate and lactate peak intensities to the modified Bloch equations for two site exchange (16) (Equations 2 – 4).

$$L \underset{k_P}{\overset{k_L}{\rightleftharpoons}} P \quad [2]$$

$$dLz/dt = -\rho_L(L_z - L_\infty) + k_P Pz - k_L Lz \quad [3]$$

$$dPz/dt = -\rho_P(P_z - P_\infty) + k_L Lz - k_P Pz \quad [4]$$

Lz and *Pz* are the z magnetizations of the ^{13}C nucleus in the lactate and pyruvate carboxyl carbons, p_L and p_P are the spin lattice relaxation rates and L_∞ and P_∞ are the equilibrium magnetizations (i.e. at $t = \infty$). In some studies an arterial input function has also been included (26). This simple analysis provides a robust estimate of k_P that is relatively insensitive to the values of p_L or p_P, or to assumptions that may be made about their values in order to facilitate data fitting, for example that $p_L = p_P$ (27). In many studies only a value for the first order rate constant, k_P, has been reported rather than the biochemically relevant flux (in mM s^{-1}) since the latter requires an estimate of the pyruvate concentration in the image voxel. In the future considerable effort will be required to derive robust estimates of concentration and thus flux, particularly in a clinical setting.

Any drug that affects the concentration of LDH or its substrates, or affects the levels of the monocarboxylate transporters (MCTs), which transport pyruvate and lactate across the cell membrane, or affects pyruvate delivery to the tumor (28,29) will have an effect on the kinetics of lactate labeling by hyperpolarized [1-^{13}C]pyruvate. In lymphoma tumors treated with a chemotherapeutic drug, loss of exchange was shown to be due to a number of factors, including loss of NAD(H) and decreases in tumor lactate and LDH concentrations. A small molecule MCT inhibitor has been shown to inhibit the exchange (25), as have drugs that modulate LDH concentration through inhibition of the PI3K-Akt pathway (17,24). The hyperpolarized [1-^{13}C]pyruvate experiment thus offers a novel way of monitoring PI3K-Akt pathway inhibition non-invasively *in vivo*, which has become an important drug target (30). Since the central pathways of metabolism are so highly interconnected, in a so called "scale-free" network, where perturbation of any part of the network is communicated as metabolite changes throughout the network (31), it is likely that the hyperpolarized [1-^{13}C]pyruvate experiment will be exquisitely sensitive to the effects of many different drugs.

Monitoring treatment response with hyperpolarized [1-^{13}C]pyruvate is analogous to monitoring treatment response with FDG-PET and the two methods have been compared directly (32,33). In an etoposide-treated murine lymphoma model the amplitude of the changes in FDG uptake and lactate labeling were comparable suggesting that the two techniques would have comparable sensitivities for detecting treatment response in the clinic (32). If this were the case then what would be the advantage(s) of using the polarized pyruvate experiment? The FDG-PET experiment does not work well in some tumor types; the prostate shows relatively low levels of FDG uptake and high levels in the adjacent bladder make quantitation of the signal more difficult and in the brain high levels of FDG uptake in normal surrounding brain tissue can mask tumor uptake. Both are tumor types where the hyperpolarized pyruvate experiment has been shown to work well (15,34,35).

Hyperpolarized [U-^{2}H, U-^{13}C]glucose

Both the hyperpolarized pyruvate and FDG-PET experiments monitor aspects of the elevated aerobic glycolysis that characterizes tumors. The hyperpolarized pyruvate experiment depends on the rate of vascular delivery, and on the monocarboxylate transporter and LDH activities, whereas the FDG-PET experiment depends on vascular delivery and on the activities of the glucose transporters and hexokinase. The activities of both the transporters and the enzymes are elevated in tumors. However, neither experiment can assess flux through the entire glycolytic pathway. Recently injection of hyperpolarized [U-^{13}C, U-^{2}H]glucose was shown to lead to detectable labeling of tumor lactate *in vivo* and this flux of hyperpolarized ^{13}C label through the glycolytic pathway was markedly decreased at 24 h after treatment with a chemotherapeutic drug (36). Labeled lactate was only observed in tumors, and not in surrounding normal tissue nor in other tissues in the body, not because glycolytic flux is low in these tissues but because they do not accumulate lactate. Measurements of ^{13}C label flux through the entire glycolytic pathway, which had been observed previously in *E. coli* (37), yeast (38) and breast cancer cells *in vitro* (39) was made possible by extending the lifetime of the polarization through deuteration of the glucose molecule. The ^{13}C T_1s increase from ~2 to ~10 s with deuteration.

Hyperpolarized [1,4-^{13}C]fumarate – Has the drug killed any cells?

Polarized pyruvate and glucose-MRS and FDG-PET measurements can indicate whether a drug has hit its target, but not necessarily whether the treatment has killed any tumor cells. A decrease in lactate labeling or FDG uptake might indicate a loss of cells within the tumor, but equally could reflect some metabolic change, for example a decrease in LDH activity through changes in its substrate concentrations, or down-regulation of the glucose transporters at the plasma membrane (32). Since cell death soon after treatment can be a good prognostic indicator for treatment outcome considerable effort has gone into the development of imaging methods that detect cell death more specifically (40). Necrotic cell death can be detected using hyperpolarized [1,4-^{13}C]fumarate (41). In viable cells there is no detectable uptake within the relatively short lifetime of the polarization. However, in dying or necrotic cells, where the plasma membrane permeability barrier has been compromised, there is rapid uptake of labeled fumarate and subsequent hydration, in the reaction catalyzed by the enzyme fumarase, to form hyperpolarized [1,4-^{13}C]malate. A linear correlation between the levels of cell necrosis and the rate of malate formation has been demonstrated in tumor cells *in vitro* and tumors *in vivo* (28,29,41,42). Since fumarase is ubiquitous in biological systems and the only other substrate required is water this could be a general method for detecting cell death *in vivo*. It has the advantage over other methods of detecting cell death in that there is no background. If labeled malate is observed then current evidence suggests that there must be dead cells present within the image voxel. The technique has already been demonstrated in different tumor types and with different types of drugs, including anti-vascular drugs, such as combretastatin A4 phosphate, where measurements of hyperpolarized pyruvate and fumarate metabolism could provide a more sustained and sensitive indicator of response to this vascular disrupting agent than dynamic contrast agent enhanced or diffusion weighted MRI respectively (28). There is also evidence that this technique could be used to detect necrosis in other tissues (43), including the kidney (44).

Concluding remarks

Metabolic imaging with hyperpolarized ^{13}C-labelled cell substrates has, with the first clinical trial of pyruvate in prostate cancer (18), taken its first steps on the road to clinical translation. If the technique is to become widely used in the clinic then we will need to find compelling applications; things which it can do much better than existing imaging technologies, which

provide robust, readily interpretable and clinically meaningful results and at a reasonable cost. It seems likely that finding the important clinical applications for hyperpolarized ^{13}C imaging will only be found through widespread clinical utilization of the technology at multiple research centers and this could take some time, perhaps 10 – 15 years. However, the exciting developments in the pre-clinical arena, which have driven rapid translation of the technique into the clinic, give reason to be optimistic that the technique will have an impact on the practice of radiology in the future.

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