Pegylated Phosphotidylethanolamine Inhibiting P-Glycoprotein Expression and Enhancing Retention of Doxorubicin in MCF7/ADR Cells

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ABSTRACT: The failure of the clinical treatment of cancer patients is often attributed to drug resistance of the tumor to chemotherapeutic agents. P-glycoprotein (P-gp) contributes to drug resistance via adenosine 5′-triphosphate (ATP)-dependent drug efflux pumps and is widely expressed in many human cancers. Up to date, a few of nanomaterials have shown the effects on P-gp function by different ways. To study the mechanism of the increased cytotoxicity of doxorubicin (DOX) by pegylated phosphotidylethanolamine (PEG-PE) in drug-resistant cancer cells, a series of *in vitro* cell assays were performed, including identification of P-gp function, quantitative studies on uptake and efflux of DOX, inhibitory effects of blank PEG-PE micelles on mRNA and protein levels of P-gp, and intracellular ATP content alteration. Finally, combining MDR-1 RNA interference (siRNA) with DOX encapsulated in PEG-PE micelles (M-DOX) to improve cytotoxicity of DOX was also studied. M-DOX showed fivefold lower the concentration that caused 50% killing tumor cellthan that of free DOX in the P-gp-overexpressing MCF-7 breast cancer (MCF-7/ADR) cells. M-DOX enhanced the cellular uptake and retention of DOX in MCF-7/ADR cells. PEG-PE block molecules can inhibit P-gp expression through downregulating *MDR-1* gene. Cytotoxicity of M-DOX was further improved by knocking down the *MDR-1* gene using siRNA in the multidrug-resistant cells. We conclude that the increased cytotoxicity of DOX encapsulated in PEG-PE micelle is due to the reduced P-gp expression by PEG-PE block molecules, and accordingly enhancing the cellular accumulation of DOX. To overcome drug resistance of tumor cells, the combination of nanotechnology and biotechnology could be an effective strategy such as PEG-PE formed micelles and siRNA. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2267–2277, 2011

Keywords: P-glycoprotein; PEG-PE; Polymeric drug carrier; Micelle; multidrug resistance; MDR-1 siRNA; doxorubicin; Cancer chemotherapy.

INTRODUCTION

The failure of the clinical treatment of cancer patients is often attributed to drug resistance of the tumor to chemotherapeutic agents.[1] Multidrug resistance (MDR) has become a severe barrier for chemotherapy to exert an antineoplastic effect in most common malignancies. Among various mechanisms of MDR, the reduced accumulation of antitumor drug is most commonly seen.[2] P-glycoprotein (P-gp), together with MDR-associated proteins (MRP1 and MRP2), the breast cancer resistance protein (BCRP), and so

Abbreviations used: PEG-PE, pegylated phosphotidylethanolamine; DOX, doxorubicin; P-gp, P-glycoprotein; M-DOX, doxorubicin encapsulated in PEG-PE micelles; F-DOX, free doxorubicin; siRNA, RNA interference; MCF-7/ADR, P-gp-overexpressing MCF-7 breast cancer; RT-PCR, reverse transcriptase-polymerase chain reaction.

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on, contributes to drug resistance via adenosine 5′-triphosphate (ATP)-dependent drug efflux pumps and is widely expressed in many human cancers, including cancers of the gastrointestinal tract, cancers of the hematopoietic system, cancers of the genitourinary system, and childhood cancers.[3,4]

As the most common membrane drug efflux transporter, P-gp significantly reduces intracellular levels of various structurally differed compounds, including doxorubicin (DOX), a broad-spectrum cytotoxic anticancer drug commonly included in the regimens of breast cancer treatment. Clinical data show that this transporter has been detected in 63% of patients with untreated breast cancer.[5] An increase in P-gp expression in breast cancer after chemotherapy has also been correlated with lower clinical response rates.[6,7] These observations indicate that the activity of P-gp efflux pump seems to be one of the most influential factors in resistant breast cancer treatment.

In the past years, nano-sized formulation strategies, such as folate-targeted liposomes,[8,9] pH-sensitive micelles,[10,11] and solid lipid nanoparticles, have been developed to potentially address P-gp-mediated drug resistance.[12–16] These delivery systems have been shown to decrease the resistance of P-gp-expressing cells *in vitro*, which is due to increased cellular accumulation of the drug. However, intracellular drug was still pumped by P-gp efflux because these formulations did not affect P-gp function. Up to date, a few of nanomaterials have shown the effects on P-gp function by different ways. Polyethyleneglycol-660 hydroxystearate inhibited P-gp-related drug transport.[17,18] Fatty acid–PEG diesters interfered with P-gp substrate binding.[19,20] Pluronic micelles composed of poly(oxyethylene)–poly(oxypropylene) and Brij 78 (polyoxyethylene 20-stearylether)-based nanoparticals have been used to selectively inhibit the P-gp function by ATP depletion.[21,22] Proposed mechanisms for some conjugates, such as N-(2-hydroxypropyl) methacrylamide, have included internalization by endocytosis and partial inhibition of *P-gp* gene expression.[23]

Here, we described a new strategy to overcome MDR by DOX loaded into pegylated phosphotidylethanolamine (PEG-PE) micelles (M-DOX) that showed higher cytotoxicity than free DOX (F-DOX), without any chemical ligand decoration, and had an ability to partially reverse DOX resistance in MCF-7/ADR cells. As it was shown in our previous study,[24] this micelle delivery system effectively enhanced *in vitro* and *in vivo* antitumor efficacy of DOX in nonresistant lung cancer. The evaluation of M-DOX performance in resistant breast cancer cells and exploration of related mechanism will help us better understand the micelle intracellular activity and provide useful reference for further design of nano-sized drug delivery system in resistant cancer treatment.

EXPERIMENTAL

Materials

PEG2000-DSPE was purchased from Avanti Polar Lipids (Alabaster, Alabama); DOX was kindly provided by HaiZheng Corp. (Taizhou, China); triethylamine, chloroform, rhodamine123, and verapamil were purchased from Sigma–Aldrich (St. Louis, Missouri); and methanol was purchased from Merck (Darmstadt, Germany).

Cell Culture

Sensitive human breast cancer cell line (MCF-7) and multidrug-resistant cell line (MCF-7/ADR) were granted by Academy of Military Medical Sciences. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, New York) supplemented with 10% fetal bovine serum (PAA, Pasching, Austria), 100 units/mL penicillin, and 100 units/mL streptomycin.

Reverse Transcriptase-Polymerase Chain Reaction

To compare the mRNA expression of the multidrug-resistant genes in different types of cancer cells, total RNAs were isolated from MCF-7 and MCF-7/ADR cells using TRIzol (Invitrogen, California). To address the effect of blank PEG-PE micelles on the mRNA expression of the multidrug-resistant genes, RNAs were also isolated from MCF-7/ADR cells treated with blank micelles 6.8 or 34 μM, respectively. The mRNAs were reversely transcribed to complementary DNAs by M-MLV reverse transcriptase (RT) (Invitrogen). All the primers were purchased from Invitrogen. Primers for human MDR1: 5′-AAAGCTGTC AAGGAAGCCAA-3′ and 5′-TGACTCC ATCATCGAAACCA-3′, MRP1: 5′-ATGTCACGTGG AATACCAGC-3′ and 5′-GAAGACTGAACTCCCTT CCT-3′, MRP2: 5′-ACAGAGGCTGGTGGCAACC-3′ and 5′-ACCATTACCTTGTCACTGTCCATGA-3′, and BCRP: 5′-TGGCTGTCATGGCTTCAGTA-3′ and 5′-GCCACGTGATTCTTCCACAA-3′ were used. The primers for β-actin were sense primer 5′-CATGTACGTTGCTATCCAGGC-3′ and antisense primer 5′-CTCCTTAATGTCACGCACGAT-3′. After initial denaturation at 95°C for 5 min, polymerase chain reaction (PCR) was performed for 30 cycles (30 s at 94°C, 30 s at annealing temperature, and 40 s at 72°C) using Taq polymerase (TaKaRa, Dalian, China). Reaction products (20 μL per lane) were electrophoresed in 1% agarose, stained with ethidiumbromide and photographed.