Comparing the Chemotherapeutic Response of Prostate Cancer Cells using MR-visible Lipids and Fluorescent Fatty Acid Incorporation

D-J. Leung[1,2], and E. J. Delikatny[2]

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

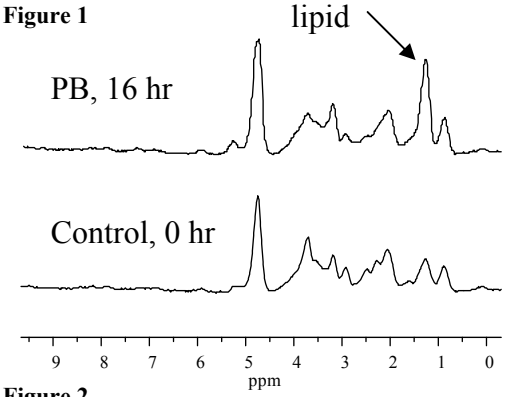
Introduction

Observations of intracellular lipid (particularly triglyceride) accumulation in the form of MR-visible mobile lipids and lipid droplets have been associated with apoptosis and necrosis in a variety of cancer models [1-4]. It has previously been shown that the action of phenylbutyrate (PB) can be monitored by increases in mobile lipids and total choline (tCho) in proton MR spectroscopy and in glycerophosphocholine (GPC) in phosphorous MR spectroscopy on DU145 prostate tumors [1]. These data indicate that mobile lipid accumulation may represent a central hallmark of the cellular response resulting from pharmacological challenge with anticancer drugs, however the mechanisms underlying these changes remain poorly understood. It has been proposed that intracellular phospholipases may be responsible for membrane and phospholipid content turnover [5-7] during cellular stress as well as triglyceride accumulation acting as a defense mechanism against lipid cytotoxicity [8, 9]. However, in addition to membrane remodeling, the process of lipid accumulation involves multiple steps that include fatty acid transport, activation, and esterification into triglycerides or cholesterol esters in the lipid droplets. Here we use MRS in combination with fluorescent fatty acids to assess the contribution of these steps to the process of mobile lipid formation in DU145 prostate cancer cells. We then employ this assay to screen DU145 cells against a range of anticancer drugs. Our goal is to assess the degree and universality of lipid accumulation as a response to cytotoxic drug treatment.

Methods

Diffusion-Weighted NMR Spectroscopy: Biosilon microcarriers (1.8 grams) were inoculated with 5.0 x 10^6 DU145 cells/ml and cultured for 48 hr. 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 R2I 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 (Gdiff), 9 G/cm; spectral width, 4 kHz; data size, 4K; NS, 512). ^{1}H and ^{31}P MR spectra were alternately acquired for 16 hr and integrated resonance intensities compared to baseline. PB (10 mM) was added at the end the second hour of MR acquisition. *Lipid Accumulation:* Fatty acid transport was assessed by the uptake of fluorescent fatty acids via the QBT Fatty Acid Uptake kit using a bottom-read Molecular Devices SpectraMax plate reader. Cells were serum starved for 1 hr prior to the PB or 10 μM of the Approved Oncology Drugs set (NCI, NIH).

Figure 1



Results and Discussion

In Figure 1, proton MR spectra of perfused DU145 cells show lipid accumulations at 1.3 ppm induced by 16 h of PB treatment. These mobile lipids steadily increase with time and plateau at ~12 h (data not shown). Fatty acid incorporation measured by the QBT assay (Figure 2) show comparable results, with the uptake in DU145 cells being greater at shorter exposure times of PB (* $p < 0.01$ compared to control; ** $p < 0.015$ compared to 6 h). This indicates that the recruitment of fatty acid during PB-induced cytotoxic stress is an early response, with peak fatty acid uptake after 2 hr of treatment. To determine the universality of lipid droplet formation, DU145 cells, were treated with 10 μM of the drugs in the Approved Oncology Drugs set for 12 hr. The fluorescent fatty acid was added directly to the cells and immediately measured for fluorescent incorporation into the cell as shown in Figure 3 (refer to the set list; the highest responses are listed). The four-fold observed variation in fluorescence indicates a range of lipid response for the respective drugs. We believe that this approach used as a screening tool in conjunction with MRS, will aid in the functional dissection of the contributing pathways involved in lipid formation in cancer cells undergoing chemotherapy.

Figure 2

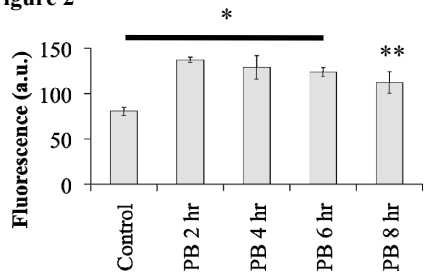
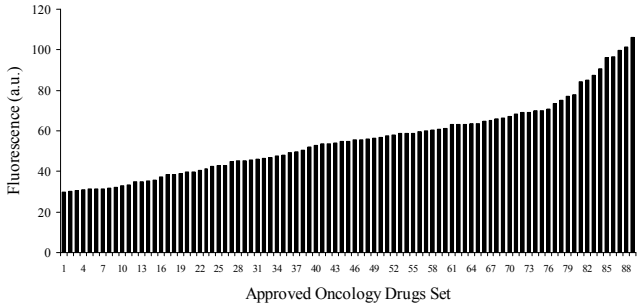


Figure 3



80. Bleomycin
81. Docetaxel
82. Ixabepilone
83. Fulvestrant
84. Valrubicin
85. Plicamycin
86. Everolimus
87. Paclitaxel
88. Vinblastine Sulfate
89. Nilotinib

References

1. Milkevitch, M., et al., *Increases in NMR-visible lipid and glycerophosphocholine during phenylbutyrate-induced apoptosis in human prostate cancer cells.* Biochim Biophys Acta, 2005. **1734**(1): p. 1-12.
2. Delikatny, E.J., et al., *Nuclear magnetic resonance-visible lipids induced by cationic lipophilic chemotherapeutic agents are accompanied by increased lipid droplet formation and damaged mitochondria.* Cancer Res, 2002. **62**(5): p. 1394-400.
3. Brisdelli, F., et al., *Two-step formation of 1H NMR visible mobile lipids during apoptosis of paclitaxel-treated K562 cells.* Biochem Pharmacol, 2003. **65**(8): p. 1271-80.
4. Al-Saffar, N.M., et al., *Apoptosis is associated with triacylglycerol accumulation in Jurkat T-cells.* British Journal of Cancer., 2002. **86**(6): p. 963-70.
5. Cummings, B.S., J. McHowat, and R.G. Schnellmann, *Phospholipase A(2)s in cell injury and death.* J Pharmacol Exp Ther, 2000. **294**(3): p. 793-9.
6. Leung, D.-J., T. Mawn, and E.J. Delikatny, *Comparison of MRS with Fluorescence for Molecular Imaging and Determination of Phospholipase Isoforms.* Proc Int Soc Magn Reson Med, 2009. **17**: p. 45.
7. Liimatainen, T.J., et al., *1H MR spectroscopic imaging of phospholipase-mediated membrane lipid release in apoptotic rat glioma in vivo.* Magn Reson Med, 2008. **59**(6): p. 1232-8.
8. Listenberger, L.L., et al., *Triglyceride accumulation protects against fatty acid-induced lipotoxicity.* Proc Natl Acad Sci U S A, 2003. **100**(6): p. 3077-82.
9. Wright, L.C., et al., *The origin of 1H NMR-visible triacylglycerol in human neutrophils. Highfatty acid environments result in preferential sequestration of palmitic acid into plasma membrane triacylglycerol.* Eur J Biochem, 2000. **267**(1): p. 68-78.