Reversal of Chemoresistance in Ovarian Cancer Cells By Liposomal Co-delivery of Tariquidar and Paclitaxel

Shravan Kumar Sriraman and Vladimir P.Torchilin

Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, Boston, MA 02115
sriraman.s@husky.neu.edu

ABSTRACT SUMMARY
Chemotherapy with taxanes and platinum-based drugs still remains the current standard of care for a variety of cancers. However, in most cases, the tumor is able to develop resistance by over-expressing P-gp, a drug efflux pump. In order to overcome these barriers, we have developed a novel liposomal formulation encapsulating paclitaxel (PCT) and tariquidar (XR), a 3rd generation P-gp inhibitor. The co-loaded formulation was targeted with folate to reduce the off-target toxicity. The formulation was able to effectively overcome drug-resistance in paclitaxel-resistant ovarian cancer cells (SKOV3-TR) even at low drug concentrations.

INTRODUCTION
Ovarian cancer is one of the most lethal gynecologic malignancies. Mortality rates for ovarian cancer have not drastically been improved in almost 40 years. In a majority of cases, this can be attributed to the developments of multidrug resistance (MDR), which requires very high doses of drug to induce therapeutic effects. Over-expression of P-gp is one of the major reasons for the development of MDR.

Tariquidar, a 3rd generation P-gp inhibitor, shows great promise in reversing the MDR. However, it must be delivered specifically to tumor cells thereby preventing toxicity to P-gp-expressing normal organs. Liposomal encapsulation of such drugs allows us to circumvent such issues and effectively deliver the drugs to the target site. Various nanoparticles like liposomes tend to accumulate at sites of disease via the enhanced permeability and retention effect (EPR). Active targeting of these particles could serve to further enhance their penetration specifically into cancer cells.

EXPERIMENTAL METHODS
Liposomes were prepared by the thin film hydration method followed by extrusion through 200 nm polycarbonate membranes. The drugs to be loaded (XR and PCT) were included (in equimolar amounts) in the lipid film at a 1% (w/w) ratio to the total amount of lipid. Following extrusion, the non-encapsulated drug was removed by syringe filtration of the formulation through 0.22µ membranes. Folate was conjugated to the distal end of the polyethyleneglycol (PEG) blocks in PEG-PE copolymers and post-inserted into the liposomes by overnight incubation at 37°C. Liposomal drug concentrations were determined using the reverse phase high performance liquid chromatography (HPLC). A mixture of 10mM ammonium acetate buffer (pH 4) and acetonitrile was used as the mobile phase. The size of the liposomes was characterized using transmission electron microscopy (TEM) with uranyl acetate stain. All in vitro cytotoxicity experiments were carried out on SKOV3 sensitive and TR cells, where the cells were incubated with the formulations for 48 hrs following analysis using the Promega Cytotox assay. N=3 for all experiments.

RESULTS AND DISCUSSION
All the liposomal formulations exhibited a homogeneous size distribution of approximately 200nm (see Figure 1).

![Figure 1. TEM images of plain liposome (A), XR liposome (B), PCT liposome (C) and XR+PCT liposome (D) (scale bar = 500 nm)](image-url)
The formulations were then tested out on SKOV3 sensitive and TR cell lines (see Figures 2 and 3). At all concentrations, the XR liposomes did not cause any significant cell death (< 15% cell death) in both the SKOV3 sensitive and TR cell lines. In the TR cell line, free PCT and liposomal PCT could not induce cell death even at high concentrations of PCT (1.57 µM), while the co-delivery with XR (whether as free drug or liposome) was able to effectively reverse PCT resistance (able to produce equivalent toxicities as compared to sensitive cells) causing more than 60% cell death even at PCT concentrations as low as 50 nM (see Figure 3).

Figure 2. In vitro cytotoxicity of formulations on SKOV3 sensitive cells (Lp: untargeted liposomal formulation, F-Lp: folate-targeted liposome)

It is also interesting to note that targeting the XR+PCT liposomes with folate did not significantly improve their cytotoxic potential when tested out on both cell lines in vitro. We hypothesize that the targeting strategy will be of use in preventing off-target toxicities in non-cancerous cell lines. Currently, the efforts are underway to optimize the targeting ligand density and evaluate these on other ovarian cancer cell types.

CONCLUSION
Uniform liposomes co-loaded with XR and PCT were prepared. The co-loaded formulation was able to effectively overcome resistance to PCT in SKOV3-TR cells even at low PCT doses resulting in enhanced cytotoxic effects compared to liposomes loaded with either XR or PCT.

Figure 3. In vitro cytotoxicity of formulations on SKOV3-TR cells

REFERENCES

ACKNOWLEDGEMENTS
This work was supported by the NIH grant U54CA151881 to VT.