Detection of liposomal uptake to ovarian cancer cells using ^{1}H MR Spectroscopy

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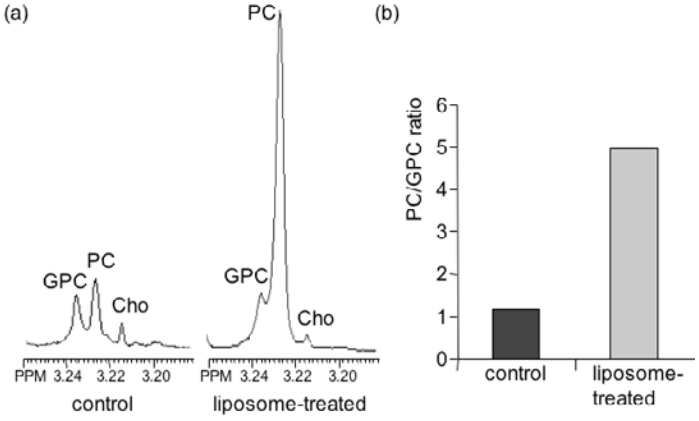
Introduction

Utilizing the ability of ^{1}H MRS to detect phosphocholine (PC) levels in cells can be an efficient method of detecting targeted cells upon uptake of liposomes composed of phosphatidylcholine. Elevated PC and total choline (tCho) levels have been detected in both breast and ovarian cancer cells, and have been used to detect cancer cells both *in vivo* and *in vitro* (1, 2). In this study, immunoliposomes labeled with the humanized monoclonal antibody trastuzumab (Herceptin), and composed of phosphatidylcholine were targeted to SKOV3 ovarian cancer cells. Following dual phase extraction of the cells, ^{1}H MRS detected elevated levels of PC compared to untreated SKOV3 cells. This is a first step study towards the potential use of ^{1}H MRS to detect liposomes targeted to ovarian cancer *in vivo.*

Methods

Mixtures of phosphatidylcholine(PtdCho):cholesterol (1:1 molar ratio) and polyethyleneglycol (PEG)-labeled lipids (6-mol percentage of total lipid) in $CHCl_3$ were dried in a rotary evaporator. The lipids were resuspended in phosphate-buffered saline (PBS) and then annealed at 55°C for 2 hr (3). To make liposomes, the lipid suspension was then taken through 21 cycles of extrusion (Liposo-Fast; Avestin) through two stacked polycarbonate filters with an 800-nm filter pore diameter. Liposomes containing maleimide-PEG were incubated with thiolated Herceptin (Traut's reagent, Pierce) overnight at room temperature. Immunoliposomes were separated from the unreacted antibody by Sepharose 4B size exclusion column. Immunoliposomes were incubated with SKOV3 cells for two hours before trypsinization and extensive cell washing to remove unbound liposomes. Cells underwent dual phase extraction (4), followed by fully relaxed ^{1}H MRS of the water-soluble and the lipid extract fractions. Fully relaxed ^{1}H MRS of the cell extracts was performed on a Bruker Avance 500 NMR spectrometer, using 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP, for water-soluble fractions) or tetramethylsilane (TMS, for lipid fractions) as internal concentration standards. Signal integrals of PC, GPC, and free choline (Cho) were analyzed from the water-soluble extract fractions, and membrane PtdCho was analyzed from the lipid extract fractions.

Figure:
(a) Expanded regions of ^{1}H MR spectra of liposome-treated versus control SKOV3 ovarian cancer cells, demonstrating a large increase in cellular PC levels following treatment with liposomes. (b) The PC/GPC ratio in liposome-treated versus control cells was 4.2-fold increased following treatment.



Results

Following incubation with liposomes composed of PtdCho, SKOV3 ovarian cancer cells contained elevated levels of PC, but not GPC, resulting in an increased PC/GPC ratio (Fig). Levels of PC were 4-fold higher in liposome-treated versus untreated cells. Only cellular PC levels were increased, whereas GPC, Cho, and membrane PtdCho remained unchanged.

Discussion

We have demonstrated that ^{1}H MRS is a useful technique for measuring liposomal delivery to ovarian cancer cells in culture. SKOV3 cells most likely incorporated immunoliposomes composed of phosphatidylcholine by receptor-mediated endocytosis. Liposomal PtdCho was broken down to PC by intracellular phospholipases, leading to the elevated cellular PC levels detected by ^{1}H MRS. This study demonstrates that delivery of liposomes to ovarian cancer cells resulted in elevated PC levels. Here, we have, for the first time, presented data that show promising results for potential future use of ^{1}H MRS to follow liposomal delivery to SKOV3 cells *in vivo.* Detection of liposomal delivery *in vivo* would be extremely useful because addition of contrast agents to the liposome composition could be avoided.

References

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