Hyperpolarized ^{13}C MRS detection of reduced pyruvate-lactate conversion following PI3K inhibition

C. S. Ward[1], H. Venkatesh[1], A. Brandes[1], M. van Criekinge[1], H. Dafni[1], J. Kurhanewicz[1], C. D. James[2], D. A. Haas-Kogan[2], and S. M. Ronen[1]

[1]Radiology and Biomedical Imaging, UCSF, San Francisco, CA, United States, [2]Neurological Surgery, UCSF, San Francisco, CA, United States

Purpose

The PI3K signaling pathway is an attractive target for novel mechanism-based anticancer treatments (1). The aim of this work is to demonstrate in a perfused cell system the efficacy of hyperpolarized ^{13}C MRS as a method to detect target modulation following treatment with the PI3K inhibitor LY294002 on two separate cell lines.

Methods

The effect of PI3K inhibition was examined in MDA-MB-231 human breast adenocarcinoma and glioblastoma multiforma (GBM) human neurosphere cells following incubation with 50 µM LY294002 for 40 hr. For MRS studies, cells were encapsulated in agarose beads and loaded into a perfusion system, modified from previously described (2). MR studies were performed on a 500 MHz INOVA spectrometer (Varian). ^{31}P spectra were acquired using a 30° pulse with 1 sec relaxation. $^{13}C_1$-pyruvate was hyperpolarized using the Hypersense DNP (Oxford Instruments) polarizer as described previously (3). Sequential injections of 3.0 mL hyperpolarized pyruvate were performed at 2 hr intervals. In experiments with MDA-MB-231 cells, final concentrations of 2 to 10 mM polarized pyruvate were attained in a volume of 8.0 mL. ^{13}C MR spectra were acquired in 3 sec intervals for 300 secs using a 5° pulse. Experiments with GBM used final concentrations of 1 to 3 mM polarized pyruvate and ^{13}C spectra acquired using a 13° pulse. The intensities of lactate peaks were quantified by integration and normalized to polarization and cell number determined prior to encapsulation. Maximum lactate values of treated cells were compared to control values of corresponding pyruvate injections. In parallel experiments, the effect of PI3K inhibitor treatment on lactate dehydrogenase (LDH) and HIF-1 levels was determined by standard Western blot techniques. Enzyme activity assays were performed to assess the effect of PI3K inhibition on LDH reaction kinetics.

Results

Sustained cell viability of perfused cells was confirmed throughout the experiment by ^{31}P spectra (Fig. 1) acquired prior to injections of hyperpolarized pyruvate. ^{13}C MR spectra (Fig. 2) revealed conversion of hyperpolarized pyruvate to lactate at all of the concentrations presented. Importantly, treatment with 50 µM LY294002 for 40 hr resulted in reduced hyperpolarized lactate formation to 70 ± 16% of control (P=0.006 n=6) in MDA-MB-231 and 45 ± 14% (P=0.0002 n=6) in GBM. Both exhibited increasing rates in response to increased substrate concentrations (Fig. 3). There was also a significant decrease of LDH activity with treatment as determined by a drop in V_{max} in cell lysates: 69 ± 12% of control in MDA-MB-231 (Fig. 4) and 35 ± 7.5% of control in GBM. The K_M remained unchanged, suggesting the reduced activity stems solely from a reduction in enzyme concentration. Reduced LDH-A levels were confirmed by Western blotting, which also revealed a decrease in HIF-1, the transcription factor controlling LDH-A expression downstream of PI3K (Fig. 4).

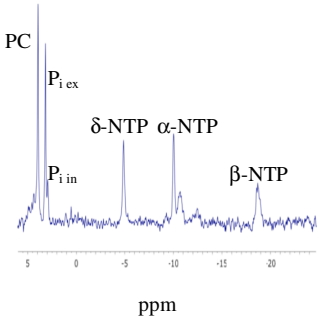

Figure 1: ^{31}P MR spectrum of control MDA-MB-231 breast adenocarcinoma cells in perfused cell system confirming cell viability throughout the experiment.

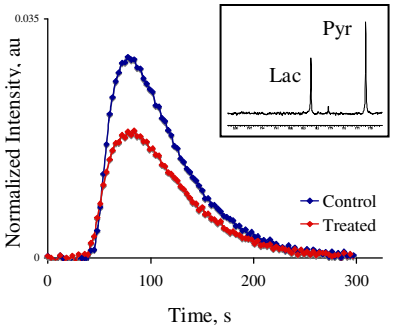

Figure 2: ^{13}C spectral array of hyperpolarized lactate after 2mM pyruvate injection in control and LY-treated GBM cells, demonstrating the reduction in lactate following PI3K inhibition. Inset: ^{13}C spectrum of control cells 150 seconds after injection.

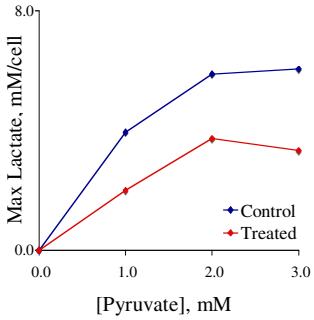

Figure 3: Apparent rates of hyperpolarized lactate formation with increasing pyruvate injection concentrations in control and LY-treated GBM cells.

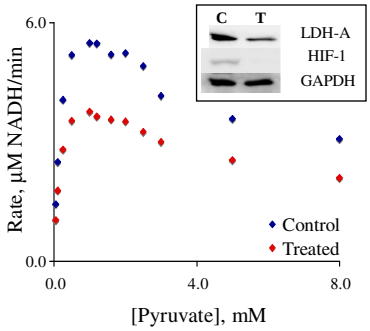

Figure 4: LDH activity assay in control and LY-treated MDA-MB-231 cells (10^7 cells per assay), demonstrating a drop in cellular activity following PI3K inhibition. Inset: Western blot of control (C) and treated (T) MDA-MB-231 cells, revealing a decrease in LDH-A and HIF-1 with treatment.

Discussion and Conclusions

This study demonstrates that the efficacy of PI3K inhibitor treatment can be studied by hyperpolarized ^{13}C MRS. Inhibition of PI3K reduced the concentration of lactate dehydrogenase, which is reflected in ^{13}C MRS by a decrease in hyperpolarized lactate formation. This work therefore suggests a promising application for hyperpolarized ^{13}C MR as a noninvasive method to monitor responses to targeted anticancer treatments.

References: [1] Workman P. Biochem Soc Trans 2004. [2] Ronen and Degani. Magn Reson Med 1989. [3] Kohler et al. Magn Reson Med 2007.

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