Essential Role of Phospholipase A2 in Phenylbutrate-Induced Activation of Phospholipid Metabolism

D-J. Leung[1,2], N. J. Beardsley[1], T. M. Mawn[1], and E. J. Delikatny[1,2]

[1]Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States, [2]Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States

Introduction

The goal of differentiation therapy is to induce terminal differentiation in tumorigenic cells. Phenylbutyrate (PB), marketed as Buphenyl, is a differentiating agent with low adverse cytotoxicity that causes G1 cell cycle arrest, subsequently driving tumor cells into either terminal differentiation or apoptosis[1-3]. The metabolic response of prostate cancer cells to PB treatment can be detected with MR spectroscopy as increases in resonances from mobile lipids, total choline (tCho), and glycerophosphocholine (GPC)[1, 4]. The observed metabolic changes appear to be intrinsic to the death response, since treatment with a PPARγ antagonist reverses both the PB-induced increases in lipid metabolites as well as cell cycle arrest and apoptosis[5]. Increases in GPC are indicative of the actions of phosphopholipase A2 (PLA2), which catalyzes the first step in the hydrolysis of phosphatidylcholine, the subsequent actions of lysophospholipase producing GPC. Changes in choline transport and/or metabolizing enzymes have been shown to affect these phospholipid metabolites in *in vivo* and cell-based models[6-9]. However, the isoform of PLA_2 responsible for PB-induced GPC release is currently unknown, as is whether this enzyme is responsible for the regulation of the downstream mobile lipid accumulation. $cPLA_2$ is a cytoplasmically-expressed phospholipase that acts on membrane phospholipids and is a critical mediator in arachidonic acid dependent cell signaling pathways. In this study, we examined the effects of trifluoromethyl ketone (AACOCF3), a selective $cPLA_2$ inhibitor, on prostate cancer cells to test the hypothesis that the PB-induced mobile lipid accumulation is mediated through the phospholipase 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. The microcarriers were transferred to a 10 mm 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 Doty 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, 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}PMR spectra were alternately acquired for 16 h and integrated resonance intensities compared to baseline. AACOCF3 (10 µM) was added to the perfused cells at 0 h prior to MR acquisition, and PB (10 mM) was added at the end the second hour of MR acquisition. *High-resolution ^{1}H MRS:* DU145 cells were treated under corresponding conditions and extracted with perchloric acid. Spectra were obtained with a pulse-acquire sequence with a 45° flip angle, TR = 8.8 s, SW =6775 Hz, NP = 65536, and NT = 128 on a Bruker Avance DMX400 spectrometer. A capillary containing sodium 3-(trimethylsilyl)-[2,2,3,3,-2H4]-1-propionate (TSP) was inserted as an external reference into the 5 mm NMR tube.

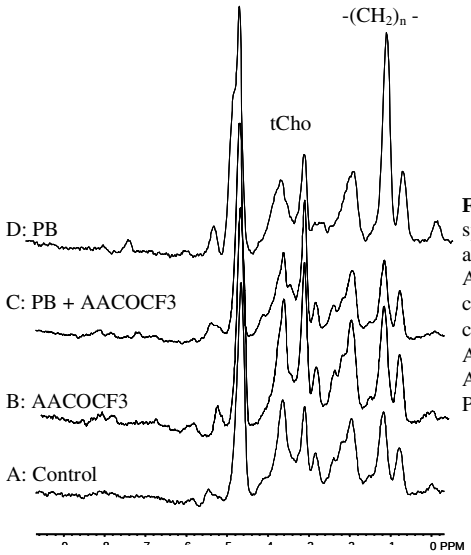

Figure 1: DW ^{1}H MR spectra of DU145 cells after 16 h perfusion with A) culture medium or culture medium containing B) 10 µM AACOCF3, C) 10 µM AACOCF3 and 10 mM PB, D) 10 mM PB.

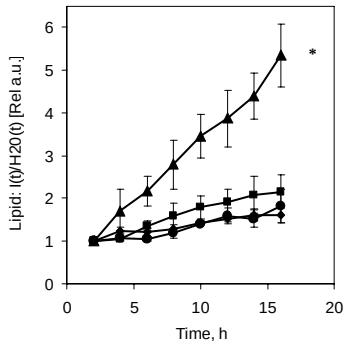

Figure 2: Time course of the fitted 1.3 ppm peak of perfused DU145 cells. Circles: control cells; diamonds: cells treated with 10 µM AACOCF3; squares: 10 µM AACOCF3 and 10 mM PB; triangles: 10 mM PB.

Results and Discussion

Spectral changes in mobile lipids and tCho caused by PB treatment can be reversed with inhibition of $cPLA_2$ (Figures 1 and 3). 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 minor contributions from the methyl group of lactate[1]. PB treatment also caused increases in the total choline (tCho) resonance at 3.2 ppm (Figures 1 and 3)[1]. Pretreatment with AACOCF3 significantly reduced PB-induced spectral changes in mobile lipids and tCho (Figures 1-3). Although changes in tCho detected in cells perfused with AACOCF3 were subtle, cell extracts analyzed with high-resolution MRS showed clear changes in choline-containing metabolites (Figure 3). Specifically, AACOCF3 inhibited the GPC formation caused by PB and decreased the GPC/PC ratio three-fold, confirming the role of $cPLA_2$ in GPC production. The observation that PB-induced mobile lipid increases are attenuated by AACOCF3 indicates that this early stage inhibition of a critical signaling phospholipase also affects downstream triglyceride synthesis. It has been shown that $cPLA_2$ is required in chemotherapeutic-induced cell death in renal carcinomas[10]. This is consistent with the current findings of altered phospholipid profiles and these results suggest that $cPLA_2$ activity may play a role in the cellular response to differentiation therapy.

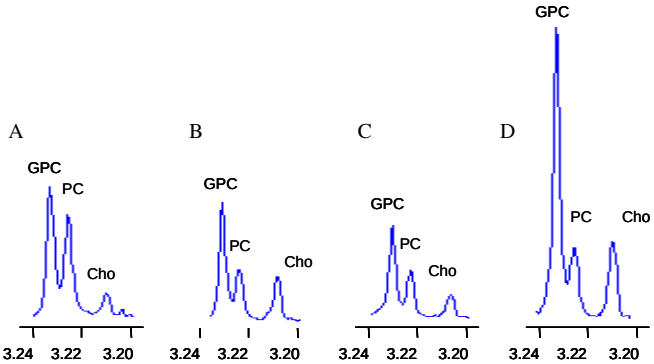

Figure 3: Expanded regions of ^{1}H MR spectra of cell extracts from A) control cells, cells treated with B) 10 µM AACOCF3, C) 10 µM AACOCF3 and 10 mM PB, D) 10 mM PB.

References

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