Published in final edited form as:

Int J Pharm. 2011 October 31; 419(1-2): 281–286. doi:10.1016/j.ijpharm.2011.07.033.

Multi-drug delivery to tumor cells via micellar nanocarriers

Usha Katragadda, Quincy Teng, Bindhu Madhavi Rayaprolu, Thripathy Chandran, and Chalet Tan*

Cancer Nanomedicine Laboratory, Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, United States

Abstract

The aim of this study was to develop micellar nanocarriers for concomitant delivery of paclitaxel and 17-allylamino-17-demethoxygeldanamycin (17-AAG) for cancer therapy. Paclitaxel and 17-AAG were simultaneously loaded into polymeric micelles by a solvent evaporation method. Two candidate nanocarrier constructs, polyethylene glycol-poly(D, L-lactic acid) (PEG-PLA) micelles and PEG-distearoylphosphatidylethanolamine/tocopheryl polyethylene glycol 1000 (PEG-DSPE/TPGS) mixed micelles, were assessed for the release kinetics of the loaded drugs. Compared to PEG-PLA micelles, entrapment of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles resulted in significantly prolonged release half-lives. The simultaneous incorporation of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles was confirmed by ¹H NMR analysis. Paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles were as effective in blocking the proliferation of human ovarian cancer SKOV-3 cells as the combined free drugs. PEG-DSPE/TPGS mixed micelles may provide a novel and advantageous delivery approach for paclitaxel/17-AAG combination therapy.

Keywords

Combination therapy; Polymeric micelles; Paclitaxel; 17-AAG; PEG-DSPE; PEG-PLA

1. Introduction

Protecting a myriad of mutated and overexpressed oncoproteins from misfolding and degradation, heat shock protein 90 (Hsp90) chaperon machine is a critical mediator of oncogene addiction and tumor cell survival. Recently Hsp90 has been recognized as a promising molecular target for the treatment of human malignancies, and over a dozen of Hsp90 inhibitors are currently being tested in the clinic (Trepel et al., 2010). 17-Allylamino-17-demethoxygeldanamycin (tanespimycin, 17-AAG) is the first Hsp90 inhibitor undergoing clinical trials in advanced cancer patients. As a monotherapy, 17-AAG has demonstrated encouraging efficacy in patients with certain cancer types such as multiple myeloma and human epidermal growth factor receptor-positive breast cancer (Richardson et al., 2010; Dean-Colomb et al., 2008).

© 2011 Elsevier B.V. All rights reserved.

*Corresponding author. Tel.: +1 678 547 6240; fax: +1 678 547 6423. tan_c@mercer.edu. .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Furthermore, clinical studies of 17-AAG combined with conventional chemotherapy or other molecularly targeted agents are also ongoing (Modi et al., 2007; Tse et al., 2008; Ramalingan et al., 2008). One of such combination strategies involves the concurrent administration of paclitaxel and 17-AAG (Ramalingan et al., 2008). A potent microtubule-stabilizing agent, paclitaxel is a first-line treatment for metastatic ovarian, breast and non-small cell lung cancers. In preclinical models of these cancer types, 17-AAG notably sensitizes tumor cells to the cytotoxicity of paclitaxel, and paclitaxel/17-AAG combination is markedly more effective than either drug alone in retarding tumor growth (Solit et al., 2003; Sain et al., 2006; Sawai et al., 2008).

However, positive patient responses observed in a phase I clinical trial of paclitaxel/17-AAG combination therapy have thus far been sparse (Ramalingan et al., 2008). In the clinical setting, paclitaxel and 17-AAG, both poorly water-soluble, are dissolved individually in large amounts of organic excipients (Cremophor EL/ethanol for paclitaxel and 17-AAG; or DMSO/egg phospholipids for 17-AAG) and administered intravenously at the maximum tolerated doses weekly and twice-weekly, respectively. The dosing levels and frequencies of paclitaxel/17-AAG combination therapy are not only limited by the systemic toxicities of both drugs, but also by the toxicities of organic solvents used to solubilize the drugs. Considering the elimination half-life of each drug being drastically shorter than its dosing interval in cancer patients, it is unlikely that following the current dosing regimens of paclitaxel and 17-AAG, the drug exposure in the tumor tissue is adequately sustained to allow for optimal combination effect. To fully realize the therapeutic potential of this combination strategy, it is desirable to develop an organic solvent-free drug delivery system for paclitaxel and 17-AAG, which will improve the accumulation and synergistic interaction of the two drugs in the tumor tissue.

Nano-sized drug carriers are emerging drug delivery systems for cancer therapy. Such nanoparticles are advantageous over conventional intravenous formulations because they have the capacity to accomplish preferential drug accumulation in tumors via the enhanced permeability and retention (EPR) effect, and concomitant delivery of multiple therapeutic agents for combination therapies (Davis et al., 2008). Among numerous intravascular nanoparticulate systems that are being investigated in clinical and preclinical studies, biodegradable and biocompatible polymeric micelles (10–100 nm in diameter) are considered as promising nanocarriers for water-insoluble drugs (Nishiyama and Kataoka, 2006). By incorporating drug molecules in the vicinity of the hydrophobic core, polymeric micelles can drastically increase the aqueous solubility of hydrophobic drugs. The safety and promise of polymeric micelles are attested by the favorable clinical outcomes of a number of micellar formulations for cytotoxins (Matsumura, 2008). Genexol-PM, a micellar formulation of paclitaxel consisting of polyethylene glycol-poly(D, L-lactic acid) (PEG-PLA) copolymer, is undergoing phases II and III clinical trials in patients with advanced cancers. Eliminating the use of Cremophor EL and ethanol in the formulation, paclitaxel-loaded PEG-PLA micelles can be administered to cancer patients at significantly higher doses and frequencies than the conventional paclitaxel formulation (Kim et al., 2004; Saif et al., 2010; Kim et al., 2007). Importantly, paclitaxel-loaded PEG-PLA micelles have been shown to elicit partial response in taxane-refractory patients, which most likely results from the elevated drug distribution into tumors (Kim et al., 2004).

In this study, we report on employing PEG-distearoylphosphatidylethanolamine/tocopheryl polyethylene glycol 1000 (PEG-DSPE/TPGS) mixed micelles for the first time to achieve concomitant delivery of paclitaxel and 17-AAG. We found that paclitaxel and 17-AAG could be efficiently and simultaneously entrapped into PEG-DSPE/TPGS mixed micelles at a fixed ratio. The cytotoxicity of paclitaxel/17-AAG combination against tumor cells remained unaltered when formulated in PEG-DSPE/TPGS mixed micelle formulation. As a

proof of concept, this work demonstrates the potential of polymeric nanocarriers for rationally designed combination cancer therapy.

2. Methods

2.1. Chemicals

Paclitaxel and 17-AAG were purchased from LC Laboratories (Woburn, MA). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG-DSPE) was obtained from Avanti Polar Lipids (Alabaster, AL). PEG-PLA [The number average molecular weight (Mn) of PEG and PLA were 4.2K and 2.1K, respectively, the polydispersity index (PDI) = 1.05] was purchased from Advanced Polymer Materials (Montreal, Canada). D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) was a gift from Eastman Chemical Company (Kingsport, TN). All other chemicals were of analytical or reagent grade.

2.2. Preparation of paclitaxel/17-AAG-loaded micellar nanocarriers

The dual drug-loaded micelles were prepared by a solvent evaporation method. Briefly, in a 5-ml round-bottom flask, paclitaxel, 17-AAG and copolymers (up to 5 mg) were dissolved in 1 ml chloroform, which was removed under vacuum at the room temperature to form a homogenous thin drug-polymer film. Next, the film was hydrated with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered saline (HBS, pH 7.4) and sonicated for 10 min at room temperature. The resulting mixture was then centrifuged at 12,000 g for 10 min to yield clear micelle dispersion. PEG-PLA micelles were prepared with 5.6 mM PEG-PLA, while PEG-DSPE/TPGS mixed micelles were prepared with 12.5 mM PEG-DSPE and 25.0 mM TPGS. Under these conditions, the resulting concentrations of both PEG-PLA and PEG-DSPE were identical at 35 mg/ml. Unless specified otherwise, the resulting concentrations of paclitaxel and 17-AAG in the micellar dispersion were 0.2 mM and 1.4 mM, respectively. The hydrodynamic diameter of the micelles was assessed by dynamic light scattering using a Nano Zetasizer (Malvern Instruments, UK).

2.3. Simultaneous quantification of paclitaxel and 17-AAG by HPLC analysis

The concentrations of paclitaxel and 17-AAG were simultaneously determined using a reverse phase HPLC methodology. The HPLC system (Waters, Milford, MA) has a Waters 2795 pump, a Phenomenex (Torrance, CA) C8 column (5 μ m, i.d. 4.6 $\times$ 150 mm), and a Waters 996 photodiode array detector. As an internal standard, α -naphthoflavone was used at a concentration of 2 μ M. The detection wavelengths for paclitaxel, 17-AAG and α -naphthoflavone were 227 nm, 333 nm and 281 nm, respectively. The isocratic mobile phase was comprised of 25% (v/v) sodium phosphate buffer (25 mM, pH 3.0) and 75% (v/v) methanol with 10 mM triethylamine, and the flow rate was 1.2 ml/min. Before being injected into the HPLC system, micelle samples were first diluted with the HPLC mobile phase. The linear range for the standard curves of paclitaxel and 17-AAG was between 0.5–10 μ M.

2.4. Release kinetics of paclitaxel and 17-AAG from micelles

The release kinetics of paclitaxel and 17-AAG from micellar nanocarriers was studied using a previously reported dialysis method (Chandran et al, 2010). The dual drug-loaded micelles were prepared as described above, and diluted about 10 times with HBS or fetal bovine serum (Invitrogen, Carlsbad, CA), and loaded into a 3 ml Slide-A-Lyzer dialysis cassette (Thermo Scientific, Rockford, IL) with a 20 KD MWCO. To ensure the sink condition for the drug release, each cassette was placed in 500 ml phosphate-buffered saline (20 mM, pH 7.4) that was changed every 2 hours. At pre-determined time points, a sample (20–40 μ l)

was collected from each cassette, for which the concentrations of paclitaxel and 17-AAG were determined by HPLC. To derive the first-order release rate constant (k), the drug concentration in the dialysis cassette (C_t) as a function of release time (t) was fitted to the equation $C_t/C_0 = e^{-kt}$, wherein C_0 was the initial drug concentration (Chandran et al., 2010). The best-fit nonlinear regression was obtained by Sigma Plot (San Jose, CA), and the release half-life ($t_{1/2, \text{release}}$) was calculated by $0.693/k$.

2.5. Storage stability of paclitaxel/17-AAG-loaded micelles

Freshly prepared paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles were stored at 4 °C or 37 °C. At pre-determined time points, the micelle samples were centrifuged at 12,000 g for 10 min. The supernatants were then analyzed by HPLC for changes in the drug content.

2.6. ^1H NMR spectroscopy

The dual drug-loaded micelles were prepared as described above, except that D_2O was used in HBS solution. As a control, identical amounts of drugs and copolymers were dissolved in DMSO-d_6 . NMR data were collected on a 600 MHz NMR spectrometer (Agilent, Palo Alto, CA) at 20 °C with 2048 scans and a spectral width of 7.2 kHz. ^1H chemical shifts were referenced to DMSO at 2.50 ppm for the DMSO-d_6 sample and to internal standard sodium 3-(trimethylsilyl) propionate-2, 2, 3, 3- d_4 (TSP) at 0.0 ppm for the micelle sample. The residual HDO signal in the micelle sample was suppressed by a saturation pulse with a field strength of 40 Hz.

2.7. Cell proliferation assay

Human ovarian cancer SKOV-3 cells (ATCC, Manassas, VA) were cultured in DMEM (Invitrogen) and maintained at 37 °C with 5% CO_2 in a humidified incubator. Cells were seeded in 96-well plates and treated with paclitaxel (2.5–10 nM), 17-AAG (17.5 nM–70 nM), combinations of the two drugs at a 1:7 molar ratio as free drugs or in micelles. The dual drug-loaded micelles were prepared as described above. Stock solutions of free paclitaxel and 17-AAG were prepared in DMSO. At 72 h, cells were stained with 0.1% crystal violet, which were dissolved in 10% acetic acid and analyzed for their absorbance at 595 nm using a microplate reader (Bio-Tek, Winooski, VT). The relative cell number was calculated as the ratio of the absorbance of the treated well versus that of the untreated control (Roforth et al., 2008). The student's t -test was used for the statistical analysis (Sigma Plot, San Jose, CA). A p value < 0.5 was considered statistically significant.

3. Results

3.1. Preparation and size measurement of paclitaxel/17-AAG-loaded micellar nanocarriers

17-AAG has previously been shown to sensitize breast, ovarian and lung cancer cells to the cytotoxicity of paclitaxel (Solit et al., 2003; Sain et al., 2006; Sawai et al., 2008). In human ovarian cancer SKOV-3 cells, we observed enhanced cytotoxicity when paclitaxel and 17-AAG were combined at a 1:7 molar ratio (see data in cytotoxicity study below). We therefore selected this ratio for all our subsequent formulation and biological studies involving the dual drugs. To develop a safe formulation for combined delivery of paclitaxel/17-AAG without employing organic solvents, we hypothesized that both drugs could be simultaneously entrapped into micellar nanocarriers. We chose to study PEG-PLA micelles and PEG-DSPE/TPGS mixed micelles for the following reasons: (1) PEG-PLA micelles are being evaluated in the clinic for delivering paclitaxel (Kim et al., 2004; Saif et al. 2010; Kim et al., 2007); (2) we have recently shown that PEG-DSPE/TPGS mixed micelles may serve as a promising drug carrier for 17-AAG (Chandran et al., 2010). Here, we noticed that, the

dual drug-loaded micelles of both types were formed with minimal precipitation of either polymers or drugs. Exhibiting unimodal and narrow distribution, the average hydrodynamic diameters of paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles and PEG-PLA micelles were 11 ± 1 nm and 32 ± 1 nm, respectively (Fig. 1).

3.2. Release of paclitaxel and 17-AAG from micellar nanocarriers

To serve as true drug carriers and promote drug extravasation into tumors via the EPR effect, it is crucial for micelles to be able to entrap the loaded drug molecules for an extended period of time during circulation. To this end, we examined the release profiles of paclitaxel and 17-AAG from PEG-DSPE/TPGS mixed micelles and PEG-PLA micelles. As controls, the release of free paclitaxel and 17-AAG was studied to verify that the diffusion of drug molecules across the dialysis membrane was not a rate-limiting step during the release process. As illustrated in Fig. 2A and Table 1, the incorporation of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles resulted in significantly prolonged release half-lives ($t_{1/2, \text{release}}$) for both drugs (14.17 h and 6.85 h, respectively), compared to those from PEG-PLA micelles (6.36 h and 1.64 h, respectively). The release of both free drugs into the sink was much faster than those from either micellar formulation, which indicates that during the entire process of the drug release from the dialysis cassette, the liberation of drugs from micelles is the rate-limiting step, and the obtained release kinetics thus reflects the rate of drug release from micelles.

Next, we carried out the release study in the presence of 90% serum to mimic *in vivo* circulation environment. As illustrated in Fig. 2B and Table 1, the release of both paclitaxel and 17-AAG was again significantly slower from PEG-DSPE/TPGS mixed micelles ($t_{1/2, \text{release}} = 7.09$ h and 4.83 h, respectively) than from PEG-PLA micelles ($t_{1/2, \text{release}} = 4.23$ h and 1.92 h, respectively). The presence of serum made the release of drug molecules from both micelle types faster overall, suggesting that serum proteins may have weakened the hydrophobic interactions between drug molecules and micelle-forming polymers. The only exception was that, the presence of serum in the PEG-PLA micelle sample appeared to slightly deter the release of 17-AAG. This may be attributed to the strong binding of 17-AAG to serum proteins (Egorin et al., 2001), which became a rate-limiting step in this case. Meanwhile, the release rate of free paclitaxel and 17-AAG was both reduced, owing to the extensive binding of both drugs to serum proteins. Taken together, the above results indicate that PEG-DSPE/TPGS mixed micelles can function as better drug carriers than PEG-PLA micelles for combined delivery of paclitaxel and 17-AAG. And we therefore focused on paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles for the remainder of this work.

3.3. Incorporation of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles

^1H NMR analysis was carried out to verify the simultaneous incorporation of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles. When drug molecules are loaded into the core-shell micelles formed by the amphiphilic copolymers, the molecular motion within the micellar core is significantly restricted, leading to broadened line width of ^1H signals from protons of drug molecules and the hydrophobic segments of the copolymers located within the micelle core. As a control, we first obtained the ^1H spectrum of a mixture of paclitaxel, 17-AAG, PEG-DSPE and TPGS, that were dissolved in DMSO- d_6 (Fig. 3A). As expected, the detected ^1H signals from all molecules in the control sample had narrow line widths, because these protons experience rapid molecular motions in DMSO solution. In contrast, following the formation of micelles (Fig. 3B), the ^1H NMR peaks from both DSPE (0.87 ppm and 1.28 ppm) and the vitamin E portion of TPGS (1.90–2.00 ppm) were much broader compared to those in the DMSO sample. These results are indicative that the micellar core is formed by DSPE and the lipophilic portion of TPGS. On the other hand, the micelle formation had minor impact on the ^1H NMR line width at 3.71 ppm, which originated from

oxyethylene (O-CH₂-CH₂) protons of PEG, compared to its counterpart in DMSO-d₆. This indicates that the PEG moiety resides in the corona region of the micelle, where the local motion of the protons is largely unrestricted by the micelle formation. Importantly, the fact that ¹H signals of paclitaxel and 17-AAG in the aqueous micelle sample were undetectable (Fig. 3B) clearly demonstrates that both drugs are buried within the hydrophobic core of the micelles.

3.4. Maximum loading of paclitaxel and 17-AAG into PEG-DSPE/TPGS mixed micelles

By modulating the input concentrations of both drugs, a combination of paclitaxel and 17-AAG could be loaded into PEG-DSPE/TPGS mixed micelles at a fixed ratio precisely. Aimed at a molar ratio of 1:7, paclitaxel and 17-AAG could be maximally incorporated into PEG-DSPE/TPGS mixed micelles at about 1.81 ± 0.04 mM and 12.57 ± 1.42 mM, respectively. At these concentrations, the aqueous solubility of each drug was increased by more than 5,000- and 700-fold. The loading efficiency, defined as the ratio of the incorporated versus the input drug concentrations, was about 95% for paclitaxel and 90% for 17-AAG.

3.5. Storage stability of paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles

We examined the storage stability of the dual drug-loaded micelles by monitoring the drug concentrations in the micelle dispersion. We found that, maximally loaded micelles remained stable at 4 °C for two weeks with both drug concentrations nearly unaltered (< 10% loss in the drug content). At 37 °C the same micelles were stable and maintaining above 95% of initial drug concentrations for 48 h. By 1 week, however, there were nearly 35% reductions in the concentrations of both drugs.

3.6. Cytotoxicity of paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles

In order to evaluate the cytotoxicity of paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles, cell proliferation assay was performed in SKOV-3 cells. For comparison purpose, we also examined the cytotoxicity of individual drugs and their combination in free forms. At a 1:7 molar ratio, combination of free paclitaxel (5–10 nM) and 17-AAG (35–70) exhibited significantly enhanced cytotoxicity in SKOV-3 cells, compared to those of individual drugs ($p < 0.05$, Fig. 4). When both drugs were simultaneously formulated into PEG-DSPE/TPGS mixed micelles, paclitaxel/17-AAG remained as cytotoxic to SKOV-3 cells as the combined free drugs. As a vehicle control, empty PEG-DSPE/TPGS mixed micelles had little inhibition (<10 %) on the proliferation of SKOV-3 cells at concentrations present in the above drug-loaded micelles. These results demonstrate that PEG-DSPE/TPGS mixed micelle formulation retains the antiproliferative activity of paclitaxel/17-AAG combination.

Discussion

The development of novel therapeutic agents that target specific molecular signaling pathways responsible for tumor growth has offered unprecedented opportunities for exploring new combination strategies. Combining molecularly targeted agents with each other, or with conventional cytotoxic drugs, holds great promises to substantially improve long-term prognosis of common human malignancies. The major challenge when administering combination therapies, however, is the safe and definitive delivery of the correct drug doses to tumors.

One potentially robust and effective approach to address this challenge is to employ a single, programmable nanocarrier system to deliver a combination of drugs. As a proof of concept, we described in the present work the feasibility of utilizing polymeric micelles for combined

delivery of paclitaxel and 17-AAG. Our goal was to develop an organic solvent-free nanocarrier that can encapsulate the two drugs at clinically relevant levels, and retain the drug molecules during circulation before reaching the tumor site. We have previously shown that by modulating concentrations of PEG-DSPE and TPGS, PEG-DSPE/TPGS mixed micelles can serve as drug carriers for 17-AAG (Chandran et al., 2010). In the current study, we demonstrate that paclitaxel and 17-AAG can be simultaneously and efficiently loaded into PEG-DSPE/TPGS mixed micelles, and the drug ratio precisely controlled by adjusting the loading input of the two drugs. The release of both drugs from these micelles was protracted, comparable to those of respective single drug-loaded micelles, indicating no adverse acceleration in drug release resulting from the co-encapsulation of the two drugs. In comparison, paclitaxel and 17-AAG were released much more rapidly from PEG-PLA micelles, which is consistent with an earlier report (Shin et al., 2009). This may be attributable to the less perfect compatibility between the hydrophobic PLA segment and the incorporated drugs, 17-AAG in particular. Although PEG-PLA micelles are being evaluated clinically for delivering paclitaxel with promising results, PEG-DSPE/TPGS mixed micelles appear to be more suitable for paclitaxel/17-AAG combination therapy because of prolonged retention of both drugs within micelles.

Evaluation of *in vitro* drug release profiles is an important aspect of developing long-circulating drug carriers. It is essential to conduct the release study under conditions that resemble *in vivo* scenario, so that the obtained *in vitro* profile can predict the release kinetics *in vivo*. In this study, we compared the release profiles of paclitaxel and 17-AAG from micelles in the absence and presence of serum, and found that the dispersion of micelles in serum made drug release faster overall. This is consistent with previous reports, that serum proteins, α - and β -globulins in particular, destabilize PEG-PLA micelles as well as PEG-DSPE micelles, leading to rapid release of the incorporated drugs (Chen et al., 2008; Diezi et al., 2010). Therefore, the release kinetics of micelles in the presence of serum should be a more accurate reflection of what happens to drug-loaded micelles during circulation *in vivo*. Our release results suggest that paclitaxel and 17-AAG have *in vivo* release half-lives from PEG-DSPE/TPGS mixed micelles close to 7 h and 5 h, respectively. Given the fact that PEG-DSPE micelles extravasate to the tumor tissue rapidly and reach a plateau by 4–5 h (Lukyanov et al., 2002), it is conceivable that PEG-DSPE/TPGS mixed micelles will be able to retain a significant proportion of loaded drug molecules *in vivo* and preferentially accumulate in tumors via the EPR effect, improving the efficacy of paclitaxel/17-AAG combination therapy for cancers.

In conclusion, we have demonstrated that PEG-DSPE/TPGS mixed micelles may serve as nanocarriers for concomitant delivery of paclitaxel and 17-AAG to tumor cells. Further evaluation of paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles in animal models will be essential to validate and optimize this delivery approach.

Acknowledgments

This work was supported by the National Cancer Institute CA152860 (to C. Tan).

References

- Chandran T, Katragadda U, Teng Q, Tan C. Design and evaluation of micellar nanocarriers for 17-allylamino-17-demethoxygeldanamycin (17-AAG). *Int. J. Pharm.* 2010; 392:170–177. [PubMed: 20363305]
- Chen H, Kim S, He W, Wang H, Low PS, Park K, Cheng JX. Fast release of lipophilic agents from circulating PEG-PDLLA micelles revealed by *in vivo* Förster resonance energy transfer imaging. *Langmuir*. 2008; 24:5213–5217. [PubMed: 18257595]

- Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov.* 2008; 7:771–782. [PubMed: 18758474]
- Dean-Colomb W, Esteva FJ. Emerging agents in the treatment of anthracycline- and taxane-refractory metastatic breast cancer. *Semin. Oncol.* 2008; 35:S31–38. [PubMed: 18410797]
- Diezi TA, Bae Y, Kwon GS. Enhanced stability of PEG-block-poly(N-hexyl stearate L-aspartamide) micelles in the presence of serum proteins. *Mol. Pharm.* 2010; 7:1355–1360. [PubMed: 20575526]
- Egorin MJ, Zuhowski EG, Rosen DM, Sentz DL, Covey JM, Eiseman JJ. Plasma pharmacokinetics and tissue distribution of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) in CD2F1 mice. *Cancer Chemother. Pharmacol.* 2001; 47:291–302. [PubMed: 11345645]
- Kim D-W, Kim S-Y, Kim H-K, Kim S-W, Shin SW, Kim JS, Park K, Lee MY, Heo DS. Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer. *Ann. Oncol.* 2007; 18:2009–2014. [PubMed: 17785767]
- Kim T-Y, Kim D-W, Chung J-Y, Shin AG, Kim S-C, Heo DS, Kim NK, Bang Y-J. Phase I and pharmacokinetic study of Genexol-PM, a Cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin. Cancer Res.* 2004; 10:3708–3716. [PubMed: 15173077]
- Lukyanov AN, Gao Z, Mazzola L, Torchilin VP. Polyethylene glycol–diacyllipid micelles demonstrate increased accumulation in subcutaneous tumors in mice. *Pharm. Res.* 2002; 19:1424–1429. [PubMed: 12425458]
- Matsumura Y. Poly(amino acid) micelles nanocarriers in preclinical and clinical studies. *Adv. Drug Deliv. Rev.* 2008; 60:899–914. [PubMed: 18406004]
- Modi S, Stopeck AT, Gordon MS, Mendelson D, Solit DB, Bagatell R, Ma W, Wheler J, Rosen N, Norton L, Cropp GF, Johnson RG, Hannah AL, Hudis CA. Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2–overexpressing breast Cancer: a Phase I dose-escalation study. *J. Clin. Oncol.* 2007; 25:5410–5417. [PubMed: 18048823]
- Nishiyama N, Kataoka K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol. Ther.* 2006; 112:630–648. [PubMed: 16815554]
- Ramalingam SS, Egorin MJ, Ramanathan RK, Remick SC, Sikorski RP, Lagattuta TF, Chatta GS, Friedland DM, Stoller RG, Potter DM, Ivy SP, Belani CP. A Phase I study of 17-allylamino-17-demethoxygeldanamycin combined with paclitaxel in patients with advanced solid malignancies. *Clin. Cancer Res.* 2008; 14:3456–3461. [PubMed: 18519777]
- Richardson PG, Chanan-Khan AA, Alsina M, Albitar M, Berman D, Messina M, Mitsiades CS, Anderson KC. Tanespimycin monotherapy in relapsed multiple myeloma: results of a Phase 1 dose-escalation study. *Br. J. Haematol.* 2010; 150:438–445. [PubMed: 20618337]
- Roforth MM, Tan C. Combination of rapamycin and 17-allylamino-17-demethoxygeldanamycin abrogates Akt activation and potentiates mTOR blockade in breast cancer cells. *Anti-Cancer Drugs.* 2008; 19:681–688. [PubMed: 18594209]
- Saif MW, Podoltsev NA, Rubin MS, Figueroa JA, Lee MY, Kwon J, Rowen E, Yu J, Kerr RO. Phase II clinical trial of paclitaxel loaded polymeric micelle in patients with advanced pancreatic cancer. *Cancer Invest.* 2010; 28:186–194. [PubMed: 19968498]
- Sain N, Krishnan B, Ormerod MG, De Rienzo A, Liu WM, Kaye SB, Workman P, Jackman AL. Potentiation of paclitaxel activity by the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin in human ovarian carcinoma cell lines with high levels of activated AKT. *Mol Cancer Ther.* 2006; 5:1197–1208. [PubMed: 16731752]
- Sawai A, Chandarlapaty S, Greulich H, Gonen M, Ye Q, Arteaga CL, Sellers W, Rosen N, Solit DB. Inhibition of Hsp90 down-regulates mutant epidermal growth factor receptor (EGFR) expression and sensitizes EGFR mutant tumors to paclitaxel. *Cancer Res.* 2008; 68:589–596. [PubMed: 18199556]
- Shin H-C, Alani AWG, Rao DA, Rockich NC, Kwon GS. Multi-drug loaded polymeric micelles for simultaneous delivery of poorly soluble anticancer drug. *J. Control. Release.* 2009; 14:294–300. [PubMed: 19409432]

- Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N. Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to taxol. *Cancer Res.* 2003; 63:2139–2144. [PubMed: 12727831]
- Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer.* 2010; 10:537–549. [PubMed: 20651736]
- Tse AN, Klimstra DS, Gonen M, Shah M, Sheikh T, Sikorski R, Carvajal R, Mui J, Tipian C, O'Reilly E, Chung K, Maki R, Lefkowitz R, Brown K, Manova-Todorova K, Wu N, Egorin MJ, Kelsen D, Schwartz GK. A Phase I dose-escalation study of irinotecan in combination with 17-allylamino-17-demethoxygeldanamycin in patients with solid tumors. *Clin. Cancer Res.* 2008; 14:6704–6711. [PubMed: 18927314]

Fig. 1. The hydrodynamic diameters of paclitaxel/17-AAG-loaded PEG-PLA micelles and PEG-DSPE/TPGS mixed micelles. Each result shows representative data obtained from 3 independent experiments.

Fig. 2.

The release kinetics of paclitaxel and 17-AAG from the dual drug-loaded PEG-PLA micelles and PEG-DSPE/TPGS mixed micelles (**A**) in HBS, and (**B**) in 90 % fetal bovine serum. The initial concentrations of paclitaxel and 17-AAG were identical in all samples. Each data point was the average + SD from at least 3 independent experiments. The lines represent the respective best-fit regression line for each data set.

Fig. 3. ^1H NMR spectra of (A) a mixture of PEG-DSPE, TPGS, paclitaxel and 17-AAG in DMSO-d_6 ; (B) paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles prepared in HBS/ D_2O buffer. The inserts in the regions of 0.0–2.9 ppm and 5.0–9.0 ppm are the intensity-scale expansions, which are plotted with the same intensity magnification in A as their counterparts in B. The ^1H signals of paclitaxel and 17-AAG were not present in the inserts of B.

Fig. 4.

Paclitaxel/17-AAG-loaded PEG-DSPE/TPGS mixed micelles retain antiproliferative effect. Human ovarian cancer SKOV-3 cells were incubated with paclitaxel and/or 17-AAG at concentrations as indicated for 72 h. Results show representative data obtained from at least 3 independent experiments and are reported as the means + SD.

Table 1

The release half-lives ($t_{1/2, \text{release}}$) of paclitaxel and 17-AAG from PEG-PLA micelles and PEG-DSPE/TPGS mixed micelles in comparison to free drugs.

Formulations	<u>Paclitaxel</u>		<u>17-AAG</u>	
	$t_{1/2, \text{release}}$ (h)	Goodness-of-fit (R^2)	$t_{1/2, \text{release}}$ (h)	Goodness-of-fit (R^2)
Free drugs (no serum)	0.53	0.988	0.45	0.996
Free drugs (with serum)	1.04	0.988	1.02	0.984
PEG-PLA micelles (no serum)	6.36	0.988	1.64	0.998
PEG-PLA micelles (with serum)	4.23	0.953	1.92	0.998
PEG-DSPE/TPGS micelles (no serum)	14.17	0.982	6.85	0.997
PEG-DSPE/TPGS micelles (with serum)	7.09	0.997	4.83	0.998