Hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate magnetic resonance spectroscopy can detect response to the vascular disrupting agent, Combretastatin-A4-phosphate

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Background and Motivation

Vascular Disrupting Agents (VDAs) are drugs that selectively shut down tumor blood vessels. Early response to these agents cannot be assessed using standard measures such as RECIST(1) as they rarely evoke a change in tumor size. Detection of response has focused on Dynamic Contrast Enhanced MRI (DCE-MRI) measurements of tumor perfusion or MRS measurements of metabolic changes post treatment. Previous work in our laboratory has shown that a decrease in the lactate dehydrogenase catalyzed flux of ^{13}C label between hyperpolarized [1-^{13}C]pyruvate and lactate is an early indicator of treatment response in a murine lymphoma tumor model treated with a chemotherapeutic agent(2). Furthermore, an increase in the fumarase-catalyzed hydration of hyperpolarized [1,4-^{13}C]fumarate to malate has been shown to be a marker of treatment response in the same model both *in vitro* and *in vivo*, and this corresponds to cellular necrosis *in vitro*(3). The aim of this study was to determine whether hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate can sensitively detect response to treatment with a widely used vascular targeting agent, Combretastatin A-4 Phosphate, and to compare them with DCE-MRI and Diffusion Weighted Imaging (DWI), both of which have been employed in previous studies with this agent.

Methods

[1-^{13}C]pyruvate and [1,4-^{13}C]fumarate were hyperpolarized as described previously(2,3) and administered consecutively to mice bearing EL4 murine lymphoma tumours. Animals were split into 3 groups: untreated, 6 hr treated and 24 hr treated. A single 100 mg/kg dose of Combretastatin-A4-Phosphate was given to the treated cohorts. DCE-MRI was performed following i.v. administration of GdDTPA, monitored via T_1-weighted spin-echo images prior to, then for 10 minutes after, injection. DWI used a navigated dual-echo spin echo pulse sequence with diffusion-sensitising gradients (b=0, 68, 271, 609 and 1082 s/mm^2) along the slice axis. All tumours were examined histologically.

Results and Discussion

The flux of hyperpolarized ^{13}C label between pyruvate and lactate, k_P, (Fig. 1A) was reduced by 34% within 6 hours of treatment ($p<0.01$) and remained at the same level after 24 hours (Fig. 2A). The uptake of GdDTPA contrast agent was suppressed at 6 hours (Fig 2C) indicating reduced perfusion of the tumour, whereas by 24 hours uptake had recovered and exceeded the untreated level. The production of ^{13}C labelled malate from hyperpolarized fumarate, k_F, (Fig. 1B) was increased 3.5-fold ($p=0.02$) 6 hours after treatment (Fig. 2B) and remained so at 24 hours, indicating that this may be a more sensitive marker of necrosis than DWI, which did not show any response until 24 hours after treatment (Fig. 2D). Histology confirmed this finding, showing a significant increase in necrotic areas at 6 hours ($p<0.05$) and widespread necrosis at 24 hours ($p<0.01$). We propose therefore that hyperpolarized pyruvate and fumarate could be used as imaging biomarkers of response to vascular targeted therapy.

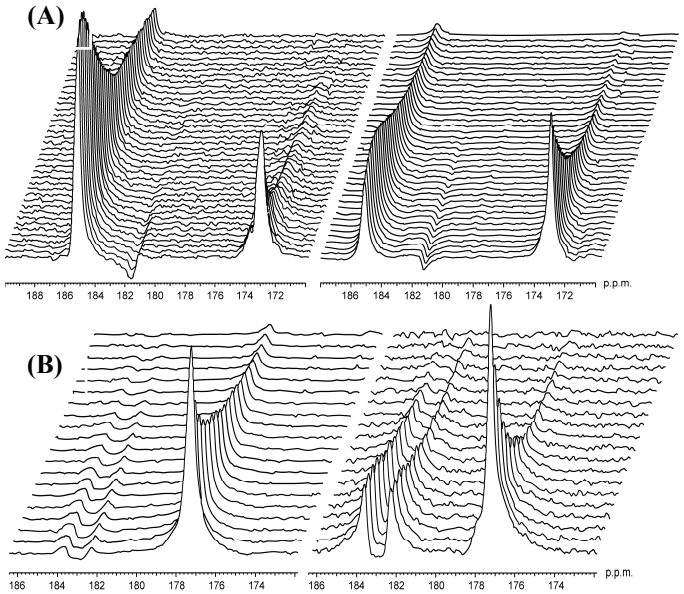

*Figure 1: Time course showing the flux of hyperpolarized ^{13}C label in a 6 mm tumour slice between **(A)** [1-^{13}C] pyruvate (172.9p.p.m.) and lactate (185.1p.p.m.) and **(B)** [1,4-^{13}C] fumarate (177.2p.p.m.) and malate (182.2, 183.6p.p.m.) in an untreated tumour (left) and 24 hours after treatment with Combretastatin (right). Only every 4th spectrum shown for clarity.*

References: (1) E A Eisenhauer *et al* 2009 *Eur J Cancer* 45 228-247 (2) S E Day *et al* 2007 *Nature Med* 13 1382-1387 (3) F A Gallagher *et al* 2009 *PNAS* in press

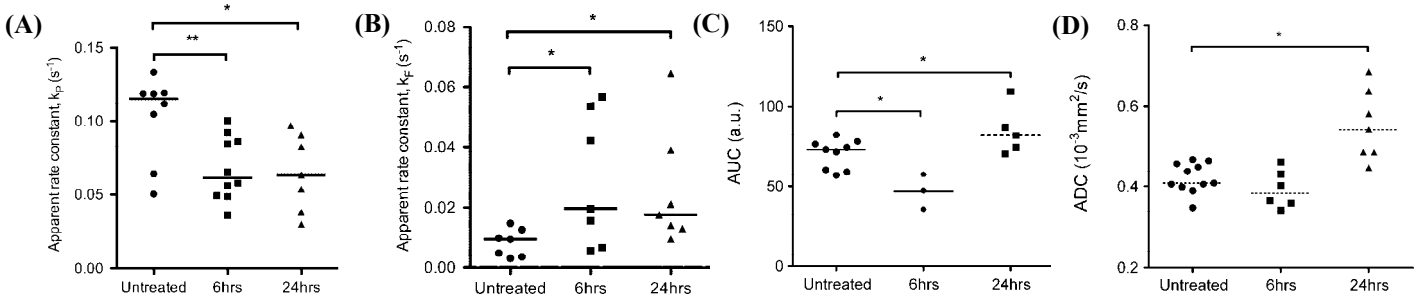

*Figure 2: The apparent rate constant, k_P, of ^{13}C label flux between hyperpolarized Pyruvate and Lactate **(A)** is decreased significantly following treatment ($p<0.01$) while k_F, which reflects the rate of production of Malate from Fumarate, increases concurrently ($p=0.02$) **(B)**. The inflow of GdDTPA, as measured by the area-under-curve AUC **(C)** is decreased significantly 6 hours after treatment, but recovers by 24 hours. The apparent diffusion coefficient ADC **(D)** is sensitive to changes in tumour cellularity and increases 24 hours post treatment ($p<0.02$) but is not significantly altered after 6 hours.*

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