Activation of Choline Kinase and Phospholipase C in HDAC Inhibition

C. S. Ward[1], J. Hwang[1], and S. M. Ronen[1]
[1]Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, California, United States

Purpose

Histone deacetylase (HDAC) inhibitors are a recently developed class of anticancer drugs that have had success in clinical trials (1). Clinical use of therapeutic drugs are significantly enhanced when a noninvasive means are available to longitudinally monitor the efficacy of treatment. Previous studies have reported increased phosphocholine levels in response to treatment with HDAC inhibitors suberoylanilide hydroxamic acid (SAHA) and LAQ-824 (2,3). Yet the mechanism remains poorly understood and requires characterization for phosphocholine to be used as a validated biomarker. The aim of this study is to examine the modulation of choline metabolism by SAHA through a combination of magnetic resonance spectroscopy and enzymatic studies.

Methods

The effect of HDAC inhibition was studied in MCF-7 breast adenocarcinoma cells following 48-hr incubation with 10 μM FSAHA (a homemade fluorinated derivative of SAHA with identical activity (2)). Prior to MR extract studies, cells were cultured in medium containing [1,2-^{13}C]-labeled choline at its normal concentration (64.1 μM) for 6 hr. Cells were extracted using the dual-phase extraction method (4). MR spectra were recorded on a 600-MHz Varian spectrometer at 25°C. ^{13}C and ^{31}P spectra were obtained using a 30° pulse-acquire sequence with proton decoupling, and a 3 sec relaxation delay. Relative metabolite concentrations were determined by integration and normalized to external reference (TMS, ^{1}H and ^{13}C; MDPA, ^{31}P). Choline kinase (ChoK) activity in cell lysates was measured by ^{1}H MR, as previously described (5). ChoKα gene expression was determined by RT-PCR using glyceraldehyde 3-phosphate hydrogenase (GAPDH) as the housekeeping gene. Phosphatidylcholine-specific phospholipase C (PLC) activity was determined using the EnzChek Direct Phospholipase C Assay (Invitrogen). Fluorescence (485 nm excitation, 535 nm emission) was measured by SpectraFluor Plus spectrofluorometer (Tecan).

Results

Following HDAC inhibition, cell proliferation was inhibited by 50 ± 5% (n=3) at 48 hr. MR data revealed metabolic changes following 48-hr treatment. ^{1}H MRS data showed an increase of total choline (tCho) to 189% relative to control. ^{31}P MRS data revealed an increase in PC to 204% and in glycerophosphocholine (GPC) to 137%. ^{13}C-labelled PC in treated cells increased to 204%. ChoK activity increased with treatment to 167 ± 27% (n=2) of control. RT-PCR indicated an increase in mRNA expression of ChoKα to 356 ± 16% (P<0.001, n=4). Additionally, phospholipase C activity increased with treatment to 127 ± 12% (P=0.01, n=3).

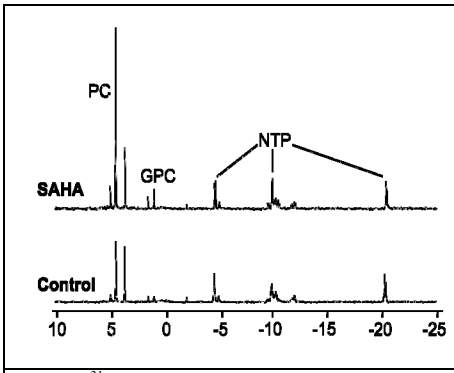

Fig. 1: ^{31}P spectra of control and FSAHA-treated MCF-7 cell extracts, illustrating increased PC and GPC levels.

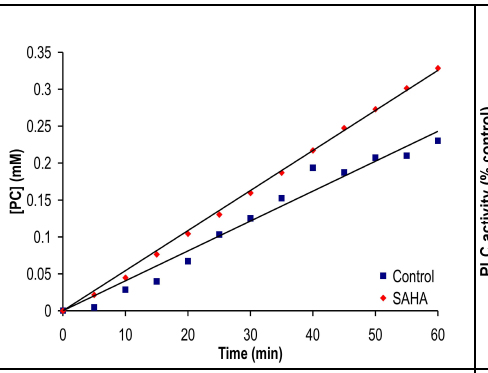

Fig. 2: Peak areas of PC in control and SAHA-treated cell lysates over a 1-hr period, showing elevated ChoK activity with treatment.

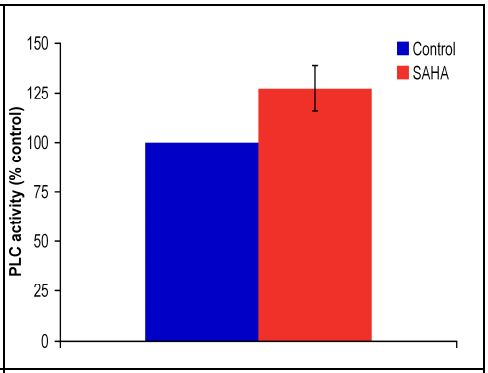

Fig. 3: PLC activity of control and SAHA-treated cell lysates, indicating higher PLC levels.

Discussion

This study confirmed previous findings of increased choline-related metabolites following HDAC inhibition, while providing new insight into the underlying mechanism. Analysis of MRS data revealed increases in tCho, PC and GPC. HDAC inhibition led to activated *de novo* PC synthesis, as ^{13}C-labelled PC increased with treatment. Increased PC synthesis suggests activated ChoK, which was verified by ChoK activity and ChoKα expression. In addition, PC-PLC activity was elevated with treatment, indicating increased breakdown of phosphatidylcholine to PC. HDAC inhibition has previously been reported to induce CTP:choline-phosphate cytidylyltransferase (CCT) expression (6); studies are currently under way to verify elevated CCT activity by MRS.

References

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Acknowledgments

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