Polyethylene Glycol–Phosphatidylethanolamine (PEG–PE)/Vitamin E Based Micelles for the Co-Delivery of Paclitaxel and Curcumin to Overcome Multi-Drug Resistance in Cancer

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ABSTRACT SUMMARY
The development of multi-drug resistance (MDR) is one of the major factors leading to the failure of many conventional chemotherapies. Curcumin, a polyphenol, has been shown to downregulate nuclear factor kappa B (NFkB) and Akt pathways thereby reversing MDR. In this study, we explored the therapeutic efficacy of PEG-PE/VitaminE micelles containing curcumin (CUR) and paclitaxel (PCL) on a PCL-resistant variant of SK-OV-3 human ovarian adenocarcinoma cells in vitro following the treatment with single and combination therapy.

INTRODUCTION
Polymeric micelles are composed of block copolymers with hydrophilic and hydrophobic domains. In aqueous solutions, these polymers form a particle with a hydrophobic core and a hydrophilic corona. Micellar drug delivery systems have been widely explored to solubilize hydrophobic drugs and several of these formulations have made their way into clinic. For example, Genexol-PM is a micellar delivery system of paclitaxel that is currently in phase II clinical trials in the US and has been approved in several countries. Micelles made from conjugates of polyethylene glycol (PEG) and phosphatidylethanolamine (PE) together with vitamin E are of particular interest because of their solubilization efficiency towards various poorly soluble drugs. Additional advantages include increased stability, a low CMC value of 10⁻⁵M, low immunogenicity, and small size 7-35nm that can utilize the enhanced permeability and retention (EPR) effect to accumulate at tumor site.

Two poorly soluble drugs, CUR and PCL, were used in this study. PCL inhibits cell division by binding to tubulin thus preventing the disassembly of microtubules. PCL also induces apoptosis by binding to and blocking the function of the apoptosis inhibitor protein Bcl-2. Prolonged treatment with PCL causes resistance to the drug through overexpression of Pgp efflux transporter. Curcumin, a polyphenol, is an active principle of the perennial herb Curcuma longa (commonly known as turmeric). CUR has been shown to bind to and inhibit the activity of various kinases, and modulate the activation of various transcription factors. Curcumin has been shown to enhance paclitaxel-induced cytotoxicity through downregulation of nuclear factor (NF)-κB and Akt pathways.

To overcome MDR, nanoparticles containing multiple drugs can be used to improve tumor accumulation and lower the apoptotic threshold. PCL has been successfully incorporated into PEG-PE/vitamin E-based micelles with high solubilization efficiency. In this work, we co-loaded PCL and CUR into the same formulation. The toxicity of these micelles was examined against SK-OV-3 and SK-OV-3-TR human ovarian adenocarcinoma cells in vitro following the treatment with single and combination therapy.

EXPERIMENTAL METHODS
Micelles (5mM micelle-forming material) were prepared by the thin film hydration method. Briefly, drug loaded micelles were prepared by combining PEG₂₀₀₀–PE, Vitamin E, and a drug. The organic solvents were removed by the vacuum rotary evaporation followed by freeze-drying for at least 4 hrs. Films were rehydrated using PBS, pH 7.4, centrifuged at 13500 rpm for 5 minutes, and filtered through a 0.2µm syringe filter to remove any unincorporated drug. Micelle size was determined using a Coulter N4 MD Submicron Particle Size Analyzer (Coulter Electronics, Miami, FL, USA). Drug loading efficiency for these micelles was evaluated using D-7000 reverse phase HPLC (Hitachi, Japan). Cytotoxicity studies on SK-OV-3 cells and the paclitaxel resistant variant SK-OV-3-TR were performed using Cell Titer Blue assay (Promega, Madison, WI, USA). Cells were cultured according to established protocols, and then seeded at 3,000 cells/well in 96-well cell-culture plates for the treatment. Plates were analyzed following 48hr treatment.

RESULTS AND DISCUSSION
PEG₂₀₀₀–PE/VitaminE micelles (89:11 molar ratio) were able to successfully incorporate PCL as previously demonstrated by Sawant et. al at a concentration of ~600µg/mL (~4.7% w/w) CUR was also effectively encapsulated at a concentration of ~2mg/mL (15.7% w/w). The same concentrations of both drugs were achieved when co-loaded into a single micellar formulation. The micelles had sizes ranging from 15 – 20nm (Table 1).

<table>
<thead>
<tr>
<th>Formulations (Molar ratio)</th>
<th>Size (nm)</th>
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<tbody>
<tr>
<td>Empty PEG₂₀₀₀–PE: Vit.E (89:11)</td>
<td>15.6 ± 1.9</td>
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<tr>
<td>CUR-loaded PEG₂₀₀₀–PE: Vit.E (89:11)</td>
<td>17.2 ± 3.0</td>
</tr>
<tr>
<td>PCL-loaded PEG₂₀₀₀–PE: Vit.E (89:11)</td>
<td>17.9 ± 1.7</td>
</tr>
<tr>
<td>CUR-PCL co-loaded PEG₂₀₀₀–PE: Vit.E (89:11)</td>
<td>19.3 ± 1.9</td>
</tr>
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Table 1: Particle size in different micellar formulations (mean diameter ± standard deviation, n = 3)

The in vitro cytotoxicity of different micellar formulations was investigated using SK-OV-3 and
SK-OV-3-TR cell lines. The cells were incubated with different drug-loaded micellar formulations for 48 hours and analyzed for their survival using the Cell Titer Blue assay.

Empty PEG–PE/vitamin E micelles had minimal cytotoxic effects on the cells at the corresponding concentrations used. The PCL IC50 on SK-OV-3 cells was determined to be ~10nM. The addition of CUR at different concentrations did not significantly enhance the cytotoxic effect of PCL as shown in Figure 1.

Figure 1: Cell viability of SK-OV-3(PCL-sensitive) after 48hrs of incubation with micellar PCL at various concentrations with and without micellar CUR. Box displays the cell viability of micellar CUR.

The PCL IC50 for the resistant cell line was ~3μM. CUR toxicity was observed to be the same for the sensitive and resistant cell lines. Co-delivery of CUR and PCL using micelles on the resistant cell line resulted in three distinct outcomes: (1) No additional cytotoxicity of PCL was observed at concentrations below 8μM of CUR; (2) at concentrations between 8μM and 10μM of CUR, PCL showed enhanced toxicity while CUR alone had negligible toxicity; (3) Minimal additive toxicity was noted with CUR concentration above 10μM. (Figure 2)

Figure 2: Cell viability of SK-OV-3-TR (PCL-Resistant) after 48hrs of incubation with micellar PCL at various concentrations with and without micellar CUR. Box displays the cell viability of micellar CUR.

MDR reversal capability of CUR was best demonstrated at concentration of 10μM. While varying the concentration of PCL in the combination micelles and keeping CUR constant, the combination treatment resulted in significantly higher toxicity compared to the additive toxicity of individual drugs except at high concentrations of PCL as demonstrated in Figure 3.

Figure 3: Comparison of cell death of SK-OV-3 TR after 48hr treatment with various concentrations of PCL, 10μM CUR, or combination treatment.

CONCLUSION
The intrinsic properties of CUR, its toxicity towards cancer cells and MDR reversal capability, could have great potential in the clinic, especially when used in combination with chemotherapeutic agents. Here, we demonstrated the effectiveness of PEG-PE/VitaminE micelles in solubilizing CUR and the ability to co-load it with PCL into the same formulation. This combination treatment can have significant advantages in vivo compared to individual therapy.

REFERENCES

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