Hyperpolarized 13C magnetic resonance spectroscopy detects early changes in tumor metabolism following treatment with the anti-angiogenic agent Bevacizumab

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Background and Motivation

Targeting of tumor vasculature is an attractive option in cancer therapy, as the development of a blood supply is a rate limiting step in tumor progression [1]. Anti-angiogenic drugs act to block the development of new tumor blood vessels so early response to these agents cannot be assessed using standard measures such as RECIST criteria [2] as they rarely evoke a change in tumor size [3]. Detection of response has therefore focused on metabolic and functional imaging, including Dynamic Contrast Enhanced MRI (DCE-MRI) measurements of tumor perfusion [4]. Previous work in our laboratory [5] has shown that a decrease in the flux of ^{13}C label between hyperpolarized [1-^{13}C]pyruvate and lactate, k_P, is an early indicator of treatment response to the vascular disrupting agent Combretastatin-A4-Phosphate in murine lymphoma tumors, while the hydration of hyperpolarized [1,4-^{13}C]fumarate to malate correlates with tumor cell necrosis in the same model [5]. In the present study, we therefore sought to determine whether hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate could be used to detect differential response to treatment in two colon cancer models treated with the anti-angiogenic drug, Bevacizumab.

Methods

[1-^{13}C]pyruvate and [1,4-^{13}C]fumarate were hyperpolarized as described previously [6] and administered consecutively to mice bearing xenograft human colon carcinoma tumors, established by s.c. injection of $5x10^6$ LoVo or HT29 cells. Animals were treated with 5mg/kg Bevacizumab at time 0 and 48 h (to simulate twice weekly dosing) and experiments were performed at 24 h after each drug dose (24, 72 h). All tumors were excised for histology; tumor enzyme activities and metabolites were measured in a separate animal cohort.

Results and Discussion

Both hyperpolarized markers were sensitive to early metabolic changes following Bevacizumab treatment. In HT29 tumors, k_P rose 1.7-fold over the treatment period (Fig 1A) while in LoVo tumors, k_P fell by nearly 40% (Fig 1C; p=0.02). The differential tumor response stems from the greater vessel density and pericyte coverage of HT29s compared to LoVos, which following VEGF withdrawal means HT29s can maintain perfusion whereas hypoxic regions in LoVos can expand (6). In support of this, biochemical assay of lactate concentration showed a 30% increase after treatment from 4.2±0.8μmol/g wet wt. to 5.5±0.8μmol/g wet wt. (p=0.07) in LoVo tumors despite the significant decline in k_P, but no change was observed in HT29s. Treatment did not induce tumor shrinkage in either model and a correlation between k_P and tumor size was observed in the non-responding HT29 tumor (Fig 1B, D). Furthermore, the ratio of malate to injected fumarate increased more than 3-fold after treatment in LoVo tumors, from a low background level (Fig 2A; p=0.05). No background malate was seen in untreated HT29 tumors, but following Bevacizumab treatment, a low level of malate was observed (Fig 2B; p<0.01). H&E stained tumor sections confirmed these findings, with both tumors exhibiting small abnormal or necrotic areas at 24 h, which were larger and had expanded substantially by 72 h in the LoVo tumors. These results suggest that hyperpolarized markers may inform on acute responses to anti-angiogenic therapy and more importantly, provide a means to differentiate responders and non-responders at an early stage in the treatment time course.

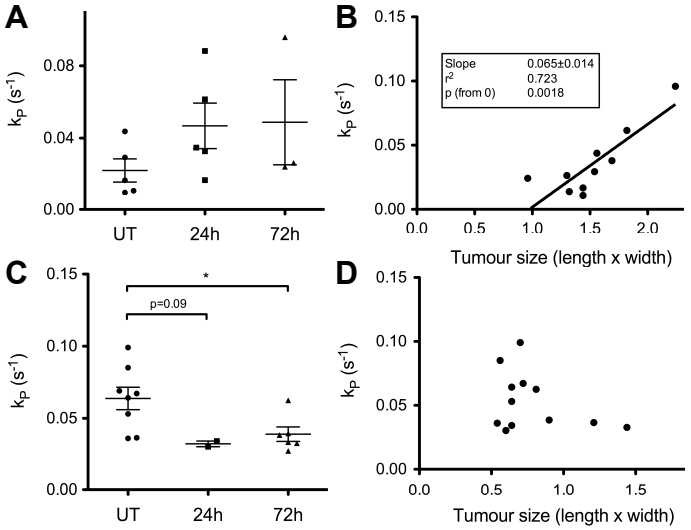

Figure 1: *The apparent rate constant k_P of ^{13}C label flux between hyperpolarized pyruvate and lactate increases after each Bevacizumab dose in the non-responding HT29 tumors (A) and correlates with the increasing tumor size (B). Conversely, k_P falls significantly by 72 hours in the responding LoVo tumors (C) and is not correlated with tumour size (D).*

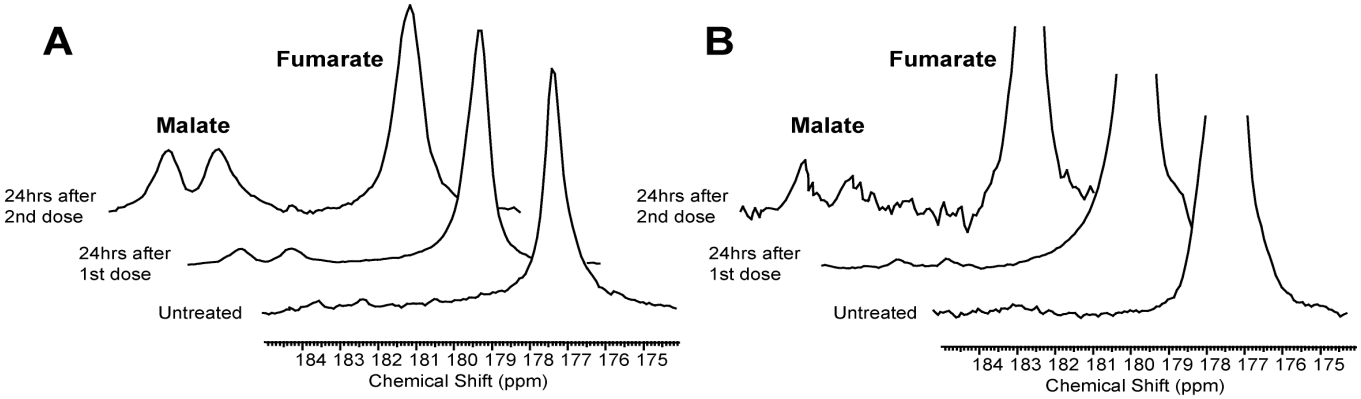

Figure 2: *The production of malate increases substantially following Bevacizumab treatment in LoVo tumors (A), while a low level of malate production is observed in HT29 tumors (B). Spectra were acquired at 25s post injection using a 60° flip angle; spectra in (B) are magnified 5-fold compared to (A)*

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