Monitoring Metabolic Shifts in TRAMP Mice Resulting from Dichloroacetate Using Hyperpolarized Pyruvate

A. K. Grant[1], P. K. Seth[2], E. Vinogradov[1], X. Wang[1], V. P. Sukhatme[2], and R. E. Lenkinski[1]
[1]Radiology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States, [2]Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States

Introduction: Many cancers preferentially metabolize glucose via fermentative glycolysis (conversion of pyruvate into lactate) rather than oxidative metabolism. Although tumor hypoxia is one common cause of enhanced glycolytic fermentation, many types of cancer exhibit enhanced rates of lactate formation even when there is sufficient oxygen available to support the TCA cycle. This feature of cancer metabolism, referred to as the Warburg phenomenon, may confer a survival advantage on tumor cells [1]. Indeed, the reduced rate of oxidative metabolism reduces levels of potentially harmful reactive oxygen species and may support the metabolic needs of rapid proliferation. A major question is whether reversal of the Warburg phenomenon can be used as a method to selectively harm cancer cells. Recent studies have shown that administration of dichloroacetate (DCA), a drug that up-regulates the activity of pyruvate dehydrogenase (PDH) and thus increases the rate of oxidative metabolism, can lead to reduced tumor growth *in vitro* and *in vivo* [2,3], including tumor regression in certain animal models. Tumors in the TRAMP [4] mouse model of prostate cancer have been shown to exhibit elevated levels of lactate following administration of hyperpolarized pyruvate [5]. The action of DCA is expected to decrease levels of endogenous lactate and the net rate of lactate formation. In addition, increased PDH activity is expected to give rise to enhanced bicarbonate formation. Here we present preliminary data to show that these expectations are borne out.

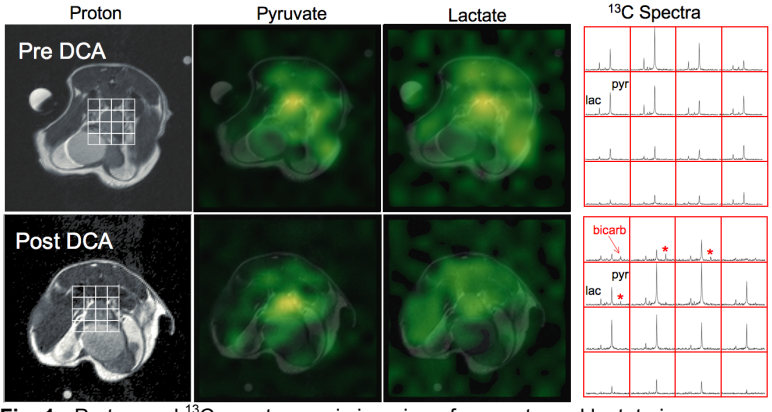

Fig. 1: Proton and ^{13}C spectroscopic imaging of pyruvate and lactate in a TRAMP mouse before (top row) and after (bottom row) administration of DCA. From left to right, each row shows: proton image and selected CSI voxels; pyruvate image; lactate image; and ^{13}C spectra from selected voxels. In the post-DCA study, small signals from bicarbonate are marked by red asterisks.

Methods: TRAMP mice underwent serial T_2-weighted imaging in order to identify potential tumors up to an age of approximately 16 weeks. Once suspect lesions had been identified, the mice underwent a pair of examinations using hyperpolarized pyruvate before and after administration of DCA. The two studies were separated by one week. Immediately prior to the second study, the animals received two daily doses of 75mg/kg DCA orally and an additional 25mg/mg intravenously 15 minutes before imaging. For imaging studies, mice were anesthetized by means of inhaled isoflurane and a narrow catheter connected to a long, thin tube was placed in the tail vein. The anesthetized mice were placed in a 4.7T animal scanner (Bruker BioSpec, Billerica MA) inside a linearly polarized ^{13}C volume coil (35mm diameter) situated within a 72mm proton coil. Body temperature was maintained at 37C using an air warming system coupled to an endorectal thermometer. Respiratory monitoring was performed and the level of anesthesia was adjusted as necessary to obtain a respiratory rate of approximately 90 cycles/minute. An 80mM solution of hyperpolarized pyruvate was prepared as described previously [6]. Following acquisition of T_2-weighted proton anatomic images, 250 microliters of the pyruvate solution were administered via the tail vein. After a waiting period of 45 seconds, 2D ^{13}C chemical shift imaging was performed with 16x16 elliptic k-space sampling, 3.5cm field of view, 10mm slice thickness, 6° tip angle, 124ppm bandwidth, 519 spectral points, 85ms TR, 16s total scan time. After apodization with a 15Hz line-broadening filter, standard Fourier reconstruction was performed and pixel-by-pixel line integrals for pyruvate, lactate, and bicarbonate were computed. Images of pyruvate and lactate were formed from these integrals and interpolated to 128-by-128 matrix size. They were then overlaid with the corresponding proton images for comparison. All procedures were approved by the Institutional Animal Care and Use Committee.

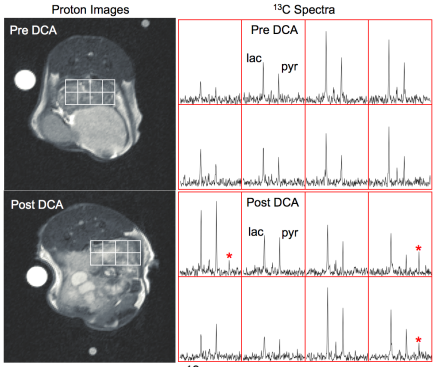

Fig. 2: Proton and ^{13}C spectra before and after administration of DCA. Lines are labeled as in Fig. 1.

Results: Fig. 1 displays a representative data set. The upper and lower rows show data acquired in a single mouse before and after administration of DCA, respectively. The left column displays a proton image, with a pixel grid overlaid on the suspect lesion in this animal. The central two panels show pyruvate and lactate images, and the rightmost panels show the ^{13}C spectra corresponding to the voxel grids at left. The lactate images have been scaled by the peak pyruvate signal in the corresponding study and displayed on a common intensity scale. The reduction in lactate image intensity is readily apparent. The ^{13}C spectra at far right show a reduction in lactate relative to pyruvate following DCA treatment: the ratio of the lactate signal, summed over the voxels indicated by the white grids in Fig. 6, to the corresponding sums for pyruvate, fell from 0.40:1 to 0.23:1 following DCA treatment, a 40% reduction. An alternative metric is the lactate SNR [4], which is reduced by 50% after DCA treatment. In addition, signals from bicarbonate, generated by the conversion of pyruvate into acetyl-CoA by PDH, are apparent following DCA treatment. The bicarbonate signal is not readily apparent in the pre-DCA study. A second study is shown in Fig. 2. In this study, a phantom containing ~3M ^{13}C labeled acetate was placed in the field of view. In this case the reduction in lactate following DCA treatment in not as readily evident. Quantitatively, however the lactate:pyruvate ratio for the indicated voxels is reduced by 20% (from 1.7 to 1.4), while the lactate signal summed over the indicated voxels and normalized to the signal in the acetate phantom is reduced by 40%. These results are relatively robust across different voxel choices in the post-DCA study. As in Fig. 1, small signals from bicarbonate are evident following DCA treatment.

Conclusions: The data show that the hyperpolarized pyruvate can be used to monitor metabolic shifts brought about by administration of DCA in TRAMP mice. DCA is expected to increase the activity of PDH, thereby shifting pyruvate metabolism away from lactate formation and increasing the rate of acetyl-CoA formation. These expectations are borne out by the data, which show both reduced lactate and the appearance of a bicarbonate signal following DCA administration.

References: [1] Vander Heiden MG *et al* Science 2009:324;1029 [2] Bonnet S *et al,* Cancer Cell 2007:11;37 [3] Cao W *et al,* Prostate 2008:11;1223 [4] Greenberg N *et al* PNAS 1995:92;3439 [5] Albers MJ *et al* Cancer Res 2008:68;20 [6] Chen AP *et al*, MRM 2009:58;1099