^{13}C MRS of hyperpolarized [1-^{13}C] pyruvate can differentiate between SAHA resistant and sensitive glioblastoma cells

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INTRODUCTION

The current standard of care for patients with glioblastoma (GBM), the most refractory form of brain cancer, is maximum safe resection, radiotherapy and Temozolomide (TMZ) treatment [1,2]. Despite this aggressive treatment, virtually all GBM recur, either during TMZ treatment or at a later time point following completion of therapy. Furthermore, many tumors are unresponsive to treatment. To date, there is no reliable, noninvasive method available either to evaluate efficacy of treatment or to predict response to therapy.

The so-called Warburg effect, or increased glycolysis and pyruvate-to-lactate conversion, are hallmarks of cancer metabolism [3]. Interestingly, several current GBM treatments have been shown to modulate the pyruvate-to-lactate conversion via different mechanisms: TMZ by affecting expression and activity of pyruvate kinase 2 (PKM2) and thus intracellular lactate levels [4], and inhibitors of the PI3K/mTOR pathway by inhibition of HIF-1-mediated transcription of the lactate dehydrogenase A (LDH-A) enzyme [5-7]. In addition to these treatments, histone deacetylase inhibitors (HDACi) are another class of drugs with promising clinical applications in GBM [8,9]. In this study, we investigated the potential of ^{13}C MRS of hyperpolarized [1-^{13}C] pyruvate to monitor response to treatment with the previously unexplored HDAC inhibitor SAHA in GBM cells. We also questioned if this imaging technique can provide prognostic information by distinguishing SAHA-sensitive from SAHA-resistant cells.

MATERIAL & METHODS

Cells and bioreactor. Resistance to SAHA was generated in GBM14 SAHA-sensitive (GBM14sens) GBM cells by serial passage in medium containing 5μM SAHA (Selleck Chemicals, Houston, TX). GBM14sens and GBM14 SAHA-resistant (GBM14res) cells were grown as monolayers in DMEM with 10% FBS, 100 units/mL penicillin and 100μg/mL streptomycine. GBM14res cells were maintained in a low dose of SAHA (2μM). For bioreactors, control and SAHA treated (10μM for 48h) cells were encapsulated in agarose beads and loaded in a 10mm NMR tube connected to a perfusion system as previously described [4-6].

Hyperpolarized [1-^{13}C] pyruvate. 6μL of [1-^{13}C] pyruvic acid (Isotec, Miamisburg, OH) containing15mM OX63 trityl radical (Oxford Instruments, Abingdon, UK) and Dotarem (Gd-DOTA, Guerbet, France) were hyperpolarized using a HyperSense\ DNP polarizer (Oxford Instruments) for approximately one hour [4-6].

^{13}C MRS acquisition and analysis. Dynamic sets of HP ^{13}C spectra were acquired on a 11.7Tesla INOVA spectrometer (Agilent Technologies) using 13° excitation pulses and 3sec TR. ^{13}C spectra were quantified using ACD/Spec Manager, and lactate levels (Lac) determined by normalizing maximum lactate signal to maximum pyruvate signal and cell number. All results represent the mean of at least 3 repeats ± sd. Two-tailed unpaired Student's *t*-test was used to establish the statistical significance of differences, with $p \leq 0.05$ considered to be statistically significant.

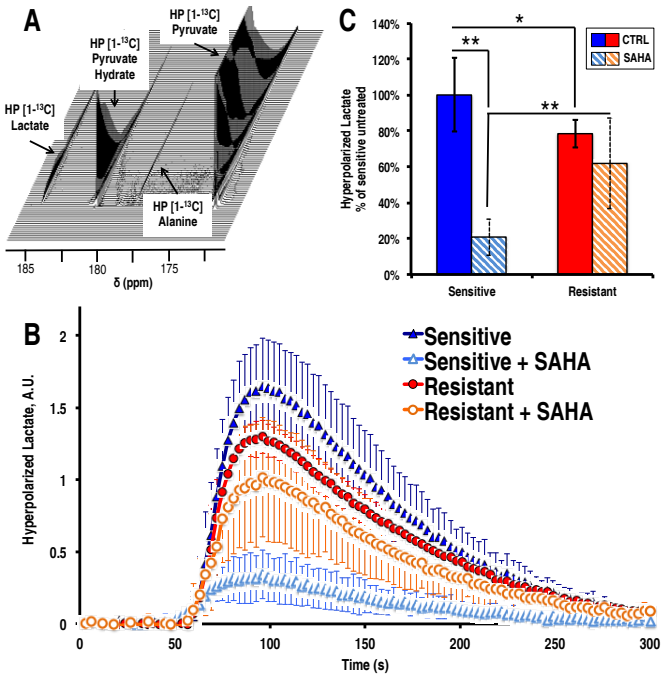

*Figure (**A**) Stack plot of HP ^{13}C spectra as measured at 11.7 Tesla in GBM14 SAHA resistant cells following injection of HP [1-^{13}C] pyruvate, showing production of HP [1-^{13}C] lactate at 184ppm. (**B**) HP lactate kinetics (A.U., normalized to maximum pyruvate and cell number) in GBM14 sensitive and resistant cells, control or after 48h of SAHA treatment. (**C**) Maximum lactate levels in GBM14 sensitive and resistant cells, control or SAHA treated. (*p<0.05; **p<0.005).*

RESULTS

Hyperpolarized [1-^{13}C] pyruvate to [1-^{13}C] lactate conversion informs on response to therapy in SAHA-sensitive GBM cells: Treatment with SAHA led to 50±3.5% cell death in GBM14sens cells, whereas only 12±0.1% cell death was observed in GBM14res cells (*p<0.05*). Following injection of hyperpolarized [1-^{13}C] pyruvate into the medium of perfused SAHA-sensitive and resistant GBM14 cells, hyperpolarized [1-^{13}C] lactate production could be detected at 184ppm (Fig. A). After treatment with SAHA, hyperpolarized [1-^{13}C] lactate production was significantly reduced in SAHA-sensitive cells at 21±10% of untreated controls (*p<0.005*; Fig. B &C). In contrast, no significant difference in hyperpolarized [1-^{13}C] lactate production could be observed between control and treated SAHA resistant cells with lactate levels for the resistant cells at 78±42% of untreated controls, (*p=0.1*; Fig. B &C).

Hyperpolarized [1-^{13}C] lactate production can differentiate between SAHA-resistant and SAHA-sensitive GBM cells, pre and post SAHA treatment: In control cells, the levels of hyperpolarized [1-^{13}C] lactate were significantly lower in SAHA-resistant GBM14 cells than in SAHA-sensitive GBM14 cells ($Lac_{GBM14res}$=79±8% of $Lac_{GBM14sens}$, *p=0.02*; Fig. B &C). In contrast, following 48h treatment with SAHA, this observation was reversed: the levels of hyperpolarized [1-^{13}C] lactate were significantly lower in SAHA-sensitive GBM14 cells than in SAHA-resistant GBM14 cells ($Lac_{GBM14sens+SAHA}$=34±30% of $Lac_{GBM14res+SAHA}$, *p<0.005*; Fig. B &C).

DISCUSSION & CONCLUSIONS

This study shows that the conversion of hyperpolarized [1-^{13}C] pyruvate to [1-^{13}C] lactate can be a useful tool in evaluating response to SAHA treatment in GBM. Cells sensitive to SAHA show a significant drop in hyperpolarized [1-^{13}C] lactate levels after 48h of SAHA treatment, whereas no change was observed in cells resistant to the agent. Furthermore, the significant difference in [1-^{13}C] lactate levels between sensitive and resistant cells pre-treatment might indicate that hyperpolarized ^{13}C MRS also has potential prognostic value. Finally, the significantly higher levels of hyperpolarized [1-^{13}C] lactate observed in resistant cells post treatment as compared to sensitive cells could potentially serve as a biomarker of acquired resistance.

In conclusion, hyperpolarized ^{13}C MRS could serve to improve existing neuro-oncology practice by enabling clinicians to rapidly determine whether GBM patients are responsive to treatment, saving valuable time while also avoiding unnecessary adverse side effects.

Acknowledgements: NIH P01 CA118816. **References**: 1. Osoba D, J Clin Oncol (2000); 2.Johnson DR, J Neurooncol (2011); 3. Warburg O, Science (1956); 4. Park and Mukherjee, Cancer Res (2014); 5.Ward, Cancer Res (2010); 6.Venkatesh, Neuro Oncology (2012); 7. Chaumeil, Neuroimage (2012); 8. Vengopal, Curr Med Chem, (2011); 9. Galanis, J Clin Oncol (2009)