Liposomal Combination Drug Delivery System for the Treatment of Drug-Resistant Ovarian Cancer

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Introduction: Ovarian cancer is the most lethal gynecological cancer, as well as the fifth leading cause of cancer mortality among women worldwide.[1] By the time a diagnosis is made, 51% of patients have stage III cancer and 29% of patients have stage IV cancer, drastically reducing their survival rate.[1] The standard of care for treating ovarian cancer is debulking surgery followed by chemotherapy treatment with cisplatin, paclitaxel, carboplatin, or a combination of drugs.[2] Approximately 75% of patients who respond positively to initial cancer treatment experience a recurrence within 2 years and fail to respond to chemotherapy.[3] After recurrence, the goal of medical care shifts from curing to providing palliative care in order to improve the patient's quality of life.[4] For these reasons, there is a need for an alternative treatment to combat chemotherapy resistance in recurrent ovarian cancer. Current research is focused on identifying novel cytotoxic agents or drugs that can overcome drug resistance through targeting apoptotic pathways, drug resistance, or cell survival.[5] In order to address chemotherapy resistance, we have developed a liposomal combination drug delivery system to deliver siRNA and paclitaxel, a hydrophobic chemotherapy drug. In our system, the siRNA will be used to silence a gene involved in ovarian cancer development and/or drug resistance while simultaneously delivering paclitaxel to kill the newly vulnerable cells. The liposome is composed of the phospholipid DPPC, the cationic lipid DOTAP, DSPE-PEG2000 for stealth, and cholesterol or cholesteryl hemisuccinate (CHEMS) for stability. CHEMS serves the same purpose as cholesterol, but it has been shown that CHEMS can trigger destabilization in the presence of acid. This pH sensitivity is desirable since tumors are associated with an acidic microenvironment.

Methodology: Ovarian cancer cells were treated with an siRNA array combined with paclitaxel treatment and followed by an MTT assay to identify an siRNA with the greatest anticancer effect after combination therapy. Cationic liposomes were synthesized via lipid film hydration. Paclitaxel was encapsulated within the hydrophobic lipid tails and siRNAs were bound to the positively charged lipids. The liposomes were characterized to determine their size using Dynamic Light Scattering (DLS) and zeta potential was determined using electrophoretic light scattering. Stability of liposomes and siRNA binding was analyzed using a gel shift assay and fluorescence spectroscopy. Loading efficiency of paclitaxel in liposomes was determined via HPLC. Cellular uptake of liposomes loaded with Nile Red and fluorescently labeled siRNA was visualized using fluorescence microscopy. The liposomes were frozen using the cryoprotectant DMSO in an effort to prolong the amount of time they are stable with maximal loading.

Results: We determined that paclitaxel-resistant OVCAR3-TR ovarian cancer cells treated with siRNAs targeting ABCB1, JAK2, and CFLAR in combination with paclitaxel treatment demonstrated the greatest anticancer effect, with viabilities of 47%, 34%, and 25%, respectively using a siRNA array/paclitaxel MTT assay. Paclitaxel-loaded liposomes formed uniformly sized nanoparticles with an average diameter of 93.6 nm with a PDI of 0.224 for cholesterol liposomes and an average diameter of 142.3 nm with a PDI of 0.245 for CHEMS liposomes. The cholesterol and CHEMS liposomes were found to have a zeta potential of 45.2 mV and 48.1 mV, respectively. After a week of freezing and subsequent thawing, the size and charge did not significantly change. The stability of both cholesterol- and CHEMS-containing liposomes and siRNA binding was confirmed using gel electrophoresis. Protection of the siRNA was evident in the gel shift assay. HPLC analysis demonstrated efficient loading of paclitaxel in both liposomal formulations, at around 80%. Cholesterol-based liposomes mediated cellular uptake of Nile Red and fluorescently labeled siRNA into OVCAR3-TR ovarian cancer cells.

Conclusion: We were able to identify three gene targets, ABCB1, JAK2, and CFLAR, based on our siRNA array, which demonstrated the greatest anticancer effects in combination with paclitaxel compared to paclitaxel treatment alone. Our results indicate that siRNA- and paclitaxel-loaded liposomes consisting of cholesterol or cholesteryl hemisuccinate (CHEMS) formed uniformly sized cationic nanoparticles. We were able to confirm efficient loading of the paclitaxel as well as protection of the siRNA. This liposomal drug delivery system shows promise in silencing genes causing chemotherapy resistance while also delivering paclitaxel in a stable particle in order to treat drug-resistant ovarian cancer.

References:

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