Phospholipase Activity Accompanies Glycerophosphocholine Production in Phenylbutyrate-Treated Prostate Cancer Cells

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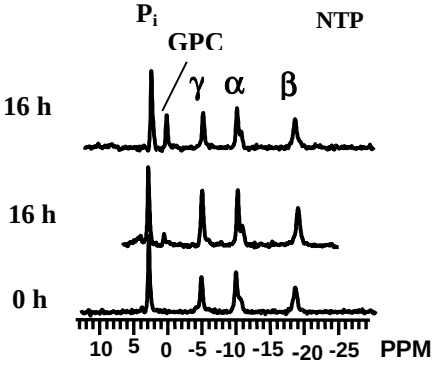
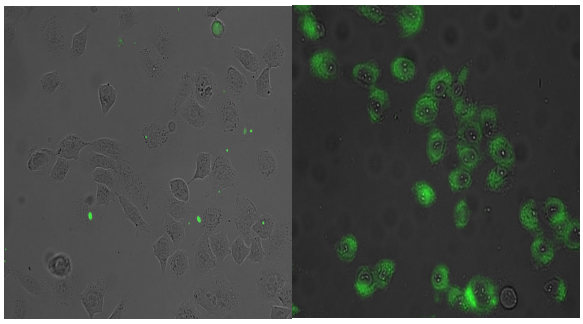
Introduction

Phospholipases catalyze phospholipid breakdown and perform a number of critical regulatory functions within cells and tissues[1,2]. Phospholipase A2 (PLA2) levels are increased during inflammation, hyperproliferation and apoptosis. PLA2 has been implicated in cancer, arthritis and Alzheimer's disease. Increases in glycerophosphocholine (GPC) have been observed in a number of cell lines and tumors treated with chemotherapeutic agents[3-6]. Treatment of the prostate cancer cell line DU145 with phenylbutyrate (PB), a differentiating agent in clinical trials for treatment of hematological and solid tissue malignancies, results in a number of alterations in lipid metabolism including an increase in GPC and total choline in ^{31}P and ^{1}H NMR spectra respectively[7]. Since one of the main pathways for GPC production is through phosphatidylcholine catabolism via the subsequent actions of phospholipase A2 and lysosphospholipase, we hypothesized that PLA2 activity could account for these observed spectroscopic changes. To verify the information obtained by MRS *in situ*, we used reporter probes that release fluorescent moieties upon phospholipase cleavage. In this study, we monitored the effects of PB with ^{31}P and diffusion-weighted (DW) ^{1}H NMR, and examination of phospholipase activity with PED-6. PED-6 is a self-quenching fluorescent phospholipid analog incorporating a BODIPY label at the *sn*-2 position of the phospholipid glycerol backbone, with a *p*-dinitrophenyl quencher attached to the ethanolamine headgroup. The molecule has low intrinsic fluorescence, but cleavage of the dye-labeled chain destroys the intramolecular quenching, leading to increased fluorescence[8-10].

Methods

DU145 human prostate adenocarcinoma cells were cultured in MEM (10% FBS in 5% CO_2 in air at 37°C). Biosilon microcarriers were inoculated with 3.0 x 10^6 cells/ml and cultured for 48 h under standard conditions. DU145 cells (2-3 x 10^7 on 3.5 ml microcarriers) were transferred to a 10 ml MR tube and perfused with medium (1.5 ml/min) equilibrated with 5% CO_2 in O_2. PB was added at 10 mM after baseline spectral acquisition. 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}), 16 G/cm, spectral width, 4 kHz; data size, 2K; NS, 256). ^{31}P MR spectra (2000 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. *Phospholipase Detection Experiments.* Liposomes were made from 1 mg of PED-6 and 50 mg of egg yolk phosphatidylcholine in chloroform, evaporated under N_2, rehydrated in PBS and vortexed (10') before extrusion 20 times through 100 nm and 50 nm filters. PED-6 liposomes (50 µl, 50 min, 37C) were added to PB-treated cells and observed with fluorescence microscopy (λ^{ex}= 505, λ^{em}= 515).





Left: Figure 1. ^{31}P MR of perfused DU145 cells. Bottom trace, untreated control. Middle trace, untreated control after 16 h of perfusion. Top trace, after 16 h perfusion with 10 mM PB. ***Middle: Figure 2a.*** Control DU145 cells treated with PED-6 liposomes. ***Right: Figure 2b.*** PB-treated DU145 cells (10 mM, 5 h) treated with PED-6 liposomes.

Results and Discussion

Treatment with PB caused a significant, time-dependent increase in the GPC resonance in ^{31}P MR spectra of DU145 cells (see Fig 1 for spectra), that correlated with an increase in total choline in DW ^{1}H spectra (data not shown). This increase was continuous over 16 h and was observed after 1h. The application of PED-6 liposomes caused a dramatic increase in fluorescence as early as 5 hours after PB treatment (Fig 2b) that was not observed in controls (Fig 2a), indicating the activation of PLA2. The observed fluorescence was cytoplasmic and not nuclear, implying that the activation was due to cytoplasmic PLA2. Increases in GPC following cytotoxic drug treatment have been observed in several studies. Koutcher showed increases in GPC in mammary tumors treated with cyclophosphamide or radiation[3,11]. Hakumaki *et al.* measured increases in lysophosphatidylcholine and polyunsaturated NMR-visble lipids during HSV-tk induced-apoptosis that were suggested to be due to the action of PLA2 activity on membrane phosphatidylcholines[4]. Delikatny showed that MR-visible lipid increases in human breast cells treated with tetraphenylphophonium chloride were accompanied by increases in GPC at the expense of phosphocholine (PC)[5,6]. Galons suggested that low pH-induced GPC at the expense of PC may be attributed to increased PLA2 and lysophopholipase[12]. This study shows unequivocally that GPC increases in this model system are correlated with increased cytoplasmic fluorescence. The results from these two imaging modalities provides evidence that metabolic changes detected by NMR can be attributed to cytoplasmic phospholipase activation, most likely cPLA2 with potential contributions from phospholipase C. This approach shows promise for deciphering the often contradictory changes in choline levels observed with MR spectroscopy. Given the importance of PLA2 to a wide range of illnesses, this study has significant implications for *in-vivo* imaging of disease processes and treatment regimens.

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