

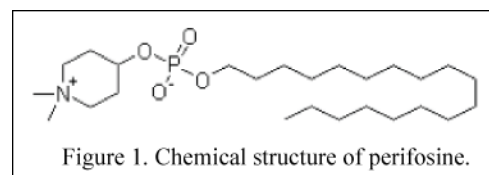
Metabolic Consequences of Perifosine Treatment

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Introduction

Perifosine (Fig. 1) is a novel anticancer alkylphospholipid that is currently being tested in phase II clinical trials for treatment of major human cancers (1). Due to its structural resemblance to naturally occurring phospholipids, perifosine can insert into plasma membrane and interfere with lipid biosynthesis. It has also been shown to affect several signaling pathways in cancer, including inhibiting PI3K signaling (2). In this study, we investigate the changes in choline metabolism modulated by perifosine treatment using magnetic resonance spectroscopy (MRS).



Materials and Methods

MCF-7 human breast cancer cells were cultured in DMEM medium supplemented as previously described (3). The cells were treated with 50 μ M perifosine (Cayman Europe, Estonia) for 48 hrs and the medium was replenished every 24 hrs. During the last 6 hrs of treatment, cells were incubated in fresh medium containing [1,2]-¹³C choline chloride (Cambridge Isotope Laboratories Inc., MA) (3). At the end of treatment, $\sim 4 \times 10^7$ cells were extracted using the dual-phase extraction (4). ¹H, ¹³C, and ³¹P MR spectra of both the aqueous and the lipid metabolite fractions were collected on a 600MHz Varian spectrometer using 90° flip angle and 3 sec relaxation delay for ¹H, and 30° flip angle and 3 sec relaxation delay for ¹³C and ³¹P. The concentrations of metabolites were determined by integration, correction for saturation, and normalization to cell number and to an external reference of known concentration.

Results

After 48 hrs of perifosine treatment, a 50% drop in phosphocholine (PC) levels, from 32 \pm 2 fmol/cell to 16 \pm 1 fmol/cell (n=3, p=0.01), was observed in ³¹P spectra of the aqueous metabolite fraction (Fig. 2A). Glycerophosphocholine (GPC) levels remained unchanged. At the same time, intracellular choline detected in the ¹H spectrum dropped by 38% (n=4, p=0.02) and total choline-containing metabolites (tCho) decreased by 44% (data not shown; n=3, p=0.02). The total cellular phosphatidylcholine (PtdCho) levels dropped 30% (n=4, p=0.05) and accumulation of perifosine could be detected in the lipid fraction of treated cells (Fig. 2B). From ¹³C spectra of the aqueous metabolite fraction, a decrease of 52% in labeled PC (n=3, p=0.03) and a decrease of 67% in labeled betaine (n=3, p=0.03) (Fig. 2C) could be detected, indicating a decrease in the *de novo* synthesis of both metabolites.

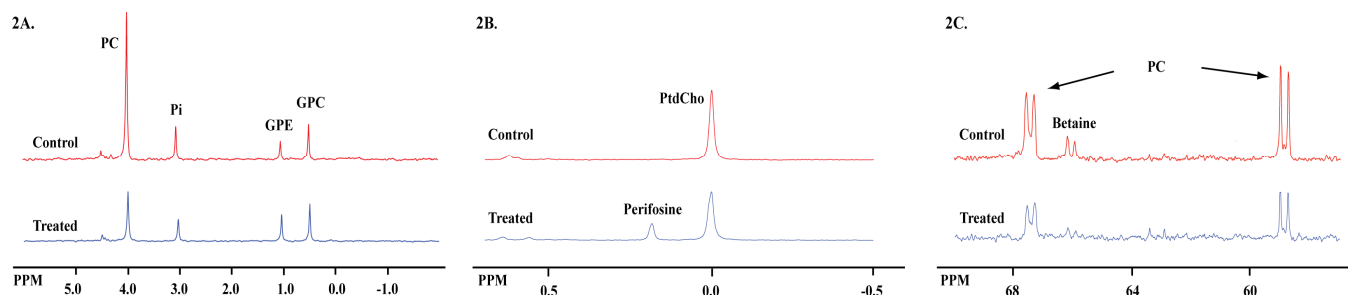


Figure 2: ³¹P spectra of (A) aqueous metabolite fraction and (B) lipid metabolite fraction for control and perifosine treated cells. (C) ¹³C spectra of aqueous metabolite fraction for control and perifosine treated cells.

Discussion and Conclusion

The drop in total PC, *de novo* PC, and total PtdCho levels is consistent with overall inhibition of PtdCho synthesis via the Kennedy pathway upon perifosine treatment. The drop in intracellular choline and decrease in ¹³C labeling of both *de novo* PC and betaine levels also suggests a lower intracellular ¹³C labeled choline pool, resulting from a decrease in choline uptake, in perifosine-treated cells. It is possible that the substantial amount of perifosine accumulated in the membrane can disturb membrane phospholipid biosynthesis and affect choline uptake by cells. Perifosine can also specifically inhibit CTP-PC cytidyltransferase, the rate limiting enzyme of the Kennedy pathway. Finally, perifosine has been shown to affect PI3K signaling, which can also modulate choline metabolism. Further studies are needed to understand whether the observed changes in choline metabolism upon perifosine treatment are linked directly to its mechanism of action or result from its effect on PI3K signaling.

References: (1) Munoz-Martinez, F.; Torres, C.; Castanys, S.; Gamarro, F. *Biochimica et Biophysica Acta* **2008**, *1778*, 530-540. (2) van Blitterswijk, W. J.; Verheij, M. *Current Pharmaceutical Design* **2008**, *14*, 2061-2074. (3) Ross, J.; Najjar, A. M.; Tong, W. P.; Kalarachchi, K.; Ronen, S. M. *Mol Cancer Ther* **2008**, *7*, 2556-2565. (4) Tyagi, R. K.; Azrad, A.; Degani, H.; Salomon, Y. *MRM* **1996**, *35*, 194-200.

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