Magnetic Resonance Spectroscopy: a Non-invasive Tool for Monitoring Histone Deacetylase Inhibition

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Introduction: Acetylation and deacetylation of nucleosomal core histones play an important role in the modulation of chromatin structure and the regulation of gene expression. The acetylation status of histones is controlled by the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). An imbalance in the equilibrium of histone acetylation has been associated with cancer progression. A number of HDAC inhibitors have been identified, which are able to activate differentiation, cell cycle arrest and induce apoptosis in transformed cancer cells. In addition, several clinical trials showed that HDAC inhibitors have potential anti-tumor activities for targeted therapy. However, the existing methods of assessing tumor response to HDAC inhibitors *in vivo* are either invasive, requiring biopsy, or indirect, based on blood derived markers. Hence, there exists a clear need to develop a noninvasive tool to optimize drug dose and to monitor tumor response to HDAC inhibitors. The present study addresses this need. We already showed that the fluorinated lysine derivative Boc-Lys(Tfa)-OH (BLT) is a substrate of HDAC. Here, we show that ^{19}F MRS of BLT combined with ^{31}P MRS of endogenous substrates can be used to monitor HDAC activity.

Materials and Methods: A fluorinated derivative of the clinically relevant HDAC inhibitor, suberoylanilide hydroxamic acid (FSAHA) was used to treat PC3 human prostate cancer cells. The cells were treated with a range of FSAHA concentrations from 2 to 10 μM with or without 1 mM BLT, for 24 hrs. Control cells were treated with 1 mM BLT or matching DMSO. The cells were extracted using the dual phase extraction method (1) and ^{19}F and ^{31}P MR spectra of the water-soluble metabolites were recorded on Avance DPX300 and DRX500 spectrometers (Bruker, Germany) respectively, using a 30° flip angle and 3 s relaxation delay. HDAC activity and cell viability were measured using *Fluor de Lys* (Biomol, USA) and WST-1 assays (Roche, USA) respectively. Protein expression was determined by Western blot analysis. The results represent mean ± S.D ($n \geq 3$).

Results and Discussion: With increasing concentration of FSAHA, the HDAC activity of PC3 cells decreased steadily and reached 53% relative to control at 10 μM FSAHA ($p<0.01$). The presence of BLT did not affect HDAC activity or cell viability over the range of FSAHA concentrations investigated. The ^{19}F MR spectra showed that the MR visible intracellular BLT concentration also increased steadily with increasing FSAHA concentration, reaching 32 ± 4 fmol/cell following treatment with 10 μM FSAHA, compared to 14 ± 4 fmol/cell in BLT-treated control cells ($p < 0.001$) (Fig.1A). A linear correlation was observed between the drop in HDAC activity and the intracellular BLT levels ($R = -0.82$, $p < 0.025$), indicating the potentiality of BLT as a ^{19}F MRS molecular marker to monitor HDAC inhibition (Fig.1B). ^{31}P MR spectra of the same samples were also measured and showed that phosphocholine (PC) levels increased to 16 ± 2 fmol/cell following treatment with 10 μM FSAHA, relative to a control value of 7 ± 1 fmol/cell ($p < 0.005$) (Fig.2A). The increase in PC levels was also correlated with the drop in HDAC activity of PC3 cells ($R = -0.82$, $p < 0.03$) (Fig.2B). No significant variation in any other metabolite was observed in the ^{31}P MR spectra. Western blot analysis showed that the Hsp90 client proteins, c-Raf-1 and cdk4 were both depleted following treatment with FSAHA. These results are consistent with our earlier studies showing an increase in PC levels associated with depletion of c-Raf-1 and cdk4 in cells treated with the Hsp90 inhibitor 17AAG (1). In conclusion, this study indicates that ^{19}F MRS of the fluorinated HDAC substrate BLT, in conjunction with ^{31}P MRS, can be used as a noninvasive tool to monitor HDAC inhibition in targeted tumor therapy.

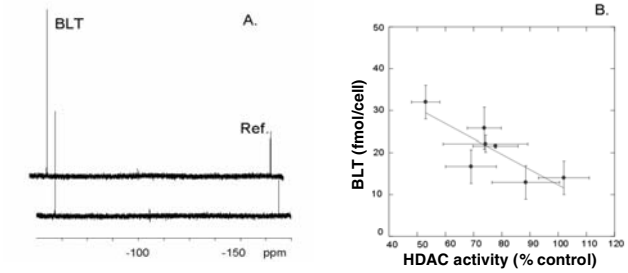

Figure 1. (A) ^{1}H decoupled ^{19}F MR spectra of the water soluble fraction of control (bottom) and FSAHA treated PC3 cells (top)

(B) Correlation between intracellular BLT levels and HDAC activity

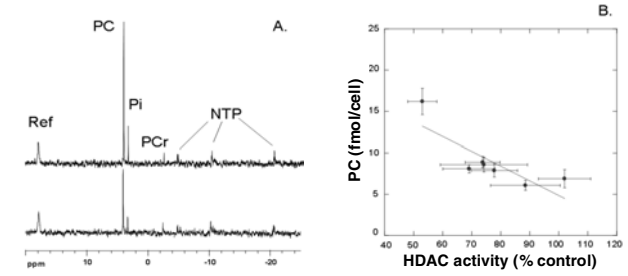

Figure 2. (A) ^{1}H decoupled ^{31}P MR spectra of the water soluble fraction of control (bottom) and FSAHA treated PC3 cells (top)

(B) Correlation between phosphocholine levels and HDAC activity

Reference: 1. Chung, Y. L., Troy, H., Banerji, U., Jackson, L. E., Walton, M. I., Stubbs, M., Griffiths, J. R., Judson, I. R., Leach, M. O., Workman, P., and Ronen, S. M. (2003) Journal of the National Cancer Institute, *95:* 1624-1633. **Acknowledgment**: We acknowledge NCI grant CA016672 for core NMR facility and thank Dr. Kumaralal K. Kaluarachchi for his help in recording NMR spectra.