Detecting response to treatment in human breast adenocarcinoma using a co-administration of hyperpolarized [1-^{13}C]pyruvate and [1,4-$^{13}C_2$]fumarate

T. H. Witney[1,2], M. I. Kettunen[1,2], D-E. Hu[1,2], F. A. Gallagher[1,2], and K. M. Brindle[1,2]

[1]Department of Biochemistry, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom, [2]Cancer Research UK Cambridge Research Institute, Cambridge, Cambridgeshire, United Kingdom

Objective: Tumor response to therapy is assessed primarily in the clinic by monitoring reductions in tumor size. However, this approach lacks sensitivity since in many cases several weeks may elapse before there is evidence of tumor shrinkage. There is therefore a pressing need to develop non-invasive imaging techniques for monitoring tumor treatment response in the clinic. It has been shown recently that the uptake and metabolism of hyperpolarized [1-^{13}C]pyruvate can be used to detect response to therapy using ^{13}C magnetic resonance spectroscopy (MRS) and spectroscopic imaging in murine models of lymphoma and prostate cancer (1,2). A new molecule, hyperpolarized [1,4-^{13}C]fumarate, has also recently been shown to be a marker of necrosis both *in vitro* and *in vivo* (3). The aim of the present study was to determine whether hyperpolarized ^{13}C MRS could be used to detect response to therapy in a model of human breast adenocarcinoma.

Methods: MDA-MB-231 human breast cancer cells were treated with doxorubicin (1 μg/ml) to induce cell death. Following treatment, the temporal changes in hyperpolarized [1-^{13}C]pyruvate - [1-^{13}C]lactate exchange and hyperpolarized [1,4-^{13}C]fumarate - [1,4-^{13}C]malate exchange were assessed. 75 mM hyperpolarized [1-^{13}C]pyruvate, 20 mM hyperpolarized [1,4-^{13}C]fumarate and 75 mM unlabeled lactate (1,3) were added to a suspension of 2-6x10^7 MDA-MB-231 cells in a 10 mm NMR tube, maintained at 37°C. ^{13}C spectra were acquired at 1 s intervals for 240 s using a low flip angle pulse in a 9.4 T vertical wide-bore magnet (Oxford Instruments). For *in vivo* experiments MDA-MB-231 tumor xenografts were grown in the lower flank of female SCID mice to a final volume of ~1.5 cm^3. Mice were treated with an intraperitoneal injection of 10 mg doxorubicin per kg body weight. A 25 mm diameter surface coil tuned to ^{13}C (100 MHz) was positioned over the tumor. The entire assembly was placed in a quadrature ^{1}H-tuned volume coil (Varian). 128 tumor spectra were acquired at 1 s intervals using a slice selective 600 μs sinc pulse, with a nominal flip angle of 5°. The exchange rate constants and spin lattice relaxation times were determined by fitting the peak intensities to the modified Bloch equations for two-site exchange. (1) In cells, apoptosis and necrosis were assessed by flow cytometry using annexin V and SYTOX Red staining respectively. Changes in the intracellular NADH concentration was assessed by measuring UV autofluorescence at 350nm. Tumor cell death was assessed histologically in tumor sections stained with hematoxylin and eosin

Results and Discussion: Addition of 75 mM hyperpolarized [1-^{13}C]pyruvate to a suspension of untreated MDA-MB-231cells resulted in detectable exchange of label with added lactate, in the reaction catalyzed by lactate dehydrogenase (LDH). Co-administration of 20 mM hyperpolarized [1,4-^{13}C]fumarate to untreated cells resulted in no detectable appearance of signal corresponding to hyperpolarized [1,4-^{13}C]malate. Treatment of cells with the topoisomerase type I inhibitor, doxorubicin, for 72h resulted in a 7.5-fold increase in annexin V staining, indicating the induction of apoptosis as measured by flow cytometry. Low levels of necrosis were also observed by SYTOX Red staining. At this time point a 48% drop in pyruvate - lactate exchange ($P < 0.05$), coinciding with a 91% reduction of intracellular NAD(H), was observed (Fig. 1A+B). Peaks corresponding to hyperpolarized [1,4-^{13}C]malate were also observed 72h post treatment (Fig.1C+D). The largest decrease in pyruvate - lactate exchange was evident at 96h when the majority of cells were necrotic. A further 220% increase in fumarate – malate exchange ($P < 0.001$) was also observed 96h post treatment. In tumors, doxorubicin treatment corresponded with a 46% decrease in pyruvate – lactate exchange ($P < 0.05$). In this study, we show that we can probe two stages of cell death, apoptosis and necrosis, as a measure of treatment response using a single injection of hyperpolarized metabolites. With clinical trials set to commence, utilization of hyperpolarized pyruvate and fumarate as markers of cell death may enable the clinician to image the complex mechanisms behind tumor treatment response.

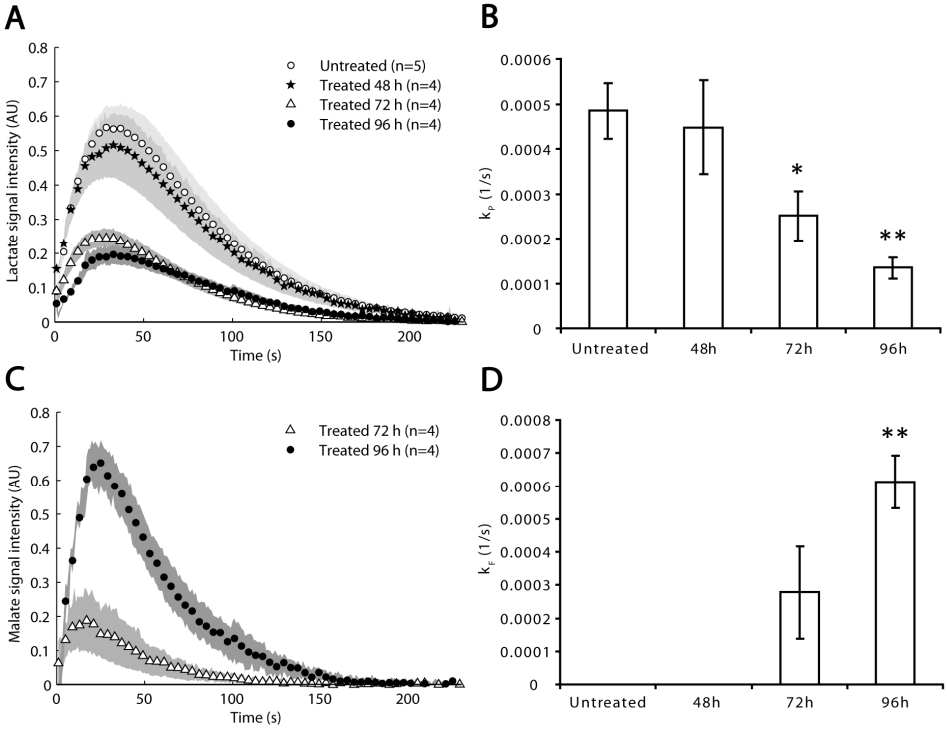

Fig.1 *– Detection of doxorubicin-induced cell death in MDA-MB-231 cells using a co-administration of hyperpolarized pyruvate and fumarate.* ***A.*** *Effect of doxorubicin treatment on hyperpolarized lactate peak intensity.* ***B.*** *Changes in hyperpolarized pyruavte - lactate exchange following treatment.* ***C.*** *Effect of doxorubicin treatment on hyperpolarized malate peak intensity.* ***D.*** *Changes in hyperpolarized fumarate - malate exchange following treatment.*

(1) Day SE et al. (2007) Nature Medicine. 13, pp.1382-1387

(2) Chen AP et al. (2008) Proc. Intl. Soc. Mag. Reson. Med. 16, pp.888

(3) In t' Zant R et al. (2009) Proc. Intl. Soc. Mag. Reson. Med. 17, pp.55

(4) Witney et al. (2009) Neoplasia. 11, pp.574-582