Lovastatin Potentiates Phenylbutyrate-Induced NMR-Visible Lipid Accumulation in Perfused DU145 Prostate Cancer Cells

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Abstract
Previous studies have shown that treatment of human prostate cells with the differentiating agent phenylbutyrate causes increases in neutral lipids as measured by diffusion-weighted 1H NMR, electron microscopy and Nile Red staining. In this study, DU145 cells were treated with PB and the HMG-CoA reductase inhibitor lovastatin, and the results monitored by 1H and 31P NMR. Lovastatin caused a significant time and dose dependent potentiation of NMR-visible lipids, evidenced by an increased 1.3 ppm resonance. Total choline at 3.2 ppm also increased with PB or PB/lovastatin treatment and 31P NMR indicated that this was due to increased glycerophosphocholine.

Introduction
Phenylbutyrate (PB) is a differentiating agent in clinical trials for the treatment of hematological and solid tissue malignancies. PB, and its prodrug phenylacetate (PA) have been shown to inhibit cholesterol synthesis, cause fatty acid accumulation in cultured cells and induce adipocyte differentiation. PA inhibits 5-pyrophosphomevalonate decarboxylase, a key enzyme in cholesterol synthesis. Lovastatin, another hyperlipidemic agent, inhibits 3-hydroxy-3-methylglutaroyl CoA reductase, which blocks production of mevalonate, a key intermediate in cholesterol synthesis. Studies have shown that lovastatin (lov), can potentiate PA-induced inhibition of proliferation of human glioma cells. Inhibition of cholesterol biosynthesis will also interfere with protein farnesylation and prenylation thereby disrupting key proteins such as G-proteins involved in signal transduction and regulation of cell replication. Cholesterol is also required for synthesis of steroid hormones. Therefore, inhibition of this key pathway may have a pronounced effect on cellular replication and regulation of metabolism. In this study, we examine the metabolic effects of concurrent treatment with PB and lov on DU145 prostate cancer cells using DW 1H and 31P NMR.

Methods
DU145 human prostate adenocarcinoma cells were cultured in MEM with 10% FBS, 2 mM glutamine and antibiotics in 5% CO2 in air at 37°C. Biosilon microcarriers were inoculated at 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 mm screw-cap NMR tube outfitted to immobilize microcarriers/cells beneath a filter to which a medium inlet tube is also situated. The cells were continuously perfused with growth medium (1.5 ml/min) equilibrated with 5% CO2 in O2. PB was added at 10 mM, or lov at 2.5 - 10 µM after 1 h acquisition. NMR spectra were acquired with a Varian 9.4 T Unity spectrometer equipped with a R1 100 G/cm gradients. Proton NMR metabolite spectra were acquired using the DW pulse sequence with a stimulated echo with CHESS water suppression applied before the 1st and 3rd rf pulses (TE, 11 ms; TM, 100 ms; TR, 3 s; duration of dephasing gradient (Gdiff), 3 ms; diffusion gradient strength (Gdiff), 8 G/cm; spectral width, 4000 Hz; data size, 2 K; number of acquisitions, 256. 31P NMR spectra (2000 scans) were acquired using a 45° rf pulse, with a repetition time of 1 s; data matrix, 2 K; spectral width, 5000 Hz; and using a line broadening of 20 Hz. Proton and 31P NMR spectra were alternately acquired for 16 h. Integrated resonance intensities from baseline corrected spectra were plotted relative to t=0.

Results and Discussion
Diffusion weighted 1H NMR spectra of treated and control DU145 cells are shown in Fig 1. PB causes a significant increase in the mobile lipids resonance at 1.3 ppm, which has been previously shown to arise predominantly from the methylene CH2 on fatty acyl chains of neutral lipids (Figure 2). There is a concentration-dependent increase in lipid resonances potentiated by the addition of lovastatin at 2.5 and 10 µM (Figure 2). Both PB and PB + lov cause a similar time dependent increase in the total choline resonance at 3.2 ppm (Fig 1). 31P NMR shows an increase in the phosphodiester, but not the phosphomonoester region of the spectrum, indicating that glycerophosphocholine (GPC) is the major contributor to the choline changes observed in 1H spectra, and that catabolism of phospholipids may be responsible. Depletion of cholesterol may inhibit proliferation by disrupting membrane structure or by increasing membrane fluidity. Both PB and lov activate peroxisome proliferator-activated receptors, ligand-activated transcription factors that regulate the expression of several genes that control fatty acid metabolism, including peroxisomal β-oxidation. Lovastatin and PA in combination may offer a novel approach to the treatment of prostate cancer.


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Left: Figure 1. DW 1H NMR of perfused DU145 cells. Lowest trace, untreated control. Middle trace, after 16 h of treatment with 10 mM PB. Top trace, after 16 h of treatment with 10 mM PB and 10 µM lovastatin. Notice increase in lipid at 1.3 ppm and cho at 3.2 ppm.

Figure 2. Time course of the integrated intensity of the 1.3 ppm peak of DU145 cells.