Comparison of MRS with fluorescence for molecular imaging and determination of phospholipase isoforms

D-J. Leung[1], T. Mawn[1], and E. J. Delikatny[1]

[1]Department of Radiology, University of Pennsylvania, Philadelphia, PA, United States

Introduction

Magnet resonance spectroscopy is a versatile tool for investigating tumor metabolism for the prediction and measurement of response to therapy. However, factors orchestrating these treatment effects are often difficult to delineate molecularly. In phenylbutyrate (PB) differentiation therapy, tumor cells are driven into G1 cell cycle arrest, followed by either terminal differentiation or apoptosis.[1, 2] The metabolic response of DU145 prostate cancer cells can be detected with MRS as increases in resonances from mobile lipids, total choline (tCho), and glycerophosphocholine (GPC).[1, 2] Choline phospholipid metabolism has been shown to be a significant factor in phenotype aggressiveness in a panel of tumor types, specifically breast and prostate cancer.[3] Though this may account for increased levels of choline transport and phosphorylation, phosphocholine catabolism to GPC while undergoing drug treatment remains unexplained. Increases in GPC are indicative of the actions of phospholipase A2 (PLA2), which catalyze the first step in the hydrolysis of phosphatidylcholine, the subsequent actions of lysophospholipase producing GPC. Although phospholipases are generally accepted to be responsible for these lipid changes, it remains unclear which isoforms facilitate the MR-visible alterations accompanied by increased tumorigencity or drug treatment. Here, we present the modulation of MR-visible metabolic response through the inhibition of the calcium-independent phospholipase A2 (iPLA2) and the cytosolic phospholipase A2 (cPLA2) isoforms and their differential roles in mediating lipid responses using the specific inhibitors BEL and AACOCF3, respectively. At the same time, a separate pathway of overall phospholipase A2 (sPLA2 isoform) activity is shown by kinetic fluorescence activation *in vitro*. This not only suggests the possibility of using MRS and optical methods to compare findings, but also the ability to characterize enzyme isoforms relevant to tumor drug response collaterally.

Methods

Cell Culture: DU145 human prostate adenocarcinoma cells were cultured in MEM (10% FBS in 5% CO_2 95% O2 at 37°C). 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. *NMR Spectroscopy:* MR spectra were acquired on a Varian 9.4 T INOVA spectrometer equipped with a 10 mm Doty multinuclear probe. ^{31}P MR spectra (2500 scans) were acquired with TR = 2 s; data size, 2K; spectral width 5 kHz. ^{31}PMR spectra were acquired for 16 h and integrated resonance intensities compared to baseline. AACOCF3/BEL (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. *Thin layer chromatography:* Cells were treated with 10 mM PB for 4 hours and then scraped for whole cell lysate preparation depending on the condition. Samples were then incubated with PED-6 for 3 hours and were spotted on TLC plates and run in a chloroform:methanol (5:1) solvent. *Fluorescence activation:* Similarly prepared cell lysate samples were measured with a Molecular Devices plate reader with an excitation and emission of 488 nm and 515 nm, respectively. *Image processing:* Spectra were analyzed with MestReC and TLC plates analyzed with ImageJ.

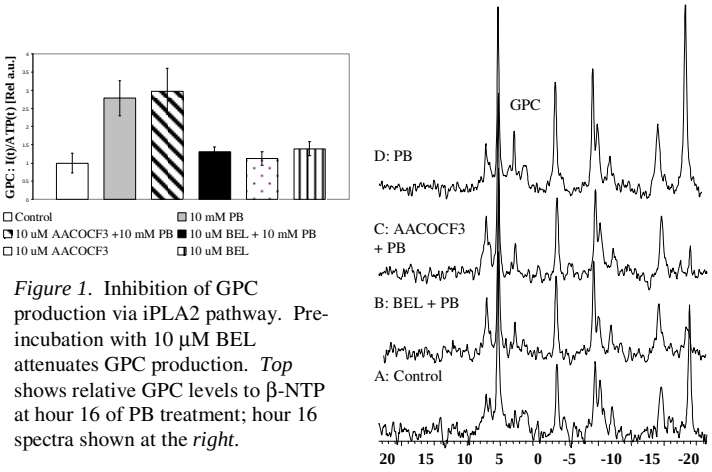

Figure 1. Inhibition of GPC production via iPLA2 pathway. Pre-incubation with 10 µM BEL attenuates GPC production. *Top* shows relative GPC levels to β-NTP at hour 16 of PB treatment; hour 16 spectra shown at the *right*.

Results and Discussion

Spectral changes in GPC caused by PB treatment can be reversed with inhibition of iPLA2 and not cPLA2, pointing to this crucial enzyme necessary for undoing the well-known GPC/PC switch (Figure 1). Also, PB caused a significant increase in the mobile lipid and total choline resonances in proton spectra.[4] However, inhibition of iPLA2 and cPLA2 caused differential changes in mobile lipid and total choline changes.[4] It appears that early stage inhibition of these critical signaling phospholipases affect downstream triglyceride synthesis in drug-induced cellular remodeling. Therefore, it is hypothesized that iPLA2 and/or cPLA2 isoforms may be upregulated and activated when PB is introduced. In contrast, commercial phospholipase A2 sensitive cocktails suggest decreased sPLA2 activity by optical detection methods (Figure 2 and 3) in PB-treated cells. Taken all together, the phospholipase family represents a diverse family of enzymes that have varied modes of action and that their potential mechanisms of regulation can be teased out by multi-modality approaches.

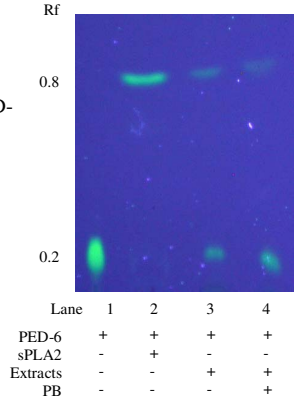

Figure 2. Thin layer chromatography of PED-6. Rf = 0.8 is the cleaved PED-6 product; rf = 0.2 is the uncleaved PED-6.

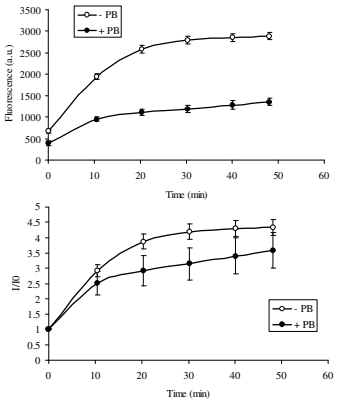

Figure 3. Phospholipase A2 activation of PED-6 by PB and non-PB treated cell extracts. *Top* shows fluorescence over time; *bottom* shows relative increases.

References

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Acknowledgements

NIH R01-CA-114347 and R25-CA-101871.