Effects of acute and chronic dichloroacetate treatment on hyperpolarized ^{13}C pyruvate metabolism, necrosis and tumor volume in P22 sarcoma bearing BDIX rats

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Introduction: Dissolution dynamic nuclear polarization (dDNP) has established itself as a preclinical technique for monitoring *in vivo* metabolism by magnetic resonance spectroscopy and imaging (MRS/MRI)[1, 2]. By massively increasing the sensitivity of MRS/MRI of ^{13}C nuclei through dDNP, real time kinetics can be followed without the interfering background signals experienced in ^{1}H MRS. Pyruvate is the end product of glycolysis prior to the TCA cycle and is a commonly used substrate in hyperpolarized (HP) MRS studies, as the rate constant for the conversion of pyruvate to lactate (k_{pl}) in tumors is a potential marker for the efficacy of anti-cancer drugs [3]. For example acute treatment of tumor-bearing animals with dichloroacetate (DCA), which disrupts pyruvate dehydrogenase kinase (PDK), resulting in an increased flux through oxidative phosphorylation, has been studied. We examine the potential efficacy of DCA over an extended time frame; from acute (30 minutes post treatment) to chronic (up to 10 days DCA treatment) on P22 sarcoma bearing BDIX rats.

Method: Two treatment cohorts were created for P22 sarcoma bearing BDIX rats. When the sarcomas reached 8-10 mm in diameter, the animals were divided into DCA treated and control groups. Cohort 1(chronic treatment) were given a daily subcutaneous injection of 200 mg/kg DCA for 7-10 days, plus an I.P. injection of 200 mg/kg DCA 1-2 hours prior to pyruvate injection on the day of MR scanning Tumor diameters were measured throughout the time-course for volume calculations. Cohort 2 (acute treatment, mean±SE tumor volume 4749±608 mm^3) were given an I.V. injection of DCA (150 mg/kg dissolved in saline, pH=7.0) ~30 minutes prior to the pyruvate injection. Control groups were given the same treatment regime substituting DCA solutions with water (acute, saline). The animal was anaesthetized for MR scanning using 1-2% isoflurane at 2 L/min via a nose cone and pyruvate delivered either via tail vein (chronic treatment) or femoral vein (acute treatment). The animal was placed in a Bruker 7T MRI system with its temperature maintained at 37°C and respiration rate monitored. The 45.5 mg of $^{13}C_1$-pyruvate (PA) was hyperpolarized using a HyperSense and dissolved in HEPES buffer solution. 5 ml/kg of HP PA buffered solution was administered over 13s using an automated injection system [4]. ^{13}C spectra were localized in the sarcoma using a 20 mm ^{13}C/^{1}H surface coil positioned over the tumor and 8 mm thick coronal slice selection (20deg flip angle Gaussian pulse, TR=1 s, SW=50ppm, 256 points). ^{13}C peak integral versus time curves for pyruvate and lactate were fitted using Matlab to a precursor product relationship [5] and k_{pl} values were extracted. After scanning, the animals were sacrificed and tumors excised and freeze clamped for biochemical assays. In a separate chronically sc/ip treated cohort tumors were excised and fixed for histology.

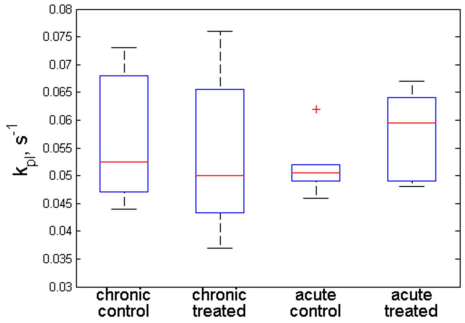

Figure 1: Estimated k_{pl} for Chronic sc/ip: treated=7, control=6, Acute treated=6, control=6 (red cross is an outlier value).

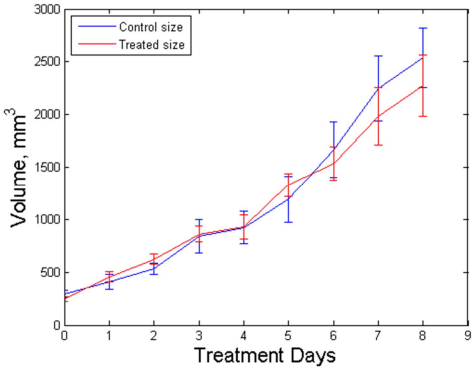

Figure 2: Mean ± SE sarcoma volume for chronic sc/ip treated and control group over an 8 day period.

Results and Discussion: For both chronic and acute treatment cohorts, DCA had no effect on k_{pl} (p=0.60 and p=0.15 respectively: Student's unpaired t-test), see figure 1. There was no significant difference for the growth in tumor volume over an 8 day period for chronically treated animals compared to controls, figure 2. Histology of the sc/ip excised sarcomas showed similar necrosis levels in the two groups (not shown). Biochemical assays of freeze clamped tumors showed a significant decrease in tumor pyruvate concentration (treated=0.034±0.007 µmol/g, control=0.12±0.02 µmol/g, mean±SE, n=5. p=0.016), but not lactate concentration (treated=8.0±0.73 µmol/g, control=7.6±1.4 µmol/g, n=4, mean±SE. p=0.82) following chronic administration DCA treatment. This could be attributed to an increased pyruvate flux through pyruvate dehydrogenase, although no hyperpolarized bicarbonate signal was observed in the MR study of our tumor model. Administration of HP $^{13}C_1$-lactate could be used to observe changes in pyruvate concentration through ^{13}C spin exchange between the lactate pool. In our study hyperpolarized MR showed that DCA treatment of P22 tumors was ineffective for disrupting tumor metabolism, although it has been shown to be effective in other models for normal rats [6]. ^{13}C MR/DNP is a suitable method for measuring acute changes in tumor metabolism and has the potential to show the effectiveness of a drug treatment before any change in tumor growth rate/size. This would be extremely useful for rapidly assessing treatment efficacy following drug administration and has the potential for aiding treatment scheduling.

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