In vivo detection of histone deacetylase inhibition by MRS

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Introduction.

Novel cancer therapies increasingly target specific molecular-genetic events associated with oncogenesis. One of these targets is the enzyme histone deacetylase (HDAC) which modulates the expression of several oncogenes and tumor suppressor genes. Treatment with HDAC inhibitors (HDACIs) results in inhibition of cell proliferation, differentiation and apoptosis. Consequently, HDACIs are currently in clinical trials. However, early detection of drug molecular action is difficult and is currently performed either indirectly by monitoring blood lymphocytes or invasively by tumor biopsies. A noninvasive method for monitoring drug action and tumor response to HDACIs is therefore needed. We have previously shown that the fluorinated probe Boc-Lys(Tfa)-OH (BLT) is a cleavable HDAC substrate and that following treatment with the clinically relevant HDACI, suberoylanilide hydroxamic acid (SAHA), intracellular BLT levels, as determined by ^{19}F MRS, correlate with HDAC inhibition. We have also shown that HDAC inhibition is associated with an increase in phosphocholine (PC) and total choline (tCho) levels in cells (1, 2). Here, we have used the same methodologies to monitor HDACI treatment in a prostate cancer xenograft model *in vivo*.

Materials and Methods.

$5x10^6$ PC3 human prostate cancer cells suspended in matrigel were injected subcutaneously in male CD-1 nude mice (n=5). When an average tumor volume of 0.2 cm^3 was reached, mice were separated into 2 groups. The treated group (n=3) was treated daily with 50mg/kg SAHA (synthesized in-house) i.p., while the control mice were treated with carrier DMSO. MRS was performed on 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 (inner diameter) dual tuned ^{1}H/^{19}F surface coil. Each MR study included T_2-weighted RARE imaging, localized ^{1}H MRS by point-resolved spectroscopy (PRESS – TE/TR=20ms/3s) with (100 averages) and without (1 average) water suppression, and ^{19}F MRS (TR=1s, 45º flip angle, 300 averages) before and after i.p. injection of 100mg/kg BLT (Advanced Chem-Tech, KY USA). BLT levels determined by ^{19}F MRS were normalized to the external reference ααα-Trifluorotoluene (Sigma-Aldrich Chemical Co., MO. USA) (TFT – in a micro-cell spherical bulb placed at a permanent location relative to the coil) and expressed as % of maximum tumor BLT levels in each study. For each mouse and also for average values, a paired t-test of BLT evolution was performed for days 2 and 7 with respect to day 0. ^{1}H MRS data was analyzed by normalizing the tCho signal either to the total ^{1}H signal or to the internal water signal.

Results & Discussion.

Following SAHA treatment, inhibition in tumor growth was observed. On day 7, the average tumor volume relative to pre-treatment values was 110% in the treated group versus 150% in control group. However, no significant difference in tumor size between control and treated tumors was observed on day 2. Fig. 1a illustrates a representative *in vivo* ^{19}F spectrum obtained from a tumor, following BLT injection i.p., in a SAHA treated mouse. The average temporal evolution of tumor BLT signal on days 0, 2 and 7 of SAHA treatment is shown in Fig. 1b, which demonstrates that steady state tumor BLT levels increased significantly following SAHA treatment compared to day 0 (p=0.0001 for day 2 and p=0.04 for day 7). In contrast, BLT levels did not increase significantly in carrier treated controls (data not shown). These results are consistent with our *in vitro* observations on PC3 cells (1), indicating higher BLT levels in SAHA-treated cells. Importantly, the increase in BLT levels was observed by ^{19}F MRS on day 2, prior to a detectable inhibition in tumor growth following SAHA treatment. The difference in the kinetics of tumoral BLT accumulation on days 2 and 7 is noteworthy. It could be indicative of vascular collapse by day 7 resulting in a slower accumulation of BLT in the tumor compared to day 2 and consistent with reported VEGF inhibition by HDACIs. Fig.2 illustrates a localized *in vivo* ^{1}H spectrum from a SAHA treated mouse, on day 0. The data indicated an increase in tCho/total signal as well as tCho/lipid (not shown) on day 2, followed by a return to pre-treatment levels on day 7. However, tCho normalized to the internal water signal remained, within experimental error, unchanged following treatment, in contrast to findings in cells. Further studies, including high resolution MRS of tumor extracts, are necessary to fully understand these observations.

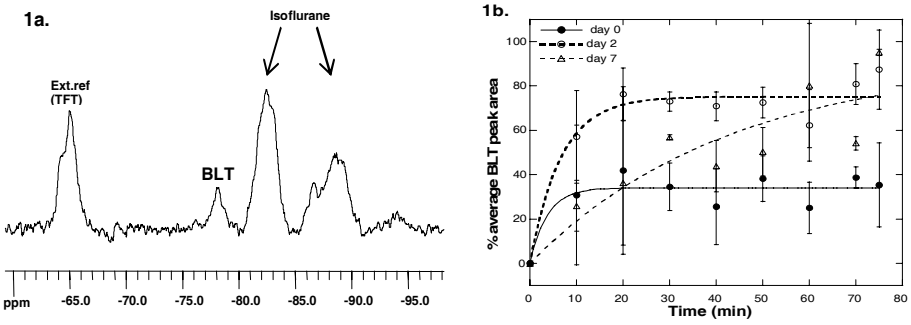

Figure 1. (a) Representative *in vivo* ^{19}F spectrum of a SAHA treated mouse after BLT injection (b) Temporal evolution of average tumor BLT signal in SAHA group (n=2)

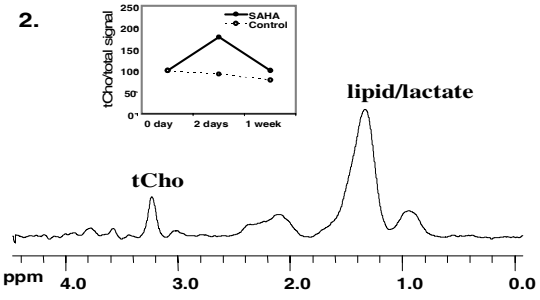

Figure 2. Representative *in vivo* ^{1}H spectrum of a SAHA treated mouse on day 0. The insert shows the tCho/total ^{1}H signal in SAHA treated and control mice (n=2).

Conclusion.

In summary, our findings confirm the potential of ^{19}F MRS of BLT as a noninvasive indicator of HDAC activity *in vivo* and highlight the importance of *in vivo* validation of observations made in cells.

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