

A relationship between hyperpolarized ^{13}C pyruvate metabolism and tumour volume in a chronic study of oral dichloroacetate treatment of P22 bearing BDIX rat

Steven Reynolds¹, Tooba Alizadeh², Becky Bibby², Matthew Fisher², Adriana Bucur¹, Samira Kazan², Martyn Paley¹, and Gillian M Tozer²

¹Academic Radiology, University of Sheffield, Sheffield, South Yorkshire, United Kingdom, ²Department of Oncology, University of Sheffield, Sheffield, South Yorkshire, United Kingdom

Introduction: Pyruvate plays a key role in several metabolic pathways including a glycolytic pathway for lactate production. There has been considerable interest over recent years in using hyperpolarised ^{13}C pyruvate for studying cancer metabolism. The methodology has been used to study the effect of drugs on tumour metabolism and in particular dichloroacetate (DCA) which disrupts pyruvate dehydrogenase kinase (PDK), resulting in an increased flux through oxidative phosphorylation. However, these studies have been acute treatments up to 24h [1, 2]. In this study we examine the effect of chronic oral DCA dosing on P22 bearing BDIX rats over a period of up to 17 days.

Method: A cohort of 11 male and female BDIX rats, with subcutaneously transplanted P22 sarcoma, were treated by administering DCA in the drinking water. When the tumours reached 8-10mm in diameter (Vernier calliper measurements) the animals were divided into DCA treated and control groups by a blocking design. The treated group received 2.5g/l of DCA in their drinking water (200mg/kg/day), adjusted to pH 6.8-7.2. The control group were given Milli Q water. Rats were treated over a period of 8-17 days and scanned on the final day. Tumour diameters were measured 2-3 times per week during the treatment period.

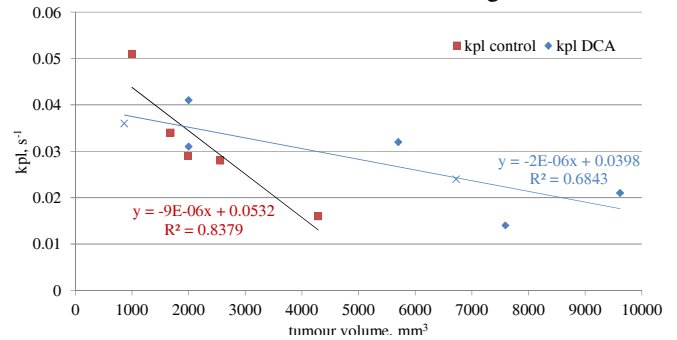


Figure 1: Estimated k_{pl} versus tumour volume for DCA treated and control BDIX rats. \times markers in DCA group indicate male within the female group. All control rats were males.

For MR scanning the animals were anaesthetised and femoral vein was cannulated for pyruvate delivery. Anaesthesia was maintained by administration of 1-2% isoflurane at 2L/min via a nose cone. The animal was placed in a Bruker 7T MRI system with its temperature maintained by an electric heating pad and rectal temperature probe. Respiration rate was also monitored. The 45mg of $^{13}\text{C}_1$ -pyruvate (PA) was hyperpolarized using a HyperSense DNP polariser and dissolved in HEPES buffer solution, pH 7. 5ml/kg of hyperpolarised PA buffered solution was administered over 13s using an automated injection system [3]. The ^{13}C metabolite signals were localised in the tumour using a 20mm $^{13}\text{C}/^1\text{H}$ surface coil positioned over the tumour and 8mm thick coronal slice selection. ^{13}C spectroscopic data were acquired using a Gaussian pulse (20deg flip angle, TR=1s). ^{13}C peak integral versus time response curves for pyruvate and lactate were fitted using Matlab to a precursor product relationship, where the tumor tissue pyruvate time-course was used as the input for the lactate time course [4], and k_{pl} values were extracted. After scanning the animals were sacrificed and tumours excised and fixed for histology.

Results and Discussion

When comparing the estimated k_{pl} values between the control (n=5) and treated (n=6) groups, a Student's unpaired T-test showed no statistically significant difference, $p=0.65$ (Figure 1). However, when the k_{pl} values were plotted against measured tumour volumes (calculated by average diameter from calliper measurements), strong linear negative correlations were found for both the control ($r=-0.92$, $p=0.03$) and treated ($r=-0.83$, $p=0.02$) groups (Figure 1). An ANCOVA statistical test showed that there was a statistically significant difference between the slopes of the two groups, $p<0.05$. Histology of the excised tumours (haematoxylin and eosin staining) showed that necrosis levels were similar in the two groups. No other linear correlations or Student's t-test significance was found between treatment time and tumour volume, Figure 2. The observed downward trend in the slopes shows that as the tumour grows the apparent estimated k_{pl} decreases, which cannot be explained by any change in necrotic levels. The relationship between k_{pl} and tumour size may be due to increased intercapillary distances as tumours grow, increasing the time for pyruvate to be transported into more remote cells. Figure 1 shows that DCA influences the apparent k_{pl} as the tumours grow, however, further work needs to be performed into understanding the mechanisms behind these effects.

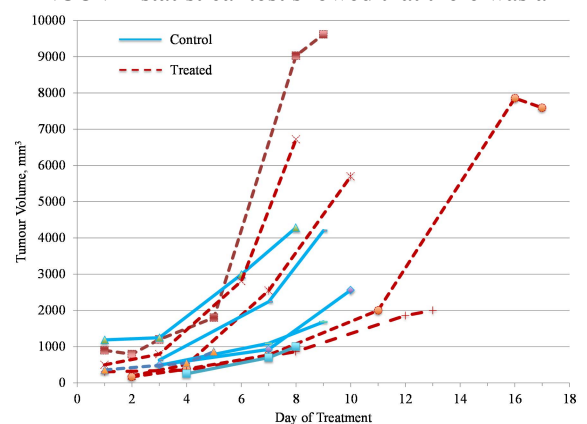


Figure 2: Growth curve v days on treatment for treated and control animals.

References

- [1] M. A. Schroeder, H. J. Atherton, M. S. Dodd, P. Lee, L. E. Cochlin, G. K. Radda, K. Clarke, D. J. Tyler, *Circulation. Cardiovascular imaging*, 5, 201-9, (2012)
- [2] P. Seth, A. Grant, J. Tang, E. Vinogradov, X. Wang, R. Lenkinski, V. P. Sukhatme, *Neoplasia*, 13, 60-71, (2011)
- [3] S. Reynolds, S. Kazan, J. Bluff, M. Port, E. Wholey, G. Tozer, M. Paley, *Applied Magnetic Resonance*, 43, 263-273, (2012)
- [4] Kazan et al, Kinetic Modeling of Hyperpolarized ^{13}C Pyruvate Metabolism in Tumors Using a Measured Arterial Input Function. *Magn. Reson. Med*, accepted paper