Measuring Tumor Glycolytic Flux in Vivo by Using Fast Deuterium MRI

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Background: Tumor cells frequently show high rates of aerobic glycolysis, which provides the glycolytic intermediates needed for the increased biosynthetic demands of rapid cell growth and proliferation. Existing clinical methods (fluorodeoxyglucose PET and carbon 13 MRI and spectroscopy) do not allow quantitative images of glycolytic flux.

Purpose: To evaluate the use of deuterium (hydrogen 2 [2H]) MR spectroscopic imaging for quantitative mapping of tumor glycolytic flux and to assess response to chemotherapy.

Materials and Methods: A fast three-dimensional 2H MR spectroscopic imaging pulse sequence, with a time resolution of 10 minutes, was used to image glycolytic flux in a murine tumor model after bolus injection of D-[6,6-2H]glucose before and 48 hours after treatment with a chemotherapeutic agent. Tumor lactate labeling, expressed as the lactate-to-water and lactate-to-glucose signal ratios, was also assessed in localized 2H MR spectra. Statistical significance was tested with a one-sided paired t test.

Results: 2H MR spectroscopic imaging showed heterogeneity in glycolytic flux across the tumor and an early decrease in flux following treatment with a chemotherapeutic drug. Spectroscopy measurements on five animals showed a decrease in the lactate-to-water signal ratio, from 0.33 ± 0.10 to 0.089 ± 0.039 (P = .005), and in the lactate-to-glucose ratio, from 0.27 ± 0.12 to 0.12 ± 0.06 (P = .04), following drug treatment.

Conclusion: Rapidly acquired deuterium (hydrogen 2) MR spectroscopic images can provide quantitative and spatially resolved measurements of glycolytic flux in tumors that can be used to assess treatment response.

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Increased glycolytic flux in tumors often results in high levels of uptake of the glucose analog fluorodeoxyglucose (FDG), detectable by using PET, compared with surrounding tissues, and tumors that respond to treatment show decreased uptake (1–4). High levels of tumor lactate resulting from increased glycolytic flux (5–6) have also enabled monitoring of disease grade, progression, and response to treatment by using carbon 13 (13C) MR spectroscopic imaging of hyperpolarized [1-13C]pyruvate metabolism (7–10). The gain in sensitivity afforded by 13C nuclear spin hyperpolarization (range, 105- to 109-fold) (11) has allowed imaging of the exchange of the hyperpolarized 13C label between injected [1-13C]pyruvate and the endogenous tumor lactate pool, where disease progression is often accompanied by an increase in lactate labeling and a positive response to treatment by a decrease. However, both FDG and hyperpolarized [1-13C]pyruvate interrogate only limited aspects of the tumor glycolytic phenotype, and neither technique measures flux through the entire glycolytic pathway from glucose to lactate. This can be important in detecting treatment response; for example, there can be a posttreatment reduction in tumor glycolytic flux in the absence of any significant decrease in FDG uptake (12).

Recently, hydrogen 2 (2H) MR spectroscopy was introduced as a tool for clinical imaging of tumor metabolism in vivo (13). The relatively low sensitivity of 2H to MRI depiction is compensated for by the fast T1 of this nucleus, which permits fast signal averaging without signal saturation. 2H spectroscopic images were acquired from glioma-bearing rats between 60 and 90 minutes after the start of [6,6-2H]glucose infusion and showed signals from labeled lactate and glutamate or glutamine in the tumor, but because images were acquired at only a single point there was no rate information. The deuterated lactate-to-glutamate ratio provides a direct measure of the partitioning of glucose carbon between glycolysis and the tricarboxylic acid (TCA) cycle. In patients with glioma, signals from deuterated lactate and glutamate were observed in tumor images acquired between 65 and 90 minutes after oral administration of [6,6-2H]glucose, demonstrating the clinical applicability of the technique, although again there was no quantitative measurement of metabolic flux.
Abbreviations

HDO = semiheavy water, TCA = tricarboxylic acid, 3D = three-dimensional

Summary

Fast deuterium (hydrogen-2) MR spectroscopic imaging provided quantitative measurements of glycolytic flux in a murine tumor model, which was spatially heterogeneous and decreased rapidly following treatment.

Key Results

- Intravenous injection of D-[6,6'-2H2]glucose followed by non-invasive deuterium MR spectroscopy of lactate labeling depicted a glycolytic flux of 0.99 mM/min ± 0.10 in a murine tumor model.
- Forty-eight hours after treatment with a chemotherapeutic drug, hydrogen-2 (2H) MR spectroscopy depicted a [3,3'-2H2]lactate-to-[6,6'-2H2]glucose signal intensity ratio decrease from 0.27 to 0.12 (P = .04) and the [3,3'-2H2]lactate-to-semiheavy water ratio decreased from 0.33 to 0.089 (P = .005).

Our study describes a fast three-dimensional (3D) 2H MR spectroscopic imaging sequence that allowed us to follow in a murine lymphoma model (EL4), with a time resolution of 10 minutes, the kinetics of [6,6'-2H2]glucose conversion to [3,3'-2H2]lactate after a bolus injection of labeled glucose. Referring to the glucose and lactate 2H signals to the natural abundance 2H signal from water allowed us to produce quantitative maps of tumor glycolytic flux in units of millimolar per minute. The purpose of our study was to evaluate the use of deuterium (2H) MR spectroscopic imaging for quantitative mapping of tumor glycolytic flux and to assess response to chemotherapy.

Materials and Methods

MR Spectroscopy and Imaging in Vivo

Animal procedures were performed with the authority of licenses issued by the UK Home Office and were approved by local ethical review bodies. A 9.4-T 20-cm bore system (Agilent, Palo Alto, Calif) was used with a 14-mm diameter home-built 2H surface transmit-receive coil and a hydrogen 1 (1H) volume transmit-receive coil (Rapid Biomedical, Rimpar, Germany). Animals were anesthetized with 1%–3% isoflurane (IsoFlo; Abbotts Laboratory, Maidenhead, England) in 75% room air and 25% O2 (2 L/min), and body temperature was maintained with warm air. 2H pulse power was calibrated to give, with a repetition time of 260 msec, a maximum steady state natural abundance semiheavy water (HDO) signal. With this repetition time and by using a literature value for the T1 of HDO of 320 msec (13), the effective flip angle is 64°. For spectroscopy, a 7-msec and 64° sinc pulse was used in the presence of a gradient to give a 10-mm slice-selective excitation. Eighty spectra (512 complex points; 256 signal averages; repetition time, 260 msec; and sweep width, 2 kHz) were acquired over 88 minutes. D-[6,6'-2H2] glucose (2 g/kg) (Sigma Aldrich, Gillingham, England) was injected 10 seconds after the acquisition of the first spectrum had finished. Resonances were fitted by using AMARES (14), implemented with a Matlab (Mathworks, Natick, Mass) toolbox (15). Prior knowledge of the relative chemical shifts and linewidths was used to restrict the fitting function. Peak amplitudes were multiplied by a factor that corrected for their different degrees of saturation, assuming the following parameters: T1 of water, 320 msec; T1 of glucose, 64 msec; and T1 of lactate, 297 msec (13). These were then normalized to the estimated labeled water concentration at t = 0, assumed to be 10.12 mM HDO (13). This value was obtained by fitting a polynomial of exponentials [f(t)=A (1 – e−t/θ)+offset], where f is concentration, t is time, A is a scaling factor, θ is the base of the natural logarithm, B is a parameter related to the rate of the increase, and offset is the concentration at t = 0, to the time course of changes in the HDO peak integral. Absolute concentrations were obtained by correcting for the numbers of deuterons per molecule.

A 2H 3D chemical shift imaging sequence was developed, with an adiabatic 50° flip angle, 2-msec B1-insensitive rotation excitation pulse (16) followed by phase-encoding gradients encoding a 9 × 9 × 3 k-space matrix. Signal was acquired into 256 complex points with a bandwidth of 2 kHz and a field of view of 27 × 27 × 27 mm3 to give a nominal spatial resolution of 3 × 3 × 9 mm3. The k-space was sampled over 4328 transients (repetition time, 140 msec; total acquisition time, 10 minutes). The choice of a 10-minute time resolution was on the basis of the spectroscopic data, which showed that this would capture the kinetics of metabolite labeling. The center of k-space was sampled with 128 signal averages, with all other k-space positions sampled with fewer averages according to a Hamming window function (Fig E1 [online]). This reduces the side lobes of the point-spread function and improves the signal-to-noise ratio (17). Sampling order was randomized to avoid sampling the center of k-space during transient metabolite concentration changes. The five-dimensional (three spatial, one spectral, and one temporal) data sets were denoised by using a Tucker decomposition with a rank reduction from 9 × 9 × 3 × 256 × 5 to 5 × 5 × 2 × 16 × 3 by using a Matlab (Mathworks) toolbox (18,19). Rank reduction in the spectral and spatial dimensions produced minimal blurring while removing noise (Figs E2, E3 [online]). The spectra in each voxel were analyzed as described.

Kinetic Analysis of Spectroscopic and Image Data

The kinetics of glucose and lactate labeling were fit to a kinetic model similar to that described previously (20) (Appendix E1 [online]).

Treatment Response

Thirty spectra were acquired before and at 48 hours after treatment with 67 mg/kg etoposide by intraperitoneal injection. Peak amplitude ratios from the summed spectra were compared by using a one-sided paired t test.

2H MR Spectroscopy Measurements of Water and Lactate Labeling in Cell Culture

Deuterium can be lost to solvent water in the pyruvate kinase-catalyzed enolization of pyruvate (21) in the glycolytic pathway...
and in several reactions in the TCA cycle (13,22,23). To investigate the relative contributions of the glycolytic pathway and the TCA cycle to water labeling, TCA cycle flux in EL4 cells growing in media containing 2 g/L D-[6,6'-2H2]glucose was modulated with dichloroacetate or rotenone. After 24 hours the cell suspensions were centrifuged, and the supernatants were used for 2H MR spectroscopy (Appendix E1 [online]).

MR Spectroscopy of Blood Samples
To determine whether labeled lactate and water in the tumor had been produced in the tumor or had been produced in other tissues and washed in via the circulation, blood samples were taken from EL4 tumor-bearing mice (n = 11) that had been injected with 2 g/kg D-[6,6'-2H2]glucose while undergoing isoflurane anesthesia. Blood was taken by cardiac puncture at 20, 40, and 60 minutes after glucose injection (n = 3). Blood from two mice was taken without previous injection of glucose. The concentrations of deuterium-labeled water, glucose, and lactate were measured by using 2H MR spectroscopy and the total concentrations of glucose and lactate, by using 1H MR spectroscopy (Appendix E1 [online]).

Statistical Analysis
Statistical significance in the treatment response study was tested with paired one-sided t tests and in the 2H measurements of cell culture media, with one-sided unpaired t tests. Calculations were performed with statistical software (Matlab version R2017a; Mathworks).

Results

Spectroscopic Measurements of Tumor Glycolysis
Tumor glucose, lactate, and water labeling after intravenous injection of 2 g/kg D-[6,6'-2H2] glucose in a tumor-bearing mouse are shown in Figure 1. Signal was acquired predominantly from the tumor and immediately adjacent tissue by placing the surface coil over the tumor and by using a slice-selective excitation pulse. Individual fitted peak amplitudes were corrected for partial signal saturation and converted into concentrations by referencing to the initial natural abundance HDO peak (13), and by correcting for the number of deuterons per molecule. Glucose washed into the tumor, with the concentration peaking at approximately 10 minutes followed by an increase in the labeled lactate concentration, which peaked at approximately 20 minutes, after which both the glucose and lactate signals declined. The labeled water signal (HDO) increased after an initial delay of approximately 15 minutes with a decrease in the rate of labeled water production after approximately 60 minutes.

Figure 1: Hydrogen 2 (2H) MR spectroscopic measurements of tumor glucose consumption, lactate production, and water labeling in an implanted murine tumor. A Sagittal hydrogen 1 (1H) reference image acquired by using a fast spin-echo sequence [repetition time sec/echo time msec, 2/2; flip angle, 90°; 12 slices; slice thickness, 2 mm; field of view, 42 X 42 mm; and 256 X 256 matrix]. B Sum of 80 2H spectra recorded in 88 minutes after injection of 2 g/kg [6,6'-2H2]glucose 10 seconds after acquisition of the first spectrum. The peaks were fitted individually by using prior knowledge. C Time course of labeled glucose and lactate concentrations and lines showing the best fit to the kinetic model, which was used to estimate the maximum glucose consumption rate [maximum rate of tumor glucose consumption in glycolysis, 0.99 ± 0.10 mM/min]. D Time course of semiheavy water (HDO) labeling resulting from the metabolism of the [6,6'-2H2] glucose.
Measurements of Water Labeling in Vivo and in Vitro

The contribution of TCA cycle flux to water labeling in the tumor was investigated by modulating TCA cycle activity in EL4 cells in culture. Cells were incubated for 24 hours with [6,6\(^{2}\)H\(_2\)]glucose and either dichloroacetate, which increases flux into the TCA cycle, or rotenone, which decreases TCA cycle flux. The concentrations of \(^2\)H-labeled glucose, lactate, and water were then determined by using \(^2\)H MR spectroscopy (Fig 2). At the concentrations used, dichloroacetate and rotenone had little effect on cell proliferation or viability (Appendix E1 [online]). Dichloroacetate decreased the glucose consumption and lactate production rates, whereas rotenone had the reciprocal effect, consistent with their expected effects on TCA cycle flux.

The ratio of the water labeling and glucose consumption rates (Fig 2, G) showed that 6.9% ± 1.8 (standard error of the mean) of the glucose label was lost to water, similar to previous estimates of 8.1% ± 2.3 (13) and 6%–14% (21) for \(^3\)H label loss in glycolysis estimated from an analysis of lactate labeling in brain tumor cells incubated with [6-\(^{13}\)C,6,6\(^{2}\)H\(_2\)]glucose. Dichloroacetate increased the ratio to 8.6% ± 1.5, whereas rotenone decreased it to 2.9% ± 2.0, such that there was a significant (P < .05) difference between the two, implying that at least some of the deuterium label was lost to water in the tumor cell TCA cycle. A similar conclusion could be reached by comparing the water labeling and lactate production rates (Fig 2, H).

The large amount of labeled water observed in the tumors in vivo, however, could not be explained by tumor cell TCA cycle activity. Measurements of the labeled lactate and water concentrations in blood samples in tumor-bearing mice before and at 20, 40, and 60 minutes after injection of 2 g/kg [6,6\(^{2}\)H\(_2\)]glucose (Table E1 [online]) showed that much of the labeled water in the tumors must have washed into the tumor from other tissues in the body rather than...
Figure 3: Dynamic hydrogen 2 (2H) MR spectroscopic imaging measurements of deuterated water, glucose, and lactate concentrations in a tumor-bearing mouse.

A, Axial slices from a reference hydrogen 1 (1H) image acquired by using a gradient echo sequence (repetition time msec/echo time msec, 78/4.3; flip angle, 20°; nine slices; slice thickness, 3 mm; field of view, 54 × 54 mm; 256 × 256 matrix). B, Metabolite concentration maps calculated from three-dimensional (3D) 2H chemical shift images (field of view, 27 × 27 × 27 mm; 9 × 9 × 3 matrix; repetition time, 140 msec; sum of 4328 transients) acquired at the specified times after injection of 2 g/kg [6,6'-2H2]glucose. The color coding represents concentrations (in millimolar) derived from the ratios of the peak amplitudes to the initial semiheavy water map and corrected for the number of 2H labels per molecule and for signal saturation. The concentration maps were calculated from the second of the three axial slices in the 3D data set.

Kinetic Analysis of Lactate Labeling

The glucose and lactate deuterium signals were fit to a kinetic model of glycolysis (20) (Appendix E1, Table E2 [online]). The maximum rate of tumor glucose consumption in glycolysis (hereafter, referred to as $V_{\text{max}}$) was estimated to be 0.99 mM/min ± 0.10, which is similar to a value of 1.57 µmol/g/min (1.43 mM/min assuming 1 mL of tissue weighs 1.1 g) measured in breast cancer xenografts (26). Assuming a cell density in the tumors of $5 \times 10^8$/mL (27), this corresponds to a rate of 1.97 ± 0.20 fmol/min per cell, which is similar to the rates of labeled lactate production (2.16 fmol/min per cell ± 0.16) and glucose consumption (2.7 fmol/min per cell ± 0.18) determined for the
Tumor Glycolytic Flux in Vivo Measured with Fast Deuterium MRI

Figure 4: Treatment of EL4 tumor-bearing mice with etoposide results in a decrease in the production of deuterated lactate from \([6,6'-2H_2]\)glucose. Hydrogen 2 (2H) spectra were acquired before and 48 hours after treatment of the animals with 67 mg/kg etoposide. The spectra were acquired immediately following the injection of 2 g/kg \([6,6'-2H_2]\)glucose by using a 7-msec 64° sinc pulse in combination with a magnetic field gradient to select a 10-mm slice covering the tumor and were the sum of 7680 signal averages acquired with a repetition time of 260 msec in 33 minutes. The 2H surface coil provided additional signal localization. There were significant decreases (\(n = 5\); one-sided paired t test) shown on the box-and-whisker plots, A, in the \([3,3'-2H_2]\)lactate-to–semiheavy water ratio and, B, \([3,3'-2H_2]\)lactate-to–[6,6'-2H_2]glucose ratio at 2 days after treatment. Axial hydrogen 1 (1H) reference images acquired, C, before and, D, after treatment with etoposide by using a gradient-echo sequence (repetition time msec/echo time msec, 78/4.3; flip angle, 20°; nine slices; section thickness, 3 mm; field of view, 54 × 54 × 54 mm; and 256 × 256 matrix). The location of the tumor and the field of view for the 2H images are indicated. Glucose consumption rate maps are shown, calculated from a series of five three-dimensional 2H chemical shift images (field of view, 27 × 27 × 27 mm; 9 × 9 × 3 matrix; repetition time, 140 msec; and number of transients, 4328) acquired immediately after injection of 2 g/kg \([6,6'-2H_2]\)glucose, E; before and, F, after etoposide treatment.

cells in vitro (Fig 2, B, D), and also to published values for human leukemic cells (2.10 ± 3.57 fmol/min per cell) (28).

Dynamic Deuterium MRI

The spectroscopic data (Fig 1) demonstrated that an imaging sequence would need a temporal resolution of approximately 10 minutes to observe the transient changes in labeled metabolite concentrations. We therefore adjusted the trade-off between temporal and spatial resolution of the 3D 2H chemical shift imaging pulse sequence to achieve a 10-minute time resolution. A series of images acquired after injection of labeled glucose, overlaid on the corresponding 1H image, are shown in Figure 3. Application of denoising to the individual 1H images improved their signal-to-noise ratio but was not essential (Figs E2, E3 [online]). Glucose appeared rapidly in the tumor and throughout the body of the animal, concentrating in a region that included the bladder, whereas labeled water and lactate, which showed a similar distribution, also appeared throughout the body of the animal but concentrated in the tumor. The relative uniformity of the early water and lactate images demonstrates the effectiveness of normalizing to the initial HDO signal in compensating for the B1 field gradient of the surface coil.

Imaging Treatment Response

Treatment of tumor-bearing animals with 67 mg/kg etoposide resulted in significant decreases in the tumor lactate-to-HDO signal ratio (0.33 ± 0.10 to 0.089 ± 0.039; \(P = .005\)) and lactate-to-glucose signal ratio (0.27 ± 0.12 to 0.12 ± 0.06, \(P = .04\)) in spectra acquired at 48 hours after treatment (\(n = 5\); pairwise one-sided t test) (Fig 4, A, B). A rate map was created by fitting a series of dynamically acquired images on a voxel-by-voxel basis to the kinetic model described in the Materials and Methods section and in Appendix E1 (online), which showed that glycolytic flux was heterogeneous across the tumor (Fig 4, E). After treatment there was a decrease in flux (Fig 4, F), mirroring the decrease in lactate labeling observed in the spectroscopic data (Fig 4, A, B).
In a recent study, De Feyter et al (13) showed that intravenous [6,6-^{2}H]glucose infusion in a rat glioma model and oral administration in patients with glioma could be combined with hydrogen 2 (^{2}H) imaging to investigate glycolytic and tricarboxylic acid (TCA) cycle activities in glioma. However, because images were acquired at single point, there were no measurements of metabolic flux. Our study showed that by administering ^{1}H-labeled glucose as a bolus and by using a rapid three-dimensional MR spectroscopic imaging sequence we can acquire dynamic images of glucose metabolism with a time resolution of 10 minutes and derive quantitative maps of glycolytic flux from glucose to lactate. These maps showed that glycolytic flux is heterogeneous within the tumor and can be used to provide early evidence of response to a chemotherapeutic drug.

Several studies have used MR spectroscopy measurements of isotopically labeled glucose to investigate glycolytic metabolism in vivo. Infusion of [1-^{13}C]glucose at a similar concentration to that used in our study and acquisition of ^{13}C MR spectra were used to investigate glycolytic flux in an orthotopic breast cancer model (20). These ^{13}C spectra showed higher tumor glucose concentrations and lower lactate production rates than were observed in our study, although there was insufficient signal to acquire images. Estimation of glycolytic flux in this earlier study gave a \( V_{\text{max}} \) of 0.045 mM/min that was much lower than that estimated in our study by using a similar kinetic model (0.99 mM/min ± 0.10), consistent with the higher lactate concentration observed here and similar to a value of 1.43 mM/min measured in another breast cancer model (26). This higher value was also consistent with the rates of lactate production and glucose consumption that we measured in EL4 cells in vitro and to values reported previously for human leukemic cells (28). Hyperpolarized [U-^{2}H, U-^{13}C]glucose has been used to image glycolytic flux in the EL4 tumors used (29). However, the short lifetime of the hyperpolarization (~10 seconds) would make this experiment difficult to implement clinically. Furthermore, it was not possible to make a quantitative estimate of glycolytic flux from these data. More recently, Brender et al (30) showed that dynamic images of tumor glucose consumption and lactate production could be acquired with nonhyperpolarized ^{13}C-labeled glucose by using tensor decomposition, which effectively uses all of the signal collected during 90 minutes of data acquisition, to denoise the images and to generate a time series of individual images. However, again there was no quantitative estimate of the glycolytic rate. Application of a similar denoising technique to the individual ^{2}H images acquired in our study improved their signal-to-noise ratio but was not essential. Borowiak et al (31) injected mice with glucose labeled with oxygen 17 (^{17}O), which is released enzymatically into solvent water, and used ^{17}O MR spectroscopy measurements of water labeling to estimate the apparent cerebral metabolic rate of glucose in mouse brain. In a similar study, Lu et al (23) measured cerebral glucose consumption rate in rat brain by infusing animals with [6,6-^{2}H]glucose and measuring the kinetics of brain glucose, lactate, and water labeling by using ^{2}H MR spectroscopy. Their value for glucose consumption of 0.28 μmol/g/min ± 0.13 or 0.25 mM/min ± 0.12 (assuming 1 mL of tissue weighs 1.1 g) is lower than that measured here, which reflects the predominantly oxidative metabolism of brain and other nonneoplastic tissues. De Feyter et al (13) showed increased lactate labeling and decreased glutamate and glutamine labeling in the tumor compared with normal brain in a rat glioma model and in patients with glioma, which demonstrated the expected increased glycolytic activity and decreased oxidative metabolism in the tumor. The failure to observe glutamate labeling in the lymphomas used in our study can be explained by the much lower oxygen consumption rates and TCA cycle activities compared with healthy brain and glioma cells. The EL4 cell oxygen consumption rate has been measured at 0.0077 fmol/cell/sec (32) compared with 0.03–0.13 fmol/cell/sec in glioma cells (33).

Our study had limitations. We only demonstrated the technique in a single tumor model, which is highly glycolytic. However, the earlier observation of tumor lactate deuterium labeling in a glioma model and in patients with glioma (13) suggested that this quantitative metabolic imaging technique should also work in less glycolytic tissues.

The work of De Feyter et al (13) in patients with glioma has shown that deuterium imaging can be translated to the clinic, although this would require significant modifications to the majority of clinical imagers. Our study added to their work by showing that the technique can provide quantitative measurements of glycolytic flux, which can be used in assessment of tumor treatment response.

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