Histone deacetylase inhibition by MRS: Comparison of in vitro and in vivo studies

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Introduction.
Histone deacetylase (HDAC) inhibitors (HDACi) are a novel class of promising antineoplastic agents. Our goal is to develop a magnetic resonance spectroscopy (MRS) based method to directly and noninvasively detect HDAC inhibition. We have previously shown that intracellular levels of the 19F MRS-detectable Boc-Lys-TFA-OH (BLT) are correlated with HDAC activity and that this method can be used in both cells and in vivo tumors (1, 2) to monitor HDAC inhibition. We and others have determined that in cells HDAC inhibition also leads to an increase in phosphocholine (PC) and total choline (Cho) levels, due to heat shock protein 90 (HSP90) inhibition (1,3) by HDACi. However, we show here that surprisingly, this observation does not translate to our xenograft model.

Materials & Methods.

In vivo 1H and 19F MRS on PC3 human prostate cancer xenografts was performed and analyzed as described (2), on control (DMSO treated) (n=6) and 50mg/kg suberoylanilide hydroxamic acid (SAHA) treated (n=6) subcutaneous tumor bearing mice using a 4.7T Biospec (Bruker Biospin, Billerica, MA) prior to treatment (day 0) and on days 2 and 7 of treatment, using a 1.5 cm retunable 1H/19F surface coil. 19F MRS (TR=1s, 45° flip angle, 300 averages) was performed before and after i.p. injection of 100mg/kg BLT (Advanced Chem-Tech, KY USA). The 1H/19F surface coil was then replaced with a 31P coil and a 31P MR spectrum was recorded (TR=2s, 45º flip angle, 900 averages). Western blot analysis of acetylated histone-H3 (Ac-H3), Ac-H4 and HSP90 client proteins (c-Raf and cdk4) were performed on control and treated tumor tissue lysates.

Results & Discussion.

In PC3 tumor xenografts, HDAC inhibition was confirmed by the accumulation of Ac-H3 and Ac-H4 as seen in the western blot analysis of tumors treated with SAHA for 2 days (Fig.1a). Inhibition of tumor growth was significant by day 6 of SAHA treatment (Fig.1b). The sequential 19F spectra following SAHA treatment (Fig.1c.) show an increase in tumor BLT levels further confirming HDAC inhibition. Specifically, in SAHA-treated mice, tumor BLT levels were higher by 77% and 132% on days 2 and 7 of treatment compared to pre-treatment levels (p<0.05) while BLT levels remained unchanged in DMSO controls and in normal tissue (data not shown). However, in contrast to cell findings (1) and observations in a different tumor model (3) no significant difference in the PME levels was observed in the 31P MR spectra of SAHA treated mice compared to controls (Fig.2a). This observation was also confirmed by ex-vivo high resolution MRS of tumor extracts indicating unchanged PE and PC levels (Fig.2b). Interestingly however, and consistent with the 31P MRS results, HSP90 client proteins were not depleted following SAHA treatment in this tumor (Fig.2c).

Conclusion.

In summary, our results suggest that 19F MRS of BLT is a potential non invasive method of monitoring HDAC activity in vivo whereas PC is not a reliable marker of HDAC inhibition. However the increase in PC remains an indicator of HSP90 inhibition. This inhibition occurs following treatment with some HDACi, and was observed in PC3 cells in culture, but not in the PC3 tumor model investigated here. This study thus also highlights the need for validation of cell results in vivo.