Magnetic resonance imaging of tumor glycolysis using hyperpolarized ^{13}C-labelled glucose

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Target Audience: Basic scientists and radiologists who have an interest in the application of hyperpolarized ^{13}C-labelled substrates to investigate tissue metabolism *in vivo*.

Purpose

Tumor cells frequently display high rates of aerobic glycolysis, resulting in increased lactate production and correlating with a poor prognosis and increased tumor aggressiveness and metastasis (1). Drugs that target the glycolytic pathway are currently being evaluated (2). Decreased glucose uptake has been shown to be a marker of treatment response (3) and this has been exploited clinically with FDG-PET, which has been used both for tumor detection and monitoring of treatment response (4). However, ^{18}FDG-PET reports only on the delivery of the glucose analog to tumor tissue and its subsequent plasma membrane transport and intracellular phosphorylation by hexokinase. Moreover the use of ionizing radiation limits repeat measurements, in children and women of child-bearing age, and thus its role in guiding treatment. The technique also shows poor contrast in those tumors where there is high glucose uptake in neighboring tissues, such as the brain, in the case of brain tumors, and the bladder, in the case of prostate cancer. We show here that hyperpolarized [U-^{2}H, U-^{13}C]glucose allows real-time monitoring of tumor glycolysis *in vivo*, through the production of hyperpolarized [1-^{13}C]lactate, and that this flux is substantially reduced in murine EL4 lymphoma tumors by 24 h after etoposide treatment. Imaging glucose and its product lactate may have numerous applications, both in basic biological studies and in the clinic, and could provide a new way to detect tumor treatment response in the clinic, which avoids some of the limitations of FDG-PET.

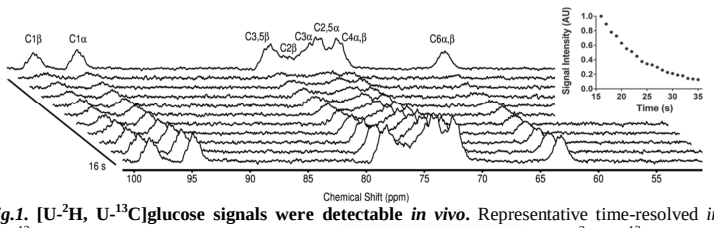

***Fig.1.* [U-^{2}H, U-^{13}C]glucose signals were detectable *in vivo*.** Representative time-resolved *in vivo* ^{13}C spectra acquired 16 s to 36 s after the intravenous injection of HP [U-^{2}H, U-^{13}C]glucose. For clarity, only every other time point is shown. Far spectrum in the stack plot is the sum of the first 20 s of acquisition.

Methods

EL4 tumor-bearing mice (n=6) were imaged before and 24 h after treatment with etoposide (67 mg/kg). [U-^{2}H, U-^{13}C]glucose (3.55 M), the radical OX063 (25.8 mM) and Dotarem (2.6 mM) were dissolved in 50 µl $^{2}H_2O$ and ^{2}H-DMSO (25 µl) was added to ensure glass formation in the solid state. Samples were polarized using a Hypersense polarizer for 120 min before dissolution at 180 °C with 3 ml of $^{2}H_2O$-saline. Experiments were performed in a 7.0-T horizontal bore magnet (Agilent) using a actively decoupled dual-tuned ^{13}C/^{1}H volume transmit coil and a 20-mm ^{13}C receiver coil placed over the tissue of interest. Hyperpolarized [U-^{2}H, U-^{13}C]glucose (100 mM, 0.35 ml) was injected i.v. over 3 s and the animal was then placed inside the magnet bore. Data acquisition started ~15 s after the start of the injection. A series of frequency-selective ^{13}C spectra were collected with 4 spectra collected from the lactate region (1 ms sinc pulse with flip angle 20º) followed by 1 spectrum collected from the glucose region (flip angle 10º). The spectral width was 4 kHz, acquired into 768 complex points, TR 0.2 s and TE 0.8 ms. In one animal, two ^{13}C chemical shift selective images were collected (FOV 40x40 mm, TR 30 ms, TE 1.3 ms, spectral width 6 kHz); the first one from the glucose resonance (8x8) and the second from the lactate resonance (16x16). Images were overlaid on ^{1}H spin-echo reference images (FOV 40x40 mm, data matrix 128x128, TR 1.8 ms, TE 20 ms, slice thickness 2 mm). For each animal, the lactate and glucose spectra were summed separately and phase- and baseline-corrected, using Matlab. The ratios of the signal integrals from lactate (183-187 ppm) and glucose (60-100 ppm) were calculated. For determination of the apparent glucose T_1, average integrals were fitted to a mono-exponential decay equation.

Results and Discussion

Injection of hyperpolarized [U-^{2}H, U-^{13}C]glucose into EL4 tumor-bearing mice resulted in readily observable signals from both anomeric forms of glucose (60-100 ppm, averaged T_1~9 s, Fig.1). At ~15 s after injection, signal from labeled [U-^{13}C]lactate was also observed in the tumor spectra (Fig.2, top left). In separate experiments, measurements obtained with the coil placed over the kidneys showed substantial signal from glucose but no observable signals from lactate in either tumor-bearing or non-tumor-bearing mice, demonstrating that the lactate is produced within the tumor. ^{13}C chemical-shift images (CSIs) from an untreated EL4 tumor-bearing mouse, showed that the glucose signal could be observed throughout the animal, within the sensitive region of the surface coil, but that the lactate signal was predominantly within the tumor (Fig. 2, bottom). The flux of ^{13}C label from [U-^{2}H, U-^{13}C]glucose to lactate was decreased at 24 h after treatment, when the lactate resonances were often barely detectable (Fig.2, top left). The labelled lactate/glucose ratio was decreased by 62% at 24 h after treatment with etoposide (1.82 ± 0.42 in untreated tumours versus 0.69 ± 0.11 in treated tumours (p = 0.026, Fig.2, top right).

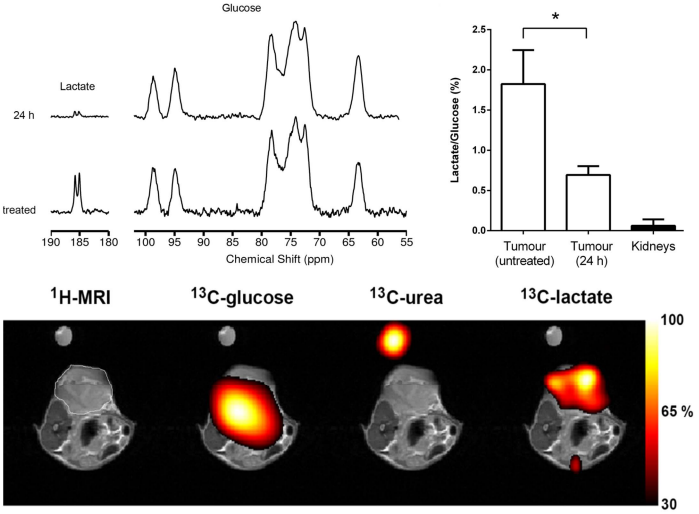

***Fig.2.* ^{13}C spectroscopic imaging with the spatial distribution of normalized labeled glucose and lactate signal (bottom) and treatment response with etoposide (top).** Representative spectra before and 24h after treatment. Lactate/glucose ratio in the tumours before and 24h after treatment, as well as in the kidneys, as measured from ^{13}C spectra (top). The ^{1}H MR images, shown in greyscale, were used to define the anatomical location of the tumour (outlined in white). A urea phantom was included to serve as a reference. Each metabolite was normalized to their own maxima (bottom).

Although the sensitivity of MR detection of hyperpolarized [U-^{2}H, U-^{13}C]glucose is much lower than PET detection of FDG, it has some significant potential advantages for detecting tumor treatment response. Detection of the labeled lactate produced should be advantageous in detecting response in brain tumors since the lactate concentration is much higher in the tumor than in the surrounding brain tissue (5) and the absence of labeled lactate in the kidney indicates that detection of treatment response in prostate cancer should be possible since there will be little or no labeled lactate in the bladder. Furthermore, since the method does not use ionizing radiation repeated measurements could be used to guide treatment. The decrease in lactate production following etoposide treatment is comparable with the decrease in FDG uptake we have observed previously in this tumor model following etoposide treatment, where we showed that decreased FDG uptake is due to down-regulation of the glucose transporters (6). Since the polarized glucose experiment measures flux through the entire glycolytic pathway, this result implies that the most of the control over flux in the glycolytic pathway in this tumor resides at the level of glucose transport. Therefore, while the FDG PET experiment works well in this case, this may not be the case with drugs that affect flux further down the glycolytic pathway, whereas the polarized glucose experiment should be sensitive to the effects of drugs that affect any step in the pathway.

Conclusion

The capacity to non-invasively measure the real-time glycolytic pathway may provide more profound understanding of the altered metabolism of cancer and probably empower the identification of targets for diagnostic imaging, prognosis or therapy.

References: (1) Gatenby, RA (2004) Nat Rev Cancer 4:891; **(2)** Cairns, RA (2011) Nat Rev Cancer 11:85; **(3)** Plathow, C (2008) JNM 49:43S; 9; **(4)** Gambhir, SS (2002) Nat Rev Cancer 2:683; **(5)** Day et al, Nat (2011) 65:557; **(6)** Witney, TH (2009) Neoplasia 11:574. **Acknowledgements:** This study was funded by CRUK, a Marie Curie Fellowship (TBR) and an EMBO fellowship (TBR).