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Combination Drug Delivery Strategy for the Treatment of Multidrug Resistant Ovarian Cancer

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Abstract: The onset of multidrug resistance (MDR) in ovarian cancer is one of the main causes of treatment failure and low survival rates. Inadequate drug exposure and treatment-free periods due to intermittent chemotherapy select for cancer cells overexpressing drug efflux transporters, resulting in resistant disease. The present study examines the sustained administration of the chemotherapeutic agent docetaxel (DTX) alone and in combination with cepharanthine (CEP), a potent drug efflux transporter inhibitor. DTX and CEP were delivered via the intraperitoneal route in a sustained manner using an injectable polymer−lipid formulation. *In vitro*, the combination strategy resulted in significantly ($p < 0.05$) more apoptosis, greater intracellular accumulation of DTX, and lower DTX efflux in ovarian cancer cells showing the MDR phenotype. *In vivo*, sustained treatment with DTX and CEP showed significantly greater ($p < 0.05$) tumor inhibition (91 ± 4%) in a murine model of multidrug resistant ovarian cancer compared to sustained DTX treatment (76 ± 6%) and was more than twice as efficacious as intermittent DTX treatment. Overall findings from these studies highlight the impact of sustained delivery of monotherapy and combination therapy in the management of refractory ovarian cancer displaying the MDR phenotype.

Keywords: Combination therapy; docetaxel; multidrug resistance; ovarian cancer; efficacy

Introduction

Conventional treatment for ovarian cancer includes cytoreductive surgery followed by intermittent chemotherapy with platinum and taxane chemotherapeutic agents administered at their maximum tolerated doses (MTD).[1] This standard regimen results in a complete response rate of 40−60%; however, more than 90% of patients are believed to relapse after a median period of 18 months due to the emergence of multidrug resistance (MDR).[2,3] The MDR phenotype is a phenomenon by which cancer cells develop the ability to survive in the presence of structurally and functionally different chemotherapeutic agents.[4] Cells that either innately possess or acquire MDR are selected for during the course of intermittent chemotherapy. The most common mechanism responsible for the MDR phenotype is the overexpression of ATP-binding cassette (ABC) drug efflux transporters such as P-glycoprotein (Pgp).[5,6] This

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(1) Hennessy, B. T.; Coleman, R. L.; Markman, M. Ovarian cancer. *Lancet* **2009**, *374*, 1371–1382.

(2) Batch, C.; Huang, T. H. M.; Brown, R.; Nephew, K. P. The epigenetics of ovarian cancer drug resistance and resensitization. *Am. J. Obstet. Gynecol.* **2004**, *191*, 1552–1572.

(3) Agarwal, R.; Kaye, S. B. Ovarian cancer: Strategies for overcoming resistance to chemotherapy. *Nat. Rev. Cancer* **2003**, *3*, 502–516.

(4) Lage, H. An overview of cancer multidrug resistance: A still unsolved problem. *Cell. Mol. Life Sci.* **2008**, *65*, 3145–3167.

(5) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discovery* **2006**, *5*, 219–234.

(6) Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer* **2002**, *2*, 48–58.

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overexpression results in greater efflux of chemotherapeutic agents, leading to intracellular drug levels below cytotoxic concentrations. Tumors from patients with MDR have been shown to overexpress Pgp.[7] Different approaches to overcome MDR have been pursued including use of drug efflux transporter inhibitors, synthesis of more active drug analogues, development of drug conjugates or prodrugs and utilization of advanced drug delivery systems.[8] To improve the effectiveness of chemotherapy in resistant disease, some investigators have explored combination delivery of chemotherapeutic agents and drug efflux transporter inhibitors via advanced delivery systems such as nanoparticles.[9−13]

Treatment-free periods are required during traditional intermittent chemotherapy at the MTD to allow healthy tissues to recover from exposure to high doses of cytotoxic agents. These drug-free periods allow for selection of cells with the MDR phenotype between each treatment cycle, leading to progressively resistant disease.[3,14] Studies have demonstrated that low doses of chemotherapeutic agents administered on a more frequent, prolonged or sustained schedule can increase tumor responsiveness when compared to intermittent dosing at the MTD.[15−19] As well Ho et al. reported that sustained delivery of paclitaxel (PTX) did not induce Pgp overexpression in ovarian cancer tumors, whereas significant upregulation was observed following intermittent chemotherapy.[20]

A polymer−lipid formulation (PoLigel) that provides sustained peritoneal delivery of docetaxel (DTX), a semisynthetic analogue of PTX, has been developed.[21] Sustained intraperitoneal (ip) delivery of DTX via PoLigel−(DTX) was found to be more efficacious than intermittent ip therapy in a SKOV3 xenograft model and a murine ID8 syngeneic model, resulting in reduced tumor burden and ascites fluid, respectively.[16] The present study investigated whether DTX delivered via the PoLigel in a sustained manner can overcome MDR in ovarian cancer, and whether the addition of cepharanthine (CEP), a drug efflux transporter inhibitor, to the PoLigel−(DTX) formulation can further enhance this effect. CEP, a biscoclaurine alkaloid, competitively binds to the efflux transporters Pgp[22] and MRP7,[23] which have been shown to confer resistance to DTX when overexpressed in cancer cells.[24−27]

(7) Kaye, S. B. Reversal of drug resistance in ovarian cancer: Where do we go from here. *J. Clin. Oncol.* **2008**, *26*, 2616–2618.

(8) Zunino, F.; Cassinelli, G.; Polizzi, D.; Perego, P. Molecular mechanisms of resistance to taxanes and therapeutic implications. *Drug Resist. Update* **1999**, *2*, 351–357.

(9) Ganta, S.; Amiji, M. Coadministration of paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. *Mol. Pharmaceutics* **2009**, *6*, 928–939.

(10) Liu, Z.; Ballinger, J. R.; Rauth, A. M.; Bendayen, R.; Wu, X. Y. Delivery of an anticancer drug and a chemosensitizer to murine breast sarcoma by intratumoral injection of sulfopropyl dextran microspheres. *J. Pharm. Pharmacol.* **2003**, *55*, 1063–1073.

(11) Patil, Y.; Sadhukha, T.; Ma, L. N.; Panyam, J. Nanoparticle-mediated simultaneous and targeted delivery of paclitaxel and tariquidar overcomes tumor drug resistance. *J. Controlled Release* **2009**, *136*, 21–29.

(12) Song, X. R.; Cai, Z.; Zheng, Y.; He, G.; Cui, F. Y.; Gong, D. Q.; Hou, S. X.; Xiong, S. J.; Lei, X. J.; Wei, Y. Q. Reversion of multidrug resistance by co-encapsulation of vincristine and verapamil in PLGA nanoparticles. *Eur. J. Pharm. Sci.* **2009**, *37*, 300–305.

(13) Wong, H. L.; Bendayan, R.; Rauth, A. M.; Wu, X. Y. Simultaneous delivery of doxorubicin and GG918 (Elacridar) by new Polymer-Lipid Hybrid Nanoparticles (PLN) for enhanced treatment of multidrug-resistant breast cancer. *J. Controlled Release* **2006**, *116*, 275–284.

(14) Gottesman, M. M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **2002**, *53*, 615–627.

(15) Kamat, A. A.; Kim, T. J.; Landen, C. N.; Lu, C. H.; Han, L. Y.; Lin, Y. G.; Merritt, W. M.; Thaker, P. H.; Gershenson, D. M.; Bischoff, F. Z.; Heymach, J. V.; Jaffe, R. B.; Coleman, R. L.; Sood, A. K. Metronomic chemotherapy enhances the efficacy of antivascular therapy in ovarian cancer. *Cancer Res.* **2007**, *67*, 281–288.

(16) De Souza, R.; Zahedi, P.; Moriyama, E. H.; Allen, C. J.; Wilson, B. C.; Piquette-Miller, M. Continuous docetaxel chemotherapy improves therapeutic efficacy in murine models of ovarian cancer. *Mol. Cancer Ther.* **2010**, *9*, 1820–1830.

(17) Vassileva, V.; Allen, C. J.; Piquette-Miller, M. Effects of sustained and intermittent paclitaxel therapy on tumor repopulation in ovarian cancer. *Mol. Cancer Ther.* **2008**, *7*, 630–637.

(18) Vassileva, V.; Grant, J.; De Souza, R.; Allen, C.; Piquette-Miller, M. Novel biocompatible intraperitoneal drug delivery system increases tolerability and therapeutic efficacy of paclitaxel in a human ovarian cancer xenograft model. *Cancer Chemother. Pharmacol.* **2007**, *60*, 907–914.

(19) Vassileva, V.; Moriyama, E. H.; De Souza, R.; Grant, J.; Allen, C. J.; Wilson, B. C.; Piquette-Miller, M. Efficacy assessment of sustained intraperitoneal paclitaxel therapy in a murine model of ovarian cancer using bioluminescent imaging. *Br. J. Cancer* **2008**, *99*, 2037–2043.

(20) Ho, E. A.; Soo, P. L.; Allen, C.; Piquette-Miller, M. Impact of intraperitoneal, sustained delivery of paclitaxel on the expression of P-glycoprotein in ovarian tumors. *J. Controlled Release* **2007**, *117*, 20–27.

(21) Zahedi, P.; De Souza, R.; Piquette-Miller, M.; Allen, C. Chitosan-phospholipid blend for sustained and localized delivery of docetaxel to the peritoneal cavity. *Int. J. Pharm.* **2009**, *377*, 76–84.

(22) Hirai, M.; Tanaka, K.; Shimizu, T.; Tanigawara, Y.; Yasuhara, M.; Hori, R.; Kakehi, Y.; Yoshida, O.; Ueda, K.; Komano, T.; Inui, K. I. Cepharanthin, a multidrug-resistant modifier, is a substrate for P-glycoprotein. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 73–78.

(23) Zhou, Y.; Hopper-Borge, E.; Shen, T.; Huang, X. C.; Shi, Z.; Kuang, Y. H.; Furukawa, T.; Akiyama, S.; Peng, X. X.; Ashby, C. R.; Chen, X.; Kruh, G. D.; Chen, Z. S. Cepharanthine is a potent reversal agent for MRP7(ABCC10)-mediated multidrug resistance. *Biochem. Pharmacol.* **2009**, *77*, 993–1001.

(24) Galletti, E.; Magnani, M.; Renzulli, M. L.; Botta, M. Paclitaxel and docetaxel resistance: Molecular mechanisms and development of new generation taxanes. *ChemMedChem* **2007**, *2*, 920–942.

(25) Sharom, F. J. The P-glycoprotein efflux pump: How does it transport drugs. *J. Membr. Biol.* **1997**, *160*, 161–175.

(26) Hopper-Borge, E.; Chen, Z. S.; Shchaveleva, I.; Belinsky, M. G.; Kruh, G. D. Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): Resistance to docetaxel. *Cancer Res.* **2004**, *64*, 4927–4930.

Experimental Section

Materials. DTX was purchased from Jari Pharmaceutical Co. (Jiangsu, China). Tritium labeled DTX (^{3}H-DTX) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). CEP was purchased from LKT Laboratories Inc. (St. Paul, MN, USA). Taxotere (40 mg/mL), the clinically available formulation of DTX, was purchased from Sanofi Aventis (West Laval, Canada). Chitosan was purchased from Marinard Biotech Inc. (Quebec City, Canada). Egg phosphatidylcholine (ePC), glycidyltrimethylammonium chloride (GTMAC), lauric aldehyde (LA), 3-[4,5-dimethylthiazolyl]-2,5-diphenyltetrazolium bromide (MTT reagent), 1.0 N sodium hydroxide solution (NaOH) and Bradford Reagent were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). Primer sequences were purchased from Sigma Genosys (Oakville, ON, Canada). Ready Safe Scintillation Cocktail was purchased from Beckman Coulter (Mississauga, ON, Canada). TRIzol, DNaseI, RPMI1640 cell culture medium, fetal bovine serum and penicillin−streptomycin were purchased from Invitrogen (Burlington, Canada). Carboxyfluorescein FLICA Apoptosis Detection Kit Caspase Assay was purchased from ImmunoChemistry Technologies (Bloomington, MN, USA). ABI High Capacity cDNA Reverse Transcription Kit was purchased from Applied Biosystems (Streetsville, ON, Canada).

Cell Culture. The human ovarian cancer cell line HeyA8 and its taxane-resistant counterpart HeyA8-MDR were purchased from M. D. Anderson Cancer Center (Houston, TX, USA). HeyA8 and HeyA8-MDR cells were maintained in RPMI 1640 cell culture medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin−streptomycin (100 U/mL penicillin G and 100 mg/mL streptomycin). Both cell lines were kept as a monolayer at 37 °C in 5% CO_2 and 90% relative humidity.

Determination of *mdr1* and *mrp7* mRNA Expression. The mRNA levels of *mdr1*, which encodes for Pgp, and *mrp7*, which encodes for MRP7, in HeyA8 and HeyA8-MDR cells were measured by quantitative real-time PCR (RT-PCR). Cells grown in a monolayer were washed with PBS pH 7.4 three times and lysed with 1 mL of TRIzol, and total RNA was isolated from cell lysates as per manufacturer's instructions. Following treatment with DNaseI, single-stranded cDNA was synthesized from 2 μg of RNA using the ABI High Capacity cDNA Reverse Transcription Kit as per the manufacturer's protocol. Amplification using quantitative RT-PCR was performed on an Applied Biosystems 7900HT machine equipped with a 384-well reaction block, using SYBR green chemistry with 20 ng of cDNA product and specific primers following a method previously described.[28] Primer sequences used were as follows: *mdr1*-forward, 5′-TGCTCAGACAGGATGTGAGTTG-3′; *mdr1*-reverse, 5′-AATTACAGCAAGCCTGGAACC-3′; *mrp7*-forward, 5′-CATGCAAGCCACGCGGAACG-3′; *mrp7*-reverse, 5′-AAGCTGGGCTGGTGGAGGGT-3′. The mRNA levels for each gene were normalized to cyclophilin, and mRNA ratios are presented as a percentage of control values.

Formulation Preparation and Characterization. Polymer−lipid formulations (PoLigel) were prepared as described elsewhere with minor modifications.[21] In summary, a water-soluble chitosan derivative (WSC) was synthesized by conjugating GTMAC onto the chitosan backbone.[29] The WSC was dissolved in distilled deionized water to prepare a 4.2% (w/v) solution. DTX and various ratios of DTX plus CEP (DTX+CEP) were dissolved in anhydrous ethanol and dried under nitrogen to form a thin layered film and then placed under vacuum for 24 h to remove any residual solvent. An ePC-LA solution (1:4 w/w) was used to resuspend the DTX and DTX+CEP films. Finally the WSC solution was added to the ePC−LA−(DTX or DTX+CEP) solution and vortexed for 1 min. The final DTX and DTX+CEP to material ratios were 1:8 (w/w). All formulations were sterilized under UV light (Sterilizer T209, Intercosmetics, Canada) for 3 h prior to use.

Computational software was employed to estimate the octanol to water partition coefficient (log $P_{o/w}$) and solubility parameters (δ) for DTX, CEP and material components of the PoLigel (i.e., WSC, LA and ePC) as outlined previously.[30] *In vitro* formulation stability was assessed by placing PoLigel formulations in vials containing 0.01 M PBS (pH 7.4) with 2 mg/mL lysozyme stored at 37 °C and taking turbidity measurements over time. Turbidity analysis was conducted using a UV spectrophotometer at $\lambda = 700$ nm (Cary 50 UV−vis spectrophotometer, Varian Inc., USA). *In vitro* drug release was performed by placing PoLigel formulations in vials containing 0.01 M PBS (pH 7.4) with 0.2% lysozyme and 4% albumin (vol %) incubated at 37 °C. At set time points aliquots were removed for analysis by high-performance liquid chromatography (HPLC) and replaced with fresh release media. A detailed outline of sample preparation for HPLC analysis can be found elsewhere.[21] The wavelengths of detection used for CEP and DTX were 284 and 227 nm, respectively. A mobile phase of 52% water and 48% acetonitrile was used for DTX and 20% water and 80% acetonitrile for CEP.

(27) Naramoto, H.; Uematsu, T.; Uchihashi, T.; Doto, R.; Matsuura, T.; Usui, Y.; Uematsu, S.; Li, X. Q.; Takahashi, M.; Yamaoka, M.; Furusawa, K. Multidrug resistance-associated protein 7 expression is involved in cross-resistance to docetaxel in salivary gland adenocarcinoma cell lines. *Int. J. Oncol.* **2007**, *30*, 393–401.

(28) Bookout, A. L.; Cummins, C. L.; Mangelsdorf, D. J.; Pesola, J. M.; Kramer, M. F. High-throughput real-time quantitative reverse transcription PCR. *Curr. Protoc. Mol. Biol.* **2006**, *73*, 15.8.115.8.28.

(29) Cho, J.; Grant, J.; Piquette-Miller, M.; Allen, C. Synthesis and physicochemical and dynamic mechanical properties of a water-soluble chitosan derivative as a biomaterial. *Biomacromolecules* **2006**, *7*, 2845–2855.

(30) Huynh, L.; Leroux, J. C.; Allen, C. Enhancement of docetaxel solubility via conjugation of formulation-compatible moieties. *Org. Biomol. Chem.* **2009**, *7*, 3437–3446.

Cytotoxicity Studies. HeyA8 and HeyA8-MDR cells were seeded onto 96-well plates or 6-well plates at a cell density of 5×10^3 cells/well or 1×10^6 cells/well, respectively. After 24 h the cell culture medium was aspirated and replaced with fresh cell culture medium containing varying concentrations of DTX, CEP or a combination of DTX and CEP (10:1, 1:1 and 1:10 w/w) in solution form and DTX or DTX and CEP (10:1 w/w) in the PoLigel. Following a 72 h incubation period, cell viability was assessed using the MTT assay. For the MTT assay, each well was aspirated and replaced with 5 mg/mL MTT reagent (in 0.01 M PBS, pH 7.4), followed by a 3 h incubation period. Extraction buffer was added to each well to solubilize the MTT crystals. Cell viability was measured by optical absorbance at $\lambda = 570$ nm using a Spectra Max Plus microplate reader (Molecular Devices, USA). Cells incubated with cell culture medium alone (i.e., untreated) were considered to be 100% viable and used as controls. The IC_{50} values or the concentrations at which the cell growth inhibition was 50% compared to untreated controls were estimated from the dose−response curves.

Determination of Combination Index (CI). Using a method developed by Chou and Talalay[31,32] the CI for DTX and CEP combination treatments in solution form and in the PoLigel were determined. First, the growth inhibitory results from each treatment were plotted using the median-effect plot [i.e., log(fraction of nonviable cells/fraction of viable cells) versus log(treatment concentration)] to determine whether the treatment combinations were mutually nonexclusive (i.e., agents acting independently or having different actions) or mutually exclusive (i.e., agents share similar modes of action). The CI values were calculated as follows:

For mutually nonexclusive combination

$$\mathrm{CI} = \frac{D_1}{(D_\mathrm{m})_1} + \frac{D_2}{(D_\mathrm{m})_2} + \frac{D_1 D_2}{(D_\mathrm{m})_1 (D_\mathrm{m})_2}$$

For mutually exclusive combination

$$\mathrm{CI} = \frac{D_1}{(D_\mathrm{m})_1} + \frac{D_2}{(D_\mathrm{m})_2}$$

$(D_\mathrm{m})_1$ and $(D_\mathrm{m})_2$ represent the IC_{50} of treatments 1 and 2 applied separately, while, D_1 and D_2 are IC_{50} of treatments 1 and 2 applied as a combination. CI < 1, CI = 1, and CI > 1 indicate synergistic, additive, and antagonistic effects, respectively.[32]

Apoptotic Activity. For quantitative analysis of apoptosis, HeyA8 and HeyA8-MDR cells were seeded onto 6-well plates at a density of 1×10^6 cells per well. Apoptosis was induced by treating cells with DTX or a combination of DTX and CEP (10:1 w/w) in solution form or in the PoLigel. The concentration of each treatment was set to equal the IC_{50} concentration of free DTX (i.e., 1.35 nM for HeyA8 cells and 264 nM for HeyA8-MDR cells). After 72 h the cells were washed twice with PBS, trypsinized and resuspended at a cell density of 5×10^3 cells/μL in PBS. Ten microliters of FLICA 30X solution was added to 300 μL of cell solution followed by 1 h incubation at 37 °C. After incubation 1 mL of 1X wash buffer was added followed by centrifugation at 4000 rpm for 5 min at room temperature. The supernatant was aspirated and the wash was repeated twice. Following removal of the final 1X wash buffer the cells were resuspended in 400 μL of PBS. Fluorescence of each cell solution was measured at an excitation wavelength of 490 nm and emission wavelength of 520 nm using a dual-scanning microplate spectrofluorometer (Spectra GeminiXS, Molecular Devices, USA). Caspase-3/7 activity was reported as fold change in activation relative to untreated control cells.

DTX Intracellular Uptake and Efflux. For uptake and efflux studies, HeyA8 and HeyA8-MDR cells were seeded onto 6-well plates at a density of 1×10^6 cells per well. Cells were treated for 4 h at 37 °C with 10 nM DTX or a combination of DTX and CEP (10:1 w/w) in solution form and in the PoLigel. All drug samples had been spiked with 0.001% of ^{3}H-DTX for analysis purposes. For uptake studies following 4 h treatment, cells were washed twice with PBS, lysed with 500 μL of 1.0 N NaOH solution, and collected in scintillation vials. For efflux studies following 4 h treatment, cells were washed twice with PBS and then fresh RPMI 1640 cell culture medium was added. At set time points (i.e., 30, 60, 90, and 120 min) cells were washed, lysed and added to scintillation vials as above. A 4 mL aliquot of scintillation cocktail was added to each vial followed by scintillation counting (Beckman Coulter LS 5000TD, Beckman Instruments Inc., USA). Final DTX concentrations in the cell lysates were determined using a standard curve and were normalized to cell protein content as determined by the Bradford protein assay.

Efficacy Studies and Levels of DTX in Tissues. All animal studies were approved by the University of Toronto Animal Care Committee and adhered to the guidelines of the Canadian Animal Care Council. Female SCID mice 6−8 weeks old (Charles River) were inoculated ip with 1×10^6 HeyA8 or HeyA8-MDR cells suspended in 200 μL of PBS (pH 7.4). Seven days later mice were randomly grouped ($n = 6$ per group) and treated with one of the following: (1) nontreated control; (2) drug free PoLigel formulation; (3) DTX PoLigel formulation (i.e., sustained DTX therapy); (4) DTX−CEP 10:1 w/w PoLigel formulation (i.e., sustained DTX−CEP therapy); and (5) Taxotere (i.e., intermittent DTX therapy). Groups 1 and 2 received one 20 μL ip injection of sterile saline or drug-free PoLigel, respectively. Groups 3 and 4 received one ip injection of PoLigel−(DTX) or PoLigel−(DTX+CEP) (total DTX dose: 32 mg/kg), respectively. Group 5 received an ip injection of Taxotere once per week for three weeks (total DTX dose: 32 mg/kg). Mice were monitored daily for signs of lethargy, weight loss, and abdominal distention. End points requiring humane eutha-

(31) Chou, T. C.; Talalay, P. Analysis of combined drug effects: A new look at a very old problem. *Trends Pharmacol. Sci.* **1983**, *4*, 450–454.

(32) Chou, T. C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol. Rev.* **2006**, *58*, 621–681.

nasia included excessive muscle wasting according to the "body conditioning scoring system",[33] abdominal distention, hypothermia, inactivity, and weight loss in excess of 20%. After a 21-day treatment period, end points for control animals were reached. Mice from all treatment groups were anesthetized and sacrificed by exsanguination via cardiac puncture. Tumors, liver, spleen, intestine, and kidneys were collected. Antitumor efficacy was calculated as % efficacy $= (W_{untreated} - W_{treated})/W_{untreated} \times 100$, where $W_{untreated}$ is the mean tumor weight of nontreated control animals and $W_{treated}$ is the mean tumor weight of treated animals. DTX levels in liver, spleen, intestine and kidneys were quantified using a previously developed HPLC assay.[21]

Statistical Data Analysis. All results were obtained from data groups of $n \geq 3$ and are expressed as mean ± standard deviation. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 16.0). A two-sample *t*-test was used to measure statistical significance between pairs of results, and $p < 0.05$ was considered to be significant. For statistical analyses among three or more groups, one-way analysis of variance (ANOVA) was used and subsequent multiple comparisons with Bonferroni correction were performed if any statistical significance was detected by the ANOVA *F*-test.

Results

Characterization of Polymer−Lipid Formulations. Three different ratios of DTX to CEP (i.e., 1:1, 1:10 and 10:1) (w/w) were formulated into the PoLigel. Turbidity measurements taken over a one week period were used to assess the effect of DTX:CEP loading ratio on *in vitro* PoLigel formulation stability. A greater ratio of CEP to DTX resulted in lower formulation stability as indicated by higher turbidity values (Figure SI1 in the Supporting Information). Both the 1:1 and 1:10 (w/w) DTX:CEP formulations showed poor stability in solution, disintegrating within 24 h post-incubation. On the other hand, the 10:1 (w/w) DTX:CEP formulation demonstrated a good stability profile, as it refrained from disintegrating over time and turbidity values remained near zero. This formulation showed a comparable stability profile to our previously developed PoLigel−(DTX) formulation.[21] Based upon these results, all subsequent studies involving the PoLigel−(DTX+CEP) formulation were done using the 10:1 (w/w) DTX:CEP ratio.

The drug release profile of DTX from the PoLigel−(DTX+CEP) formulation was assessed and compared to that from PoLigel−(DTX) (Figure 1). In both cases, a biphasic release profile was obtained, characterized by a more rapid release phase during the initial 24 h (i.e., in terms of % released per day) and a sustained, slower drug release phase for the remainder of the study period. During the initial 24 h, the PoLigel−(DTX+CEP) formulation released 7.9% of total DTX and 3.0% of total CEP. This is similar to the 8% DTX released from the PoLigel−(DTX) formulation during the initial 24 h. Following this initial phase, a sustained release rate of 1.9% ± 0.3 of DTX and 0.68% ± 0.28 of CEP per day occurred from PoLigel−(DTX+CEP). The PoLigel−(DTX) formulation released 2.4% ± 0.1 of DTX per day.

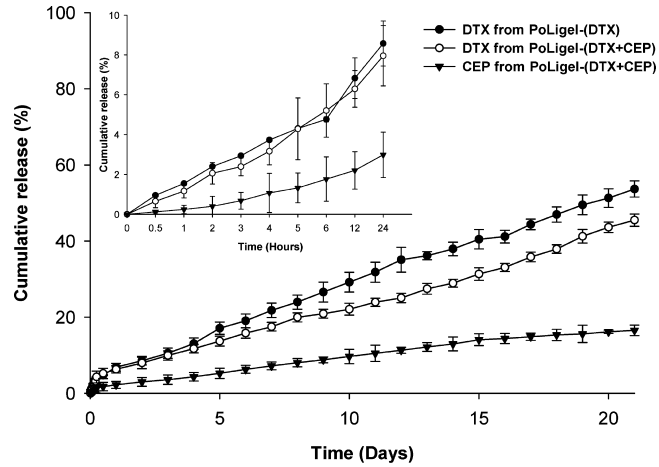

Figure 1. *In vitro* release of DTX from the PoLigel−(DTX) and DTX and CEP from the PoLigel−(DTX+CEP) (10:1 w/w). The results represent mean ± SD ($n = 3$).

Cytotoxicity Studies and Combination Effects. The 50% inhibitory concentrations (IC_{50}) of DTX and CEP as single agents and in combination in taxane-sensitive and taxane-resistant ovarian cancer cells are summarized in Table 1. Quantitative PCR was used to compare the mRNA expression of *mdr1* (which encodes for Pgp) and *mrp7* in HeyA8 and HeyA8-MDR cells (Figure SI2 in the Supporting Information). After normalization to cyclophilin expression, the mRNA concentration of *mdr1* in HeyA8-MDR cells was shown to be 3.1 times greater ($p < 0.05$) than in HeyA8 cells (1.27 ± 0.19 versus 0.41 ± 0.12), and *mrp7* expression is 3.3 times greater ($p < 0.05$) in HeyA8-MDR cells than in HeyA8 cells (16.68 ± 2.67 versus 5.02 ± 0.87). DTX IC_{50} values were found to be 1.35 ± 0.13 nM and 264 ± 38 nM, in HeyA8 and HeyA8-MDR cells, respectively. This demonstrates the high level of resistance of HeyA8-MDR cells to DTX, which require 200-fold more DTX than HeyA8 cells for the same amount of cell death. In HeyA8-MDR cells, the IC_{50} value for PoLigel−(DTX+CEP) was 440-fold, 43-fold and 3.3-fold lower than that for DTX, DTX+CEP (10:1 w/w) and PoLigel−(DTX) ($p < 0.05$), respectively. In the taxane-sensitive HeyA8 cells, exposure to PoLigel−(DTX) resulted in the lowest IC_{50} value, followed by PoLigel−(DTX+CEP). It must be noted that the drug-free PoLigel did not induce a significant cytotoxic effect over a range of concentrations tested in both cell lines (Figure SI3 in the Supporting Information). At the highest concentration of drug-free PoLigel, the cell viability was 91% and 95% in the sensitive and resistant cell lines, respectively. Overall the drug-free PoLigel showed a lack of *in vitro* cytotoxicity, which is in agreement with our previous work in L929 mouse

(33) Ullman-Cullere, M. H.; Foltz, C. J. Body condition scoring: A rapid and accurate method for assessing health status in mice. *Lab. Anim. Sci.* **1999**, *49*, 319–323.

Table 1. The 50% Inhibitory Concentration (IC_{50}) Values and Combination Index (CI) of DTX and CEP Delivered Either as Single Agents or in Combination to Taxane-Sensitive (HeyA8) and Taxane-Resistant (HeyA8-MDR) Ovarian Cancer Cells[a]

	HeyA8		HeyA8-MDR	
treatments	IC_{50} [nM]	CI[b]	IC_{50} [nM]	CI[b]
DTX solution	1.35 ± 0.13		264 ± 38	
CEP solution	$1.19 \times 10^4 \pm 0.20$		$1.33 \times 10^4 \pm 0.41$	
PoLigel−(DTX)	0.610 ± 0.02		2.01 ± 0.51	
DTX+CEP (1:1 w/w) solution	2.17 ± 0.75	0.82	50.1 ± 9.3	0.1
DTX+CEP (1:10 w/w) solution	30.5 ± 3.4	2.25	94.1 ± 2.8	0.04
DTX+CEP (10:1 w/w) solution	1.98 ± 0.39	1.34	23.7 ± 3.6	0.08
PoLigel−(DTX+CEP) (10:1 w/w)	1.09 ± 0.20	0.73	0.601 ± 0.041	0.01

[a] The results represent mean ± SD ($n = 3$). [b] CI < 0.1, very strong synergism; CI = 0.1−0.3, strong synergism; CI = 0.3−0.7, synergism; CI = 0.7−0.85, moderate synergism; CI = 0.85−0.90, slight synergism; CI = 0.90−1.10, nearly additive; CI = 1.10−1.20, slight antagonism; CI = 1.20−1.45, moderate antagonism; CI = 1.45−3.3, antagonism; CI = 3.3−10, strong antagonism; and, CI > 10, very strong antagonism.

fibroblasts and HeLa cervical cancer cells.[34] The PoLigel−(DTX+CEP) resulted in the highest synergistic effect as seen from the CI values measured by the Chou and Talalay method in both cell lines compared to DTX+CEP combination treatments in solution form (Table 1).

Cellular Apoptotic Activity. To further confirm the therapeutic potential of combination therapy, apoptosis was quantified by caspase-3/7 activity levels in both taxane-sensitive and taxane-resistant ovarian cancer cells (Figure 2). Greater caspase-3/7 activity was seen upon HEYA8 treatment with PoLigel−(DTX+CEP) or PoLigel−(DTX) in comparison to that obtained with DTX+CEP (10:1 w/w) or DTX ($p < 0.05$). When HEYA8-MDR cells were treated with PoLigel−(DTX+CEP), more caspase-3/7 activity was observed relative to that obtained following treatment with DTX, DTX+CEP (10:1 w/w) or PoLigel−(DTX) ($p < 0.05$). Treatment with PoLigel−(DTX+CEP) resulted in a 3.6-fold increase in caspase-3/7 activity when compared to DTX alone in the HEYA8-MDR cells. Both the PoLigel−(DTX+CEP) and PoLigel−(DTX) resulted in more than a 2-fold increase in caspase-3/7 activity when compared to free DTX in the HEYA8 cells.

Cellular Uptake and Efflux of DTX. The cellular uptake and efflux of DTX were quantified in both cell lines to assess transporter activity upon combination therapy. Significantly higher cellular levels of DTX resulted when both cell lines were treated with either PoLigel−(DTX+CEP) or PoLigel−(DTX) compared to DTX and DTX+CEP (10:1 w/w) ($p < 0.05$) (Figure 3). In the taxane-resistant cells incubation with PoLigel−(DTX+CEP) resulted in significantly higher accumulation of DTX in comparison to PoLigel−(DTX). The PoLigel−(DTX+CEP) resulted in a 3.1-fold, 1.9-fold and 1.3-fold higher DTX accumulation in the HEYA8-MDR cells compared to DTX, DTX+CEP and PoLigel−(DTX), respectively.

The efflux of DTX from HeyA8 cells was not significantly different irrespective of the treatment approach (Figure 4A). Overall, 2 h post-treatment, approximately 75% of the total

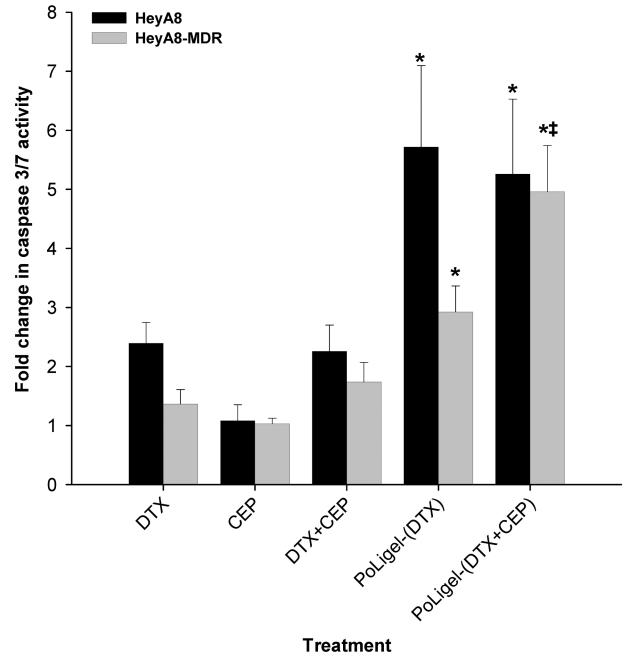

Figure 2. Caspase-3/7 activation, as measured by relative increase to control (nontreated cells), in HeyA8 and HeyA8-MDR ovarian cancer cells. Statistical significance was seen when caspase-3/7 activity associated with PoLigel−(DTX+CEP) (10:1 w/w) and PoLigel−(DTX) were compared to DTX and DTX+CEP (10:1 w/w) in both cell lines ($p < 0.05$). Within each cell line: * represents significant difference from DTX and DTX+CEP (10:1 w/w) ($p < 0.05$); ‡ represents significant difference from PoLigel−(DTX) ($p < 0.05$). The results represent mean ± SD ($n = 3$).

DTX added was still retained in the HeyA8 cells. On the other hand, the amount of DTX effluxed from the HeyA8-MDR cell line that overexpresses drug efflux transporters changed depending on treatment. After 2 h only 22% of DTX was effluxed from cells that had originally been treated with the PoLigel−(DTX+CEP), whereas 47% was effluxed from cells that had been exposed to free DTX (Figure 4B). Combination delivery of CEP and DTX via the PoLigel

(34) De Souza, R.; Zahedi, P.; Allen, C. J.; Piquette-Miller, M. Biocompatibility of injectable chitosan-phospholipid implant systems. *Biomaterials* **2009**, *30*, 3818–3824.

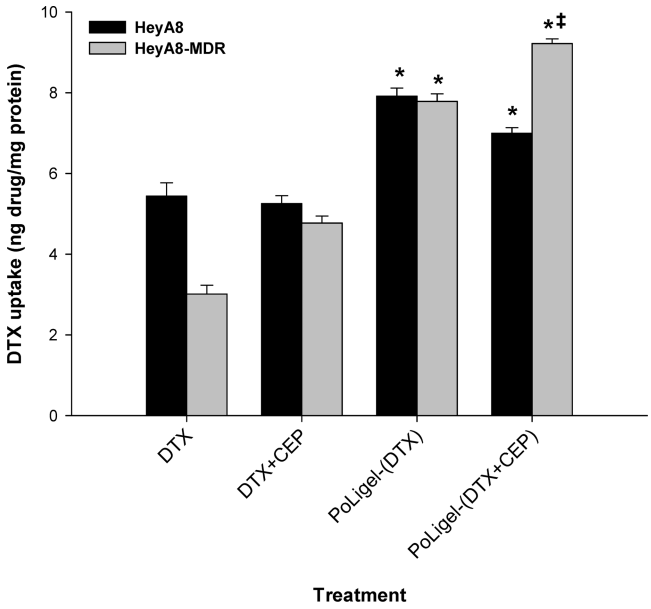

Figure 3. Cellular uptake of DTX in HeyA8 and HeyA8-MDR ovarian cancer cells. Statistical significance was seen in drug uptake when PoLigel−(DTX+CEP) (10:1 w/w) and PoLigel−(DTX) were compared to DTX and DTX+CEP (10:1 w/w) in both cell lines ($p < 0.05$). Within each cell line: * represents significant difference from DTX and DTX+CEP (10:1 w/w) ($p < 0.05$); ‡ represents significant difference from PoLigel−(DTX) ($p < 0.05$). The results represent mean ± SD ($n = 3$).

formulation significantly ($p < 0.05$) decreased DTX efflux in the HeyA8-MDR cell line compared to DTX, DTX+CEP (10:1 w/w) and PoLigel−(DTX) treatment for 2 h (Figure 4B).

Antitumor Efficacy. The ability of PoLigel−(DTX+CEP) to inhibit tumor growth was assessed in taxane-sensitive and taxane-resistant ovarian cancer models (Figure 5). In the taxane-sensitive model, a tumor growth suppression of 88 ± 3% was achieved with PoLigel−(DTX+CEP) treatment, which was not statistically different from the 94 ± 4% tumor reduction obtained with PoLigel−(DTX) treatment. Both PoLigel−(DTX+CEP) and PoLigel−(DTX) treatment resulted in greater antitumor efficacy in the taxane-sensitive model than could be achieved with intermittent Taxotere (75 ± 9%, $p < 0.05$). In the taxane-resistant model, PoLigel−(DTX+CEP) treatment resulted in a 91 ± 4% reduction in tumor burden, which was significantly greater than treatment with either PoLigel−(DTX) or intermittent Taxotere ($p < 0.05$). Treatment with PoLigel−(DTX) led to a 76 ± 6% antitumor efficacy, whereas significantly lower efficacy (36 ± 17%) was achieved with intermittent Taxotere treatment ($p < 0.05$).

Tissue Distribution. In order to verify whether combination treatment with a drug efflux transporter inhibitor caused greater DTX accumulation in healthy tissues, drug levels in liver, spleen, intestine and kidney were compared between mice in the PoLigel−(DTX) and PoLigel−(DTX+CEP) treatment groups. The average drug accumulation in liver, spleen, intestine and kidneys following 21-day treatment with

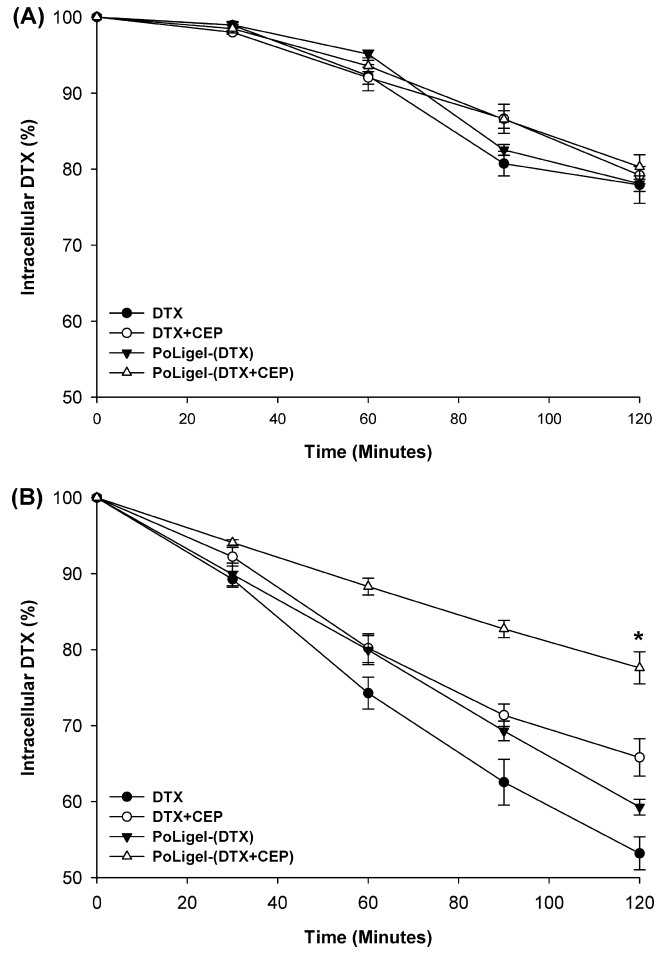

Figure 4. DTX efflux from (A) HeyA8 and (B) HeyA8-MDR ovarian cancer cells. After 120 min a statistically significant decrease in DTX efflux was seen when HeyA8-MDR cells were treated with the PoLigel−(DTX+CEP) (10:1 w/w) compared to DTX, DTX+CEP (10:1 w/w) and PoLigel−(DTX) (* = $p < 0.05$). The results represent mean ± SD ($n = 3$).

PoLigel−(DTX+CEP) were as follows: liver (0.47 ± 0.11 μg/g), spleen (0.41 ± 0.09 μg/g), intestine (0.91 ± 0.33 μg/g) and kidney (0.23 ± 0.10 μg/g). These values were not significantly different from values obtained following 21-days treatment with PoLigel−(DTX): liver (0.51 ± 0.08 μg/g), spleen (0.30 ± 0.11 μg/g), intestine (1.2 ± 0.6 μg/g) and kidney (0.13 ± 0.08 μg/g).

Discussion

Current intermittent chemotherapy used in the clinical setting for ovarian cancer therapy has been shown to eventually result in the development of MDR. Overexpression of drug efflux transporters plays a prominent role in the MDR phenotype. Previous work in preclinical models of ovarian cancer has shown that eliminating treatment-free periods using sustained drug delivery improves therapeutic outcomes in comparison to intermittent chemotherapy[16−19] and, unlike intermittent therapy, does not lead to upregulation of Pgp.[20] This study examined the impact of sustained

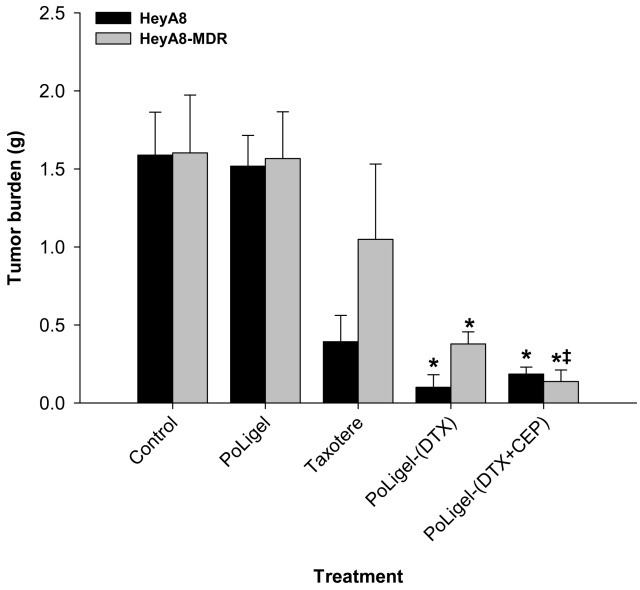

Figure 5. Antitumor efficacy of PoLigel−(DTX+CEP) (10:1 w/w) in HeyA8 and HeyA8-MDR ovarian cancer models. Significant difference was observed between the PoLigel−(DTX+CEP) (10:1 w/w) group compared to all other treatment groups in the HeyA8-MDR tumor model ($p < 0.05$). * represents significant difference from Taxotere ($p < 0.05$); ‡ represents significant difference from PoLigel−(DTX) ($p < 0.05$). The results represent mean ± SD ($n = 6$).

delivery of DTX or the combination of DTX and CEP in taxane-resistant and taxane-sensitive ovarian cancer models. CEP is a substrate of Pgp and directly interacts with and competitively binds to Pgp, thereby inhibiting its transport activity.[22] Furthermore, CEP is a competitive inhibitor of MRP7,[23] a member of the MRP family of ABC transporters.[35] MRP7 is the most structurally unrelated member of the MRP family and has a unique resistance profile, as it is the only MRP protein capable of transporting taxanes.[26] In a survey involving various natural product chemotherapeutic agents, MRP7 exhibited the greatest activity toward DTX; in fact, it has been shown to confer resistance to DTX.[26,27] MRP7 has a commonality with Pgp, in that both are the only ABC transporters with established activity toward taxanes.[26] Based on the inhibitory effect of CEP on these two efflux transporters that confer strong resistance to DTX, CEP was selected for this combination delivery strategy.

Sustained delivery of DTX alone and in combination with CEP was achieved by loading the compounds into a previously developed[21] polymer−lipid based formulation (i.e., PoLigel). The PoLigel forms a gel implant *in situ* following ip injection, allowing for sustained and localized peritoneal delivery of active agent(s). Due to greater hydrophobicity of CEP, its release rate from the PoLigel was slower than that of DTX. Both DTX and CEP showed an initial burst release phase which can be attributed to the drugs being associated with the surface of the PoLigel. This was followed by a sustained release phase, which can be attributed to DTX and CEP partitioning into the hydrophobic regions (i.e., lauric aldehyde and egg phosphatidylcholine) within the PoLigel.

The PoLigel consists of both hydrophilic and hydrophobic regions. The hydrophilic regions are composed of water-soluble chitosan, and the hydrophobic regions are made up of lauric aldehyde and egg phosphatidylcholine. Both DTX and CEP are hydrophobic in nature, with log $P_{o/w}$ values of 2.45 and 7.02, respectively. Therefore, DTX and CEP will likely partition into the hydrophobic regions of the PoLigel. CEP has an almost 3-fold higher log $P_{o/w}$ than DTX. The solubility parameter of CEP (21.03 $(J/cm^3)^{1/2}$) is closer to that of both lauric aldehyde (19.82 $(J/cm^3)^{1/2}$) and egg phosphatidylcholine (17.2 $(J/cm^3)^{1/2}$) as compared to DTX (24.26 $(J/cm^3)^{1/2}$), which indicates that CEP is likely to be more miscible with these formulation components. In this way it was expected that CEP would be more slowly released from the PoLigel formulation (Figure 1).

In both taxane-resistant and taxane-sensitive cells, delivery of DTX in a sustained manner via the PoLigel (i.e., PoLigel−(DTX)) provided a clear benefit over bolus DTX given as a single agent or in combination with CEP. *In vitro*, exposure to PoLigel−(DTX) resulted in a lower IC_{50}, higher caspase 3/7 activity indicative of more apoptosis, and greater DTX uptake than bolus DTX or bolus DTX+CEP combination in both cell lines. In the taxane-resistant cell line, greater intracellular DTX accumulation also resulted from PoLigel−(DTX) treatment. Previous studies have shown that prolonged drug delivery can overcome MDR by decreasing drug efflux when compared to bolus[36] and intermittent[20] treatment, although the underlying mechanisms are not yet well understood. The lower DTX efflux observed with PoLigel−(DTX) in taxane-resistant cells compared to bolus DTX treatment may be attributed to the materials in the PoLigel formulation. Pharmaceutical excipients such as surfactants, polymers (both natural and synthetic), and phospholipids can inhibit drug efflux transporter activity, leading to enhanced drug uptake and lower drug efflux.[37] Two components of the PoLigel formulation, specifically chitosan derivatives[38−40] and phosphatidylcholine,[41−43] have been shown to inhibit drug efflux transporters.

(35) Kruh, G. D.; Belinsky, M. G. The MRP family of drug efflux pumps. *Oncogene* **2003**, *22*, 7537–7552.

(36) Zhan, A. R.; Scala, S.; Monks, A.; Hose, C.; Bates, S.; Fojo, T. Resistance to paclitaxel mediated by P-glycoprotein can be modulated by changes in the schedule of administration. *Cancer Chemother. Pharmacol.* **1997**, *40*, 245–250.

(37) Bansal, T.; Akhtar, N.; Jaggi, M.; Khar, R. K.; Talegaonkar, S. Novel formulation approaches for optimizing delivery of anti-cancer drugs based on P-glycoprotein modulation. *Drug Discovery Today* **2009**, *14*, 1067–1074.

(38) Werle, M.; Hoffer, M. Glutathione and thiolated chitosan inhibit multidrug resistance P-glycoprotein activity in excised small intestine. *J. Controlled Release* **2006**, *111*, 41–46.

(39) Foger, F.; Hoyer, H.; Kafedjiiski, K.; Thaurer, M.; Bernkop-Schurch, A. *In vivo* comparison of various polymeric and low molecular mass inhibitors of intestinal P-glycoprotein. *Biomaterials* **2006**, *27*, 5855–5860.

In vivo, sustained DTX therapy delivered by the PoLigel resulted in greater antitumor efficacy than intermittent DTX therapy in both the sensitive and resistant tumor models. Studies have demonstrated that chemotherapy on a more frequent, prolonged or sustained manner in drug sensitive disease provides superior antitumor effects over the traditional intermittent treatment at the MTD.[16−19,44] Our results show that sustained chemotherapy can also overcome MDR disease, which confirms previous observations that show that prolonged[36] or more frequent[15] drug exposure leads to a greater response in MDR disease.

Although treatment with sustained DTX via the PoLigel provided a benefit in sensitive cells both *in vitro* and *in vivo*, the addition of CEP to the formulation did not lead to further improvement in taxane-sensitive (i.e., HEYA8) cells and tumors. In these cells, treatment with PoLigel−(DTX+CEP) resulted in a similar IC_{50} value, degree of apoptosis, intracellular DTX accumulation and efflux as treatment with PoLigel−(DTX). The level of antitumor efficacy that resulted from treatment with PoLigel−(DTX) or PoLigel−(DTX+CEP) also did not significantly differ. These results are not surprising as HEYA8 cells are almost 200 times more sensitive to DTX than their resistant counterparts, HEYA8-MDR cells. The expression of efflux pumps responsible for DTX transport is also much lower in the HEYA8 cells than in the HEYA8-MDR cells; thus, inhibition of these transporters by CEP does not provide an advantage in HEYA8 cells or the HEYA8 tumor model.

When drug efflux pumps are overexpressed, as is the case in HEYA8-MDR cells, the combination of DTX with an inhibitor of transporters that are responsible for efflux of this drug from cells should significantly improve therapeutic outcomes.[45] Indeed, we have shown that the DTX+CEP combination resulted in very strong synergism in HEYA8-MDR cells. This holds true whether the combination is administered in bolus form or sustained via the PoLigel, although treatment with the latter shows greater synergism. The IC_{50} value upon PoLigel−(DTX+CEP) exposure to HEYA8-MDR cells decreased by more than 3-fold when compared to PoLigel−(DTX), and treatment with bolus DTX+CEP resulted in an 11-fold lower IC_{50} than bolus DTX. This demonstrates that CEP significantly enhances cell kill by DTX in MDR cells. PoLigel−(DTX+CEP) resulted in more apoptosis, greater intracellular DTX uptake, and lower DTX efflux than PoLigel−(DTX) in MDR cells. A benefit was also seen *in vivo*, as greater tumor inhibition resulted from PoLigel−(DTX+CEP) therapy in the MDR ovarian cancer model. As seen in this study, sustained delivery of DTX+CEP via the PoLigel is important, as there were no differences between bolus DTX and bolus DTX+CEP in terms of apoptotic index, intracellular DTX uptake and efflux. These results demonstrate at the cellular and tumor levels that the combination of chemotherapy with an inhibitor of drug efflux transporters, when delivered in a sustained manner, is highly beneficial in the treatment of MDR disease.

The addition of drug efflux transporter inhibitors to the treatment of MDR cancers should theoretically provide a solution for recurrent disease; however, toxicities due to greater drug accumulation in healthy tissues and unfavorable pharmacokinetics have limited their use clinically.[45] Organs such as liver, kidney and intestine that express drug efflux transporters at high levels could potentially be affected by an inhibitor such as CEP when administered intraperitoneally.[46] We hypothesized that delivering the inhibitor (i.e., CEP) in a sustained manner would diminish undesirable effects in healthy tissues, which would be exposed to low doses of CEP at any given time. Indeed, there were no differences in DTX levels in liver, kidney, intestine and spleen following PoLigel−(DTX+CEP) or PoLigel−(DTX) treatment. DTX concentrations in these organs were in fact comparable to levels seen in a study conducted in healthy female CD-1 mice treated with the PoLigel−(DTX), whereby no signs of tissue toxicities were observed.[21]

Conclusions

The evolution of MDR in ovarian cancer following traditional intermittent chemotherapy at the MTD is the leading cause of treatment failure, resulting in very poor clinical outcomes. Strategies that allow for enhanced drug uptake and retention in cancer cells displaying the MDR phenotype can have a profound impact on the management of refractory disease. In this study, we have examined DTX and combination of DTX and CEP delivered in a sustained manner via a polymer−lipid based formulation for the treatment of drug-resistant ovarian cancer. Combination therapy resulted in more apoptosis, higher DTX uptake, lower efflux, and greater antitumor efficacy. Sustained delivery of DTX and CEP did not cause greater accumulation of DTX

(40) Schmitz, T.; Hombach, J.; Bernkop-Schnurch, A. Chitosan-N-acetyl cysteine conjugates: *In vitro* evaluation of permeation enhancing and P-glycoprotein inhibiting properties. *Drug Delivery* **2008**, *15*, 245–252.

(41) Onyuksel, H.; Jeon, E.; Rubinstein, I. Nanomicellar paclitaxel increases cytotoxicity of multidrug resistant breast cancer cells. *Cancer Lett.* **2009**, *274*, 327–330.

(42) Sachs-Barrable, K.; Thamboo, A.; Lee, S. D.; Wasan, K. M. Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. *J. Pharm. Pharm. Sci.* **2007**, *10*, 319–331.

(43) Veldman, R. J.; Sietsma, H.; Klappe, K.; Hoekstra, D.; Kok, J. W. Inhibition of P-glycoprotein activity and chemosensitization of multidrug-resistant ovarian carcinoma 2780AD cells by hexanoyl-glucosylceramide. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 492–496.

(44) Lennernas, B.; Albertsson, P.; Damber, J. E.; Norrby, K. Anti-angiogenic effect of metronomic paclitaxel treatment in prostate cancer and non-tumor tissue in the same animals: A quantitative study. *APMIS* **2004**, *112*, 201–209.

(45) Shukla, S.; Wu, C. P.; Ambudkar, S. V. Development of inhibitors of ATP-binding cassette drug transporters - present status and challenges. *Expert Opin. Drug Metab. Toxicol.* **2008**, *4*, 205–223.

(46) Schinkel, A. H.; Jonker, J. W. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: An overview. *Adv. Drug Delivery Rev.* **2003**, *55*, 3–29.

in healthy tissues when compared to sustained delivery of DTX alone, suggesting that this treatment strategy would not increase toxic side effects. Overall, these results demonstrate the clear benefit associated with DTX and CEP combination therapy when delivered in a sustained manner as a treatment for refractory ovarian cancer.

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Supporting Information Available: *In vitro* stability characterization of PoLigel formulations, mRNA concentrations of *mdr1* and *mrp7* genes for the HEYA8 and HEYA8-MDR cells and *in vitro* cytotoxicity evaluation of drug-free PoLigel in HEYA8 and HEYA8-MDR cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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