Detection of Early Response to Cyclophosphamide Treatment in a Myc-driven Lymphoma Model Using Hyperpolarized ^{13}C-Pyruvate and FDG-PET

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Background and Motivation

Myc oncoprotein expression is deregulated in a wide variety of human cancers. Much has been learned about the biochemistry of Myc-driven cancers through the study of lymphomas arising in the Eμ-myc transgenic mouse, a model that accurately recapitulates human Burkitt's lymphoma (1). This mouse model could also be important for evaluating treatment strategies in aggressive Myc-driven lymphomas. However, assessment of the effectiveness of a given treatment using traditional imaging approaches and evaluating reductions in tumor size remains a challenge in the clinic. Evidence of tumor shrinkage often occurs after several weeks of treatment and, in some cases, such as for several targeted therapies, even a successful outcome may not result in a reduction in tumor size. Therefore, it is important to develop non-invasive imaging method to detect changes in tumor metabolism shortly after treatment (2). It has been demonstrated that hyperpolarized [1-^{13}C]-pyruvate is an early indicator of treatment response using ^{13}C Magnetic Resonance Spectroscopy (MRS) and Spectroscopic Imaging in different murine cancer models including lymphoma (3). The aim of this study was to determine if hyperpolarized [1-^{13}C]-pyruvate and Positron Emission Tomography (PET) using 2-(^{18}F)fluoro-2-deoxy-D-glucose (FDG) can sensitively assess tumor response to treatment with the widely used genotoxic agent, cyclophosphamide, in the Eμ-myc transgenic mouse.

Methods

Experiments were carried out in Eμ-myc transgenic mice (23-28 g) when the cervical lymph nodes reached a size of approximately 6-8 mm. Tumor burden of the enlarged lymph nodes of the neck can be easily monitored by palpation. Mice were anaesthetized initially by inhalation with O_2 (1 L/min) containing 3 % isoflurane, and maintained during the experiment using a mask and 1-2 % isoflurane in O_2. Animals were taped into a holder, to minimize breathing-related motion, and were then placed in a heated probe, which maintained the core body temperature at ~37ºC, as monitored by a rectal probe. Both the respiratory rate and body temperature were monitored throughout the entire experiment by a Biotrig physiological monitor (Small Animal Instruments, Stony Brook, NY). The hyperpolarized solution of [1-^{13}C]-pyruvate was prepared as described previously (4) using a Hypersense™ polarizer (Oxford Instruments, Oxford, UK). A 20-mm surface coil (Rapid Biomedical GmbH, Rimpar, Germany) was placed over the cervical tumours and ^{13}C MRS (6-mm slice) was performed immediately after i.v. injection of 200 μl of pyruvate, using a 7 T horizontal bore magnet (Varian, Palo Alto, CA). For this purpose, we have used one cohort (n=6), both at baseline and at 24 h after treatment with a single dose of cyclophosphamide (i.p., 200 mg/kg, corresponding to 667 mg/m^2). Spectra were acquired every 2 s. The same treatment protocol was followed in a separate cohort (n=3) used for PET studies, where ~3 MBq FDG was administered at baseline and again after cyclophosphamide treatment. Data were collected from 90 to 100 min post injection using a NanoPET/CT (Bioscan/Mediso, Washington, DC) and reconstructed using a 2D OSEM single slice rebinning algorithm. PET data were normalized to injected dose and body weight and standardized uptake value means (SUVmean) were calculated in the most FDG-avid portion of the tumour (>75% of maximum). After MRS and PET studies, all the mice were euthanized and the tumors were excised for histopathological analysis. In a third cohort of animals (n=6) tumours were freeze-clamped and used for biochemical assays.

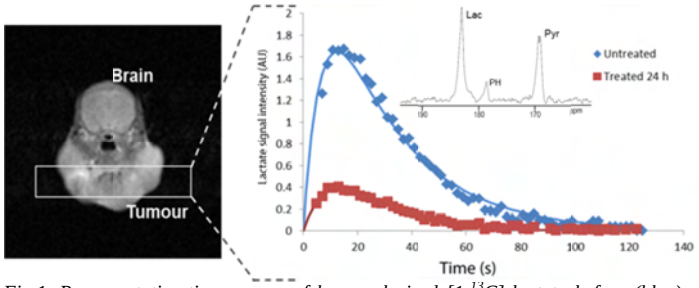

Fig.1. Representative time course of hyperpolarized [1-^{13}C]-lactate before (blue) and after (red) treatment with cyclophosphamide from a cervical lymphoma tumor. ^{1}H reference image with slice in cervical tumor showed in white (left). Fits of the [1-^{13}C]lactate peak intensities to the two-site exchange model and representative ^{13}C NMR spectrum (right). Lac: lactate, PH: Pyruvate Hydrate, Pyr: Pyruvate, AU: Arbitrary Units.

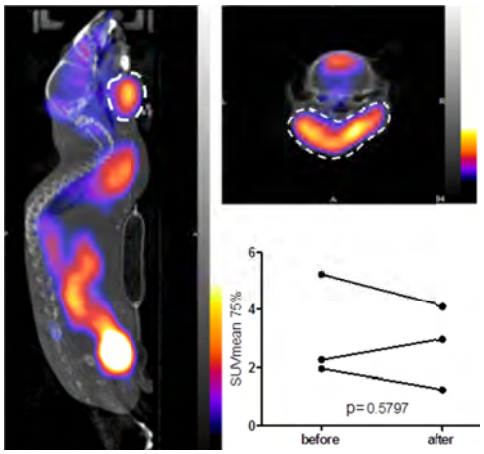

Fig.2. Representative FDG-PET performed before the administration of cyclophosphamide (left and upper right). SUVmean calculated before and after the treatment (lower right).

Results and Discussion

The use of a transgenic mouse model allowed a more realistic analysis of treatment response in spontaneous lymphomas. These tumors present genetically defined lesions that are treated at their natural site of origin. Figure 1 shows data from a representative animal that has been injected with hyperpolarized [1-^{13}C]-pyruvate. The flux of hyperpolarized ^{13}C label between pyruvate and lactate, k_{PL}, in the reaction catalyzed by lactate dehydrogenase (LDH) was 0.124±0.039 s^{-1} for the untreated mice and 0.035±0.027 s^{-1} for the mice treated with cyclophosphamide for 24 h ($P < 0.02$) , an agent used for the treatment of patients with Burkitt's lymphoma. This represents a reduction of approximately 71% in the pyruvate-lactate flux following cyclophosphamide, which is in line with previous findings in mice bearing subcutaneous EL4 lymphoma tumors treated with etoposide. Interestingly, the treatment response detected with FGD-PET imaging approach was much smaller (-7%, P =0.58) at 24 h after cyclophosphamide treatment (Figure 2). Cyclophosphamide was chosen as the cytotoxic agent because this drug is used in clinical settings; however, recent treatments with this drug can provoke an increase of activated inflammatory cells, leading to an overestimation of the viable tumor fraction because the inflammatory cells also show high FDG uptake (5). In the clinic, a decrease in the SUV from baseline of at least 30% after one cycle is necessary to obtain a partial response. On these grounds, a transient influx in inflammatory cells and its contribution to FDG uptake can be of importance. With clinical trials set to commence, utilization of hyperpolarized pyruvate may be preferentially used in pathologies and/or for drugs where the traditional approaches to assess treatment response tend to fail and an increased understanding of the underlying biological nature of the response seen with hyperpolarized pyruvate is required to help interpret the human data. This study demonstrated the feasibility of using DNP hyperpolarized ^{13}C-pyruvate to detect early response to cyclophosphamide treatment in a transgenic mouse of lymphoma which may complement FDG-PET as a clinical tool.

Acknowledgements: This study was funded by Cancer Research UK, GE Healthcare and a Marie Curie Fellowship. **References:** (1)Schmitt et al, Nat (2000) 6:1029; (2) Brindle, Nat Rev Canc (2008) 8:94; (3) Day et al, Nat Med (2007) 11:1382; (4) Day et al, MRM (2011) 65:557; (5) Kubota et al, JNM (1992) 33:197.