The PPARγ Inhibitor GW9662 Attenuates Phenylbutyrate-Induced Lipid Metabolite Accumulation in DU145 Prostate Cancer Cells

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Introduction

Differentiating agents are chemotherapeutic agents that can either cause reversion of the malignant phenotype or the triggering of apoptosis. Phenylacetate (PA) and phenylbutyrate (PB) are differentiating agents that induce G1 cell cycle arrest, cytostasis, differentiation and apoptosis in a variety of cell types, including prostate adenocarcinomas[1-4]. The mechanism of PA- and PB-induced differentiation is presently under investigation; however they have been shown to have coordinate effects on lipid metabolism. These effects include the inhibition of the cholesterol biosynthetic pathway, inhibition of protein prenylation and histone deacetylase, and the induction of peroxisome proliferator-activated receptors (PPARs) alpha and gamma[5-8]. We have previously shown that the effects of PA and PB on lipid metabolism can be detected with MRS as increases in resonances from mobile lipids, total choline and glycerophosphocholine (GPC)[1]. PPARs are ligand-activated transcription factors, implicated in growth control and differentiation[8,9]. PPARα regulates fatty acid metabolism and is highly expressed in liver, kidney and intestine. PPARγ exists in two isoforms (PPARγ1 and PPARγ2). PPARγ2 is expressed exclusively in adipose tissue and is a potent regulator of adipocyte differentiation [10]. Since one manifestation of adipocyte differentiation is increased uptake and synthesis of triglycerides, we hypothesized that PPARγ antagonists may modulate PB-induced effects on cell cycle and lipid metabolism. In this study, we examine the effects of the PPARγ inhibitor GW9662 on PB-induced mobile lipid accumulation in DU145 prostate cancer cells using ^{1}H and ^{31}P MR spectroscopy.

Methods

Cell Culture: DU145 human prostate adenocarcinoma cells were cultured in MEM (10% FBS in 5% CO_2 in air at 37°C). *Diffusion-Weighted (DW) NMR Spectroscopy:* 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. 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}), 7 G/cm, spectral width, 4 kHz; data size, 2K; NS, 256). ^{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. The PPARγ inhibitor GW9662 (1 μM) was added to the perfusate at the end of the first hour of MR acquisition, and PB (10 mM) was added at the end the second hour of MR acquisition. *Cell Cycle Analysis:* DU145 cells seeded in 6-well tissue culture plates were pretreated for 1 h with 1, 5 and 10 μM of the PPARγ inhibitor GW9662, followed by the addition of 10 mM PB and incubation for 16 h. Following staining with propidium iodide, cell-cycle analysis was preformed on a Becton-Dickinson FACScan flow cytometer, and data processed using the ModFit LT analysis software.

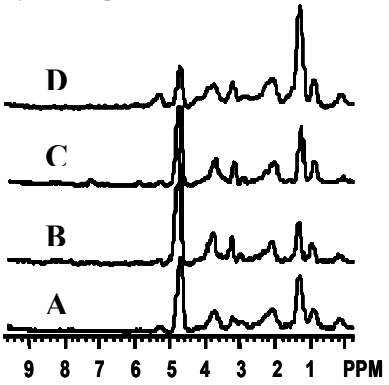

Figure 1: ^{1}H MR spectra of DU145 cells after 16 h perfusion with A) culture medium or culture medium containing B) 1 μM GW9662, C) 1 μM GW9662 and 10 mM PB, D) 10 mM PB.

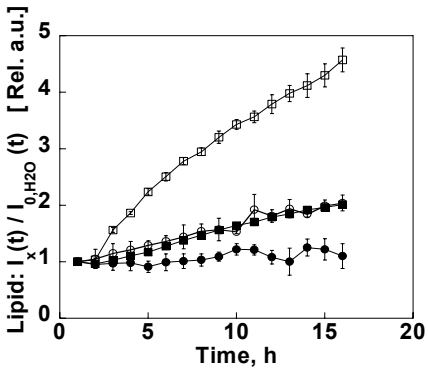

Figure 2: Time course of the integrated intensity of the 1.3 ppm peak of DU145 cells. Open squares represent cells treated with 10 mM PB, closed squares: control cells, open circles: 10 mM PB + 1 μM GW 9662, closed circles: 1 μM GW 9662.

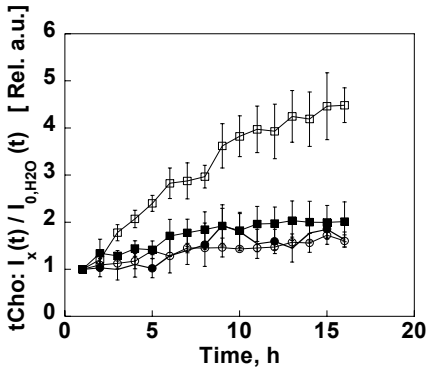

Figure 3: Time course of the integrated intensity of the 3.2 ppm peak of perfused DU145 cells. Open squares represent cells treated with 10 mM PB, closed squares: control cells, open circles: 10 mM PB + 1 μM GW 9662, closed circles: 1 μM GW 9662.

Results and Discussion

Diffusion weighted ^{1}H NMR of control and treated DU145 cells are shown in Figure 1. PB causes a significant increase in the mobile lipid resonance at 1.3 ppm (Figure 2), which has been previously shown to arise predominantly from the methylene -CH_2- on fatty acyl chains of neutral lipids[1]. PB-treatment also causes a significant increase in the total choline (tCho) resonance at 3.2 ppm (Figure 3) and in the glycerophosphocholine resonance at 0.5 ppm in ^{31}P spectra[1]. Pre-treatment with GW9662 completely prevented all PB-induced spectral changes in mobile lipids, tCho and GPC (Figures 2 and 3). Moreover, cell cycle analysis of DU145 cells pre-treated with GW9662 showed a complete reversal of PB-induced G1 arrest. GW9662 is a high affinity, irreversible antagonist binding to the PPARγ receptor[11]. PA, PB and associated analogues have been shown to be ligands to the PPARγ receptor, and their ability to bind and activate this receptor may also correspond to their chemotherapeutic potency[8]. This study strongly suggests that PB-induced spectral changes in mobile lipids and tCho occur through a PPARγ-mediated mechanism. This information is an important step in elucidating the mechanism of action of these highly useful chemotherapeutic agents.

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