Dichloroacetate treatment resulted in a dramatic drop in the conversion of hyperpolarised 1-13C labelled pyruvate to lactate in human colon carcinoma cells

Y-L. Chung¹, H. Troy¹, I. R. Judson², J. R. Griffiths³, M. O. Leach¹, and T. R. Eykyn¹

¹CR-UK and ESPRC Cancer Imaging Centre, Institute of Cancer Research, Sutton, Surrey, United Kingdom, ²CR-UK Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, United Kingdom, ³Cancer Research UK Cambridge Research Institute, Cambridge, United Kingdom

INTRODUCTION: Dichloroacetate (DCA) is a pyruvate dehydrogenase kinase (PDK) inhibitor and is found to be an anti-cancer agent [1]. It causes the activation of pyruvate dehydrogenase (PDH) and increased glucose oxidation by promoting the influx of acetyl-CoA into the mitochondria and the Krebs cycle [1]. The hyperpolarised ¹³C experiment measures the conversion of labelled pyruvate to labelled lactate which has been shown to be proportional to lactate dehydrogenase activity and the availability of cellular NADH, the enzyme co-factor [2]. Conversely, steady state measurements of eupolarised (i.e. unlabelled) lactate production report on the overall flux through glycolysis.

AIM: To use hyperpolarised 1-13C pyruvate ¹³C-magnetic resonance spectroscopy (MRS) and conventional ¹H-MRS to measure lactate formation/production in real-time and in steady state, respectively, in order to study the mechanism of action of DCA and to develop a non-invasive biomarker for reporting on drug action. Inhibition of PDK by DCA is expected to cause a drop in lactate production.

METHODS: Intact human HT29 (colon) carcinoma cells were studied after 24 hours of DCA treatment (100mM). 1-13C labelled pyruvic acid containing 15mM OX63 free radical was polarized in a HyperSense DNP polarizer at 3.35T and 1.4K. The polarized sample was dissolved in a neutralised phosphate buffered solution containing 50mM eupolarised lactate and EDTA. An aliquot (100μl) was added to a 500 μl suspension of cells (~80 to 100 million cells), final concentration 8mM polarized pyruvate, after which serial ¹³C-MRS spectra were acquired every 2 sec with a small flip angle radio-frequency pulse. Rates were derived from non-linear least squares fitting of the bi-exponential time dependence of the hyperpolarised lactate build-up and normalised to cell number in both control and treated cells. Culture media and extracts from the DCA-treated and control cells were analysed by ¹H-MRS. Cell cycle analysis was also performed.

RESULTS AND DISCUSSION: DCA treatment of HT29 cells caused a drop in cell number (~40% of controls, p<0.0001) and G1 arrest (p<0.0001). A dramatic drop in lactate formation (0.69±0.06 mmol/s/10⁶ cells in control versus 0.06±0.01 mmol/s/10⁶ cells in treated cells; p<0.0001) was measured in real-time by DNP ¹³C-MRS (Fig. 1 A-B). This could be due to the combined effect of: i) a drop in cellular NADH due to apoptosis, as DCA is known to induce apoptosis [1], which causes NADH loss [2]; and/or ii) activation of PDH by DCA increases glucose oxidation by promoting the influx of acetyl-CoA into the mitochondria and Krebs cycle and causing a decrease in NADH available for further metabolism of pyruvate to lactate. ¹H-MRS of the culture media of DCA treated cells also showed a reduction in steady state eupolarised lactate production (p<0.0001), increased alanine uptake (p<0.0001) and no difference in glucose uptake when compared with controls. Increases in alanine (p<0.0001), glucose (p<0.02), free choline (p=0.02) and glycerophosphocholine (p<0.0001) and decrease in phosphocholine (p<0.0001) were found in DCA-treated cell extracts. The phospholipid changes could be associated with modulation in membrane turnover, as DCA is known to reduce proliferation in cancer cells [1].

CONCLUSIONS: DCA treatment resulted in reduced lactate formation/production in HT29 cells, as shown by both real time and steady-state measurements. These changes have potential as non-invasive biomarkers of drug action. DCA treatment also altered phospholipid metabolism, which could provide further biomarkers of response.

Fig. 1: A) ¹³C-MRS spectra of control and DCA-treated HT29 cell suspension after the addition of hyperpolarised 1-13C pyruvate. B) Time series of ¹³C-MRS spectra of lactate from a control and a DCA-treated HT29 cell suspension recorded every 2 sec following the addition of hyperpolarised labelled 1-13C pyruvate and unlabelled lactate.


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