The Effects of Hemicholinium-3 on Phenylbutyrate Induced Changes in Lipid Metabolism

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Introduction

Differentiation therapy is based on the idea that if neoplastic transformations result from defects in cellular differentiation, then induction of tumor differentiation would result in a reversal of malignancy. Phenylbutyrate (PB) is a differentiating agent that induces G1 cell cycle arrest, subsequently driving tumor cells into either terminal differentiation or apoptosis[1-3]. We have previously shown that the effects of PB on prostate cancer cells can be detected with MR spectroscopy as increases in resonances from mobile lipids, total choline (tCho), and glycerophosphocholine (GPC)[1]. Changes in these phospholipid metabolites have been shown to be associated with increases in choline transport and/or metabolizing enzymes[4-7]. Hemicholinium-3 (HC-3) is a potent and selective choline uptake and choline kinase blocker[8]. In this study, we examined the effects of HC-3 on prostate cancer cells to test the hypothesis that the PB-induced mobile lipid accumulation is mediated through the choline kinase pathway.

Methods

Cell Culture: DU145 human prostate adenocarcinoma cells were cultured in MEM (10% FBS in 5% CO_2 95% O_2 at 37°C). *Diffusion-Weighted (DW) NMR Spectroscopy:* Biosilon microcarriers (1.8 grams) were inoculated with 5.0 x 10^6 cells/ml and cultured for 48 h under standard conditions. The microcarriers were transferred to a 10 ml MR tube and perfused with medium (1.8 ml/min) equilibrated with 5% CO_2 in O_2. MR spectra were acquired on a Varian 9.4 T INOVA spectrometer equipped with R^2I 100 G/cm gradients and a 10 mm multinuclear probe. Proton metabolite spectra were acquired using a DW pulse sequence with CHESS water suppression (TE, 21 ms; TM, 89 ms; TR, 2s; dephasing gradient (δ), 3 ms; diffusion gradient (g_{diff}), 9 G/cm; spectral width, 4 kHz; data size, 4K; NS, 512). ^{31}P MR spectra (2500 scans) were acquired with TR = 2 s; data size, 2K; spectral width 5 kHz. ^{1}H and ^{31}P MR spectra were alternately acquired for 16 h and integrated resonance intensities compared to baseline. Hemicholinium-3 (100 μM) was added to the growing cells 24 hours prior to MR acquisition, and PB (10 mM) was added at the end the second hour of MR acquisition. Each condition was performed a minimum of three times. *Caspase-3 Activity:* DU145 cells seeded in 6 cell culture petri dishes per condition were pretreated for 24 hours with 100 μM HC-3, followed by the addition of 10 mM PB and incubation for 16 h. The EnzCheck Caspase-3 Assay Kit #1 (Mol. Probes) was used to determine the activation of caspase-3 by cleavage of Z-DEVD-AMC.

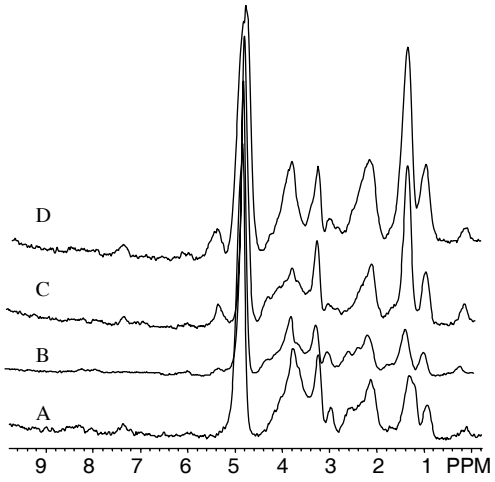

Figure 1: DW ^{1}H MR spectra of DU145 cells after 16 h perfusion with A) culture medium or culture medium containing B) 100 μM HC-3, C) 100 μM HC-3 and 10 mM PB, D) 10 mM PB.

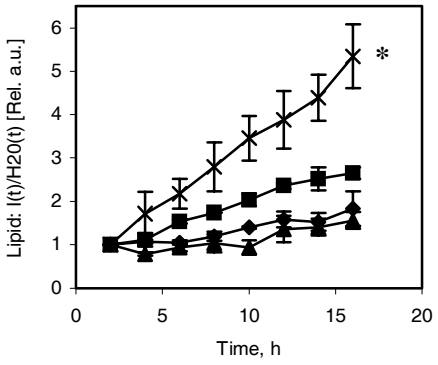

Figure 2: Time course of the fitted 1.3 ppm peak of DU145 cells. Diamonds: control cells; triangles: cells treated with 100 μM HC-3; squares: 100 μM HC-3 and 10 mM PB; crosses: 10 mM PB. * $p < 0.05$.

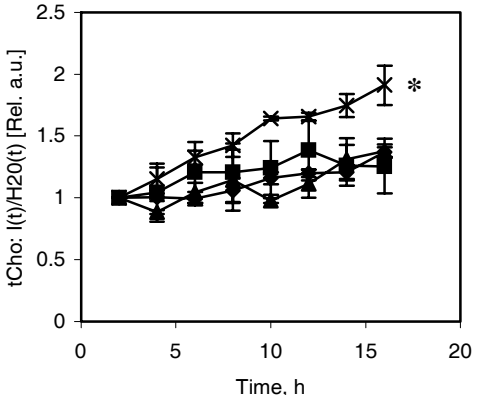

Figure 3: Time course of the fitted 3.2 ppm peak of DU145 cells. Diamonds: control cells; triangles: cells treated with 100 μM HC-3; squares: 100 μM HC-3 and 10 mM PB; crosses: 10 mM PB. * $p < 0.05$.

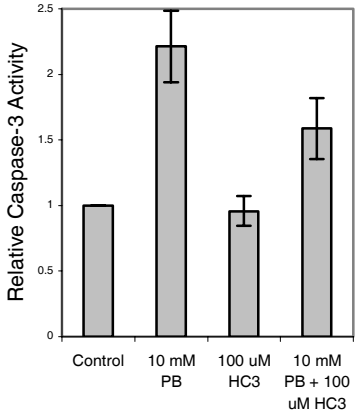

Figure 4: Caspase-3 activation.

Results and Discussion

Changes in mobile lipid and tCho metabolites are detected in DU145 prostate cancer cells when treated with PB (Figure 1). PB caused a significant increase in the mobile lipid resonance at 1.3 ppm (Figures 1 and 2). This resonance arises from the methylene -CH_2- on fatty acyl chains of neutral lipids (triglycerides) with contributions from the methyl group of lactate[1]. PB treatment also caused a significant increase in the total choline (tCho) resonance at 3.2 ppm (Figures 1 and 3)[1]. Pre-treatment with HC-3 significantly reduced PB-induced spectral changes in mobile lipids and tCho (Figures 1, 2, 3), $p < 0.05$. Decreases in tCho would be expected if HC-3 inhibited the formation of phosphocholine from exogenous choline by choline kinase[9]. However, the observation that PB-induced mobile lipid increases are decreased by HC-3 indicates that this early-stage inhibition of the Kennedy pathway of phospholipid biosynthesis may also affect downstream triglyceride synthesis. Furthermore, PB treatment of DU145 cells caused a significant increase of caspase-3 activity that is attenuated by HC-3 pre-treatment (Figure 4). These results indicate that HC-3 and other choline analogs may be useful tools in non-invasively elucidating the altered metabolic pathways in tumors undergoing apoptosis resulting from differentiation agent treatment.

References: [1]Milkevitch, M. *BBA, 1734, 1*, 2005. [2]Samid, D., *Cancer Res, 52, 1988*, 1992. [3]Liu, L., *J. Invest. Derm., 103, 335,* 1994. [4]Podo, F. *NMR Biomed,12, 7,* 1999. [5]Eliyahu, G. *Proc. ISMRM, 13, 129*, 2005. [6]Al-Saffar, N. M. *Cancer Res, 66, 1*, 2006. [7]Glunde, K. *Cancer Res, 65, 23,* 2005. [8]Hernández, R., *Oncogene, 55, 19*, 1997. [9]Daly, P. *J Biol Chem, 262, 14875*, 1987.

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