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A Cremophor-Free Formulation for Tanespimycin (17-AAG) using PEO-b-PDLLA Micelles: Characterization and Pharmacokinetics in Rats

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Abstract

Tanespimycin (17-allylamino-17-demethoxygeldanamycin or 17-AAG) is a promising heat shock protein 90 inhibitor currently undergoing clinical trials for the treatment of cancer. Despite its selective mechanism of action on cancer cells, 17-AAG faces challenging issues due to its poor aqueous solubility, requiring formulation with Cremophor EL (CrEL) or ethanol (EtOH). Therefore, a CrEL-free formulation of 17-AAG was prepared using amphiphilic diblock micelles of poly(ethylene oxide)-b-poly(D,L-lactide) (PEO-b-PDLLA). Dynamic light scattering revealed PEO-b-PDLLA (12:6 kDa) micelles with average sizes of 257 nm and critical micelle concentrations of 350 nM, solubilizing up to 1.5 mg/mL of 17-AAG. The area under the curve (AUC) of PEO-b-PDLLA micelles was 1.3-fold that of the standard formulation. The renal clearance (CL_{renal}) increased and the hepatic clearance ($CL_{hepatic}$) decreased with the micelle formulation, as compared to the standard vehicle. The micellar formulation showed a 1.3-fold increase in the half-life ($t_{1/2}$) of the drug in serum and 1.2-fold increase in $t_{1/2}$ of urine. As expected, because it circulated longer in the blood, we also observed a 1.7-fold increase in the volume of distribution (V_d) with this micelle formulation compared to the standard formulation. Overall, the new formulation of 17-AAG in PEO-b-PDLLA (12:6 kDa) micelles resulted in a favorable 150-fold increase in solubility over 17-AAG alone, while retaining similar properties to the standard formulation. Our data indicates that the nanocarrier system can retain the pharmacokinetic disposition of 17-AAG without the need for toxic agents such as CrEL and EtOH.

Keywords

Geldanamycin; Poly(ethylene oxide)-*b*-poly(D,L-Lactide); Cremophor EL; Polymeric micelle; Pharmacokinetics; 17-AAG; 17-Allylamino-17-demethoxygeldanamycin

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INTRODUCTION

Heat Shock Protein 90 (Hsp90) is becoming an important target for cancer therapy due to the key role it plays in regulating proteins involved in tumor cell proliferation. It was discovered that geldanamycin (GA), a benzoquinone ansamycin antibiotic, could bind strongly to the ATP/ADP binding pocket of Hsp90, interfering with the survival and growth of a diverse family of tumors.[1–4] GA is a promising new anticancer agent, but its clinical development has been hampered by severe hepatotoxicity and poor solubility;[4,5] therefore, the analogues tanespimycin (17-allylamino-17-demethoxygeldanamycin, 17-AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) were developed to alleviate these issues. Since 17-DMAG possesses superior aqueous solubility and greater oral bioavailability compared to 17-AAG,[6,7] several of the promising leads towards clinical translation have been directed towards development of 17-DMAG as the more pharmaceutically practical formulation.[6,8,9] However, despite its apparent advantages over 17-AAG, 17-DMAG is characterized by a large volume of distribution when administered[8] that could lead to undesired toxicity; demonstratively, the maximum tolerated dose of 17-DMAG is significantly less than 17-AAG (8 mg/m^2/day and 100–200 mg/m^2/day in dogs, respectively)[10].

The major obstacle for delivery of 17-AAG is its limited aqueous solubility (ca. 0.01 mg/mL[11]),[5] which requires complicated formulations with Cremophor EL (CrEL), DMSO, or EtOH before parenteral administration.[12] This is undesirable from a patient tolerability standpoint since CrEL is known to induce hypersensitivity reactions and anaphylaxis, and requires patient pre-treatment with antihistamines and steroids before administration.[13] Therefore, safer and more effective delivery of 17-AAG relies on the development of biocompatible delivery systems capable of solubilizing the drug without the use of harsh surfactants. The utilization of self-assembled amphiphilic block copolymer (AB) micelles has already been shown to be highly effective at encapsulating promising lipophilic drug molecules, such as paclitaxel, all without the inclusion of harmful surfactants and excipients like CrEL and EtOH.[14–17] In addition, these drug delivery systems are easily adapted to include additional features to enhance specific targeting to cancer cells, as previously shown.[18,19] Here, we report on the use of AB micelles composed of degradable amphiphilic diblock polymers of poly(ethylene oxide)-*block*-poly(D,L-lactide) (PEO-b-PDLLA) as nanocarriers for solubilizing 17-AAG, and compared its pharmacokinetic behavior with a current formulation of 17-AAG in CrEL-EtOH-PEG400.

EXPERIMENTAL PROCEDURES

Chemicals and materials were obtained from Fisher Scientific or Sigma-Aldrich unless stated otherwise, and were of the highest quality available.

17-(allylamino)-17-demethoxygeldanamycin

(17-AAG) was synthesized in the lab from Geldanamycin (GA) (LC Laboratories, Woburn, MA). Briefly, 100 mg of GA (0.2 mmol) was dissolved In 2 ml of dry CH_2Cl_2. Next, 5 equivalents of allylamine (57.1 g/mol, d = 0.763 g/ml) was added dropwise to the flask. The reaction was stirred at RT under low light until complete by TLC (approx. 2 Days) (95:5 $CHCl_3$:MeOH, R_f 0.21), precipitated with hexane (3x), centrifuged at 2000 g's for 15 min, and evaporated to dryness. Yield: 95 mg, 95%; MS *m/z* 584 (M^-); ^{1}H NMR ($CDCl_3$) δ0.99(m, 6H, 10-Me, 14-Me), 1.25 (t, 1H, H-13), 1.60–1.85 (br m, 6H, H-13, H-14, 8-Me), 2.05 (s, 3H, 2-Me), 2.46 (br M, 2H, H-15), 2.83–2.90 (br m, 3H, H-10), 3.27 (s, 3H, OMe), 3.36 (s, 3H, OMe), 3.40 (t, 1H, H-12), 3.58–3.68 (br m, 2H, H-11, H-23), 4.31 (d, 1H, H-7), 5.10 (br s, 1H), 5.21–5.55 (br m, 3H, H-9, H-24), 5.86–5.99 (br t, 2H, H-5, H-23), 6.59 (t, 1H, H-4), 6.94 (d, 1H, H-3), 7.28 (br s, 1H, H-19).

Preparation and characterization of drug loaded PEO-b-PDLLA micelles

17-AAG was formulated by dissolving it with PEO-b-PDLLA (12,000 g/mol for the PEO and 6000 g/mol for the PDLLA block or 12:6 kDa, Mw/Mn=1.3) (Polymer Source, Montreal, Canada) in dimethylacetamide (DMAc) and dialyzing against H_2O, following procedures by Kataoka and coworkers.20 For example, 5 mg of 17-AAG and 45 mg of PEO-b-PDLLA (10:90 w/w) were dissolved in 10 mL DMAc. The resulting solution was dialyzed against H_2O in 3500 MWCO tubing (SpectraPor). Resulting micelles were centrifuged at 5000 g's for 10 min to precipitate unincorporated drug. Incorporation into micelles was verified using aqueous GPC (Shodex SB-806M) by confirming equivalent retention times based on refractive index for the micelles and absorbance of 17-AAG (UV λ332). Micelle solutions were concentrated by rotary evaporation at decreased pressure and room temperature, followed by centrifugation (5000 g's for 10 min). Quantitative drug loading in micelles was determined by monitoring the area under the curve (AUC) for 17-AAG (based on a 17-AAG calibration curve) through reverse-phase HPLC (Shodex C18 column, 65–82.5 : 35-17.5 MeOH to 55% MeOH+0.2% formic acid gradient, 40°C, 332-nm detection). Effective diameters of PEO-b-PDLLA micelles, with and without drugs, were measured using a Brookhaven dynamic light scattering apparatus (100 mW, 532 nm laser) with Gaussian intensity fitting. The critical micelle concentration (CMC) for these PEO–b-PDLLA micelles were determined by measuring the 339/334-nm excitation ratio of pyrene in the presence of various concentrations of PEO-b-PDLLA (3×10^{-5} mg·mL^{-1} to 1 mg·mL^{-1}). Briefly, PEO–b-PDLLA micelles were prepared as described above in serial dilutions and incubated with 0.6 μM pyrene for 1 h at 80°C, allowed to sit in the dark for 15 h at RT, and the fluorescence emission of pyrene was measured at 390 nm (RF-5301 PC spectrofluorophotometer, Shimadzu). Pyrene undergoes well-known photophysical changes in response to its microenvironment polarity.21 A sharp increase in the ratio of 339/334-nm excitation occurs at the CMC as the pyrene preferentially partitions into the hydrophobic cores of PEO–b-PDLLA micelles.22

PEO-b-PDLLA micelle drug release studies

Release experiments were based on the methodology of Eisenberg and coworkers,23 with our previously reported modifications for temperature and pH control.24 Micelle drug solutions were prepared at 0.3 mM PEO-b-PDLLA polymer with 10% w/w drug as described above, and 2.0 mL of the micelle solution was injected into 10,000 MWCO dialysis cassettes (Pierce, Rockford, IL) (n = 3). Dialysis cassettes were placed in a well-mixed temperature-controlled water bath at 37ºC, with bath volume refreshment every 15 to 20 min. Peristaltic pumps under computer control separately injected 50-g/L solutions of dibasic and monobasic phosphate to maintain pH at 7.4 ± 0.1 (apparatus built in-house). At fixed time points, dialysis cassette volumes were made up with ddH_2O to 2 mL, if necessary, and 100-μL aliquots were withdrawn. This was mixed with 100-μL MeOH and 40 μL of the mixture was analyzed by reverse-phase HPLC (Shodex C18 column; 65–82.5 : 35-17.5 of A to B where A: MeOH and B: 55% MeOH+0.2% formic acid; 40°C; 332-nm detection).

In vitro cytotoxicity studies

PC-3 human prostate cells (ATCC CRL-1435) were grown in RPMI 1640 (Hyclone, Logan, UT) and MCF-7 human breast cancer cells (ATCC HTB-22) were cultured in DMEM (Hyclone), both supplemented with 10% Fetal Bovine Serum (Hyclone), 100 μg/mL penicillin-streptomycin (Cambrex Biosciences, Baltimore, MD), and 2 mM L-glutamine (Cambrex Biosciences). Cell lines were plated in 96-well plates at an initial density of 3000 cells per well in 90 μL of appropriate media, and maintained at 37ºC and 5% CO_2 atmosphere. After 24 h, 17-AAG in DMSO was diluted 10-fold with growth media and added to wells (3 wells in duplicate, n = 6) as 10-μL aliquots (1% v/v final DMSO

concentration). Drug-loaded micelles or micelles alone were formulated in MilliQ water (following dialysis of DMSO to form the micelles) and it was not necessary to dilute the samples further before addition to wells. Cells were incubated with all the test compounds for 72 hrs. Following, the metabolic rate of cells was determined by using the resazurin dye (Sigma-Aldrich). The concentrations inhibiting cell growth by 50% (IC_{50}) relative to controls were determined by fitting the data to a Hill-Slope curve (Sigma Plot 9.0, Systat Software, Inc.) and is reported as the average of separate measurements ± the standard deviation.

Pharmacokinetic studies

The standard vehicle for 17-AAG was formulated according to Zhong et al,25 where 15 mg/mL 17-AAG in 2:1:1 EtOH:CrEL:PEG400 was diluted to 3 mg/mL immediately before injection. The methods for incorporation and drug release for these micelles were performed as described before with slight modifications.26 Male Sprague Dawley rats were cannulated via the right jugular vein27 and dosed intravenously with either the new formulation (1.5 mg/mL 17-AAG in PEO-b-PDLLA) or the standard formulation of CrEL-EtOH-PEG400, each at 10 mg/kg (n = 5 for each treatment group). After dosing, serial blood samples (~0.30 mL) and urine samples were collected up to 24 hrs. To 100 μL of serum or urine, 100 μL of internal standard was added (25 μg/mL geldanamycin). The samples were extracted with 1 mL of ethyl acetate (EtOAc) and the organic dried. The residue was reconstituted in 400 μL of the initial mobile phase, centrifuged, and 150 μL was injected on the RP-HPLC. 17-AAG was analyzed at 332 nm with an internal standard at 305 nm on a Genesis 3 μm C18 33mm × 4.6mm column at 1 mL/min in A: 50 mM acetic acid + 10 mM triethylamine (TEA) and B: MeOH + 10 mM TEA (0–3 min 40% B, 3.01–11 min 80% B, 11.01–18 min 40%B). Pharmacokinetic parameters were calculated using WinNonlin® (ver. 5.01) and non-compartmental modeling. All animal studies were conducted in accordance with “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) and under protocols approved by the Washington State Institutional Animal Care and Use Committee.

Biodistribution studies

To assess the effect of formulation on the tissue distribution of 17-AAG, rats (n = 5 for each group, 200–240 g) were cannulated and intravenously administered with 17-AAG in CrEL-EtOH-PEG400 or 17-AAG in PEO-b-PDLLA micelles, all equivalent to the previous pharmacokinetic studies. At 3 hrs after formulation injection, each animal (n = 5 for each time point) was anaesthetized and exsanguinated by cardiac puncture. Brain, lungs, heart, liver, spleen, kidneys, urinary bladder, muscle, bone as well as samples of whole blood and serum were collected. Tissue samples were blotted with paper towel, washed in ice-cold saline, bottled to remove excess fluid, weighed and rapidly frozen in liquid nitrogen, pulverized to a fine powder with a mortar and pestle under liquid nitrogen, and stored at −70ºC until assessed for drug concentrations by HPLC analysis.

Data Analysis

Compiled data were presented as mean and standard error of the mean (mean ± SEM) or mean and standard deviation (mean ± SD) where otherwise indicated. Where possible, the data were analyzed for statistical significance using NCSS Statistical and Power Analysis software (NCSS, Kaysville, UT). Student’s t-test was employed for unpaired samples with a value of $p < 0.05$ being considered statistically significant.

RESULTS AND DISCUSSION

Geldanamycin is poorly water soluble, and formulations in typical pharmaceutical carriers are severely hepatotoxic due to the primary elimination route being the liver.28 The GA

analogues, such as 17-DMAG and 17-AAG exhibit lower hepatotoxicity than GA, but may be characterized by increased nonspecific toxicities due in part to their wide distributions;29 therefore, a nanocarrier formulation is highly desired. The expected biocompatibility, ease of manufacture, kinetic and thermodynamic stability, and ability of PEO-b-PDLLA micelles to solubilize lipophilic drugs makes for a promising nanocarrier system, as has previously been demonstrated for paclitaxel.15 Although, we did not solubilize significant quantities of 17-AAG in PEO-b-PDLLA micelles (Table 1), the enhanced solubility and lack of harsh surfactants for delivering 17-AAG may offer significant benefits over current formulations that utilize toxic CrEL.25

Micelle physical characterization

Dynamic light scattering (Brookhaven Instruments Corporation) revealed PEO-b-PDLLA (12:6 kDa) micelles with average sizes of 242 ± 5 nm without drugs and 257 ± 2 nm loaded with drugs, solubilizing up to 1.5 mg/mL of 17-AAG (Table 1). We found that PEO-b-PDLLA (12:6 kDa) had low critical micelle concentrations (CMC) of 350 nM (Figure 1). The size and CMC reported here for PEO-b-PDLLA (12:6 kDa) micelles are considerably larger than previously reported by Kataoka et al.,20 and may be due to differences in the polydispersity of the di-block polymer. We also tried to encapsulate 17-AAG in micelles using DMSO as the organic solvent, but found that DMAc worked best for forming micelles through the dialysis method. In practice, drug loading for sustained release is ideally 10–20% w/w. Therefore, the loading experiments were investigated at 10% w/w to evaluate the suitability of PEO-b-PDLLA micelles for solubilizing 17-AAG while minimizing experimental waste. The reported values of 17-AAG loading in Table 1 represent targeted 10% w/w loading of 17-AAG based on PEO-b-PDLLA. Drug incorporation into micelles was verified using aqueous GPC (Shodex SB-806M) by monitoring equivalent retention times in refractive index (micelles) and 17-AAG (UV λ332) (data not shown). The mole ratio of 17-AAG to PEO-b-PDLLA ranged from 0.47 ± 0.068 to 1, with a 17-AAG loading efficiency of 14 ± 2.0 % w/w (efficiency = drug solubilized/drug solubilization attempted). After concentration by rotary evaporation, the maximum solubility of 17-AAG in 0.3-mM PEO_{6000}-b-$PDLLA_{12000}$ was ca. 1.5 ± 0.2 mg/mL, an improvement of ca. 150-fold over 17-AAG (ca. 10 μg/mL according to the National Cancer Institute11). We also tried incorporating 1:1 ratio of alpha-tocopherol to drug to improve loading as previously reported for rapamycin,26 but resulting micelles were very large (>500 nm) and were not stable, with the drug precipitating out of solution after 4–5 hrs at 4ºC (data not shown). It was also found that drug loading in PEO-b-PDLLA (12,000 g/mol for PEO and 12,000 g/mol for PDLLA or 12:12 kDa for short) micelles was very poor (< 0.5 mg/mL) compared to PEO-b-PDLLA (12:6 kDa) micelles (1.5 mg/mL), also with and without alpha-tocopherol. We did not investigate the source of the poor loading of 17-AAG in 12:12 kDa micelles further, as the main point of this study was to develop a new formulation of 17-AAG with advantages over the current CrEL formulation.

17-AAG release studies

The *in vitro* release kinetics of 17-AAG from PEO-b-PDLLA micelles at nominal body temperature were investigated by dialysis of drug-loaded micelles against 37 ºC water. Due to the very low solubility of 17-AAG, release medium would have saturated quickly without continuous purging of the bath. Therefore, a constraint in the analysis of the release data was the inability to measure the release of 17-AAG directly from PEO-b-PDLLA micelles. Once the drug released from PEO-b-PDLLA micelles, it had to diffuse across the dialysis membrane, introducing a second diffusion barrier and complicating the analysis. To determine if the dialysis membrane contributed significantly to the overall release rate, cassettes were loaded with free 17-AAG (initially dissolved in minimal amount of MeOH before being diluted with water) and the release kinetics measured. The PEO-b-PDLLA

micelles demonstrated release with a half-life of ca. 4 hrs (Figure 2), which was similar to what was observed for the standard formulation with CrEL and the drug alone.

In vitro cytotoxicity studies

Growth inhibitory activity of the micelle-encapsulated 17-AAG against MCF-7 human breast cancer and PC-3 prostate cancer cells were determined *in vitro* using the resazurin dye to assay for metabolic activity. 17-AAG was active at sub-micromolar concentrations with IC_{50} of 22 ± 14 nM in MCF-7 and IC_{50} 74 ± 14 nM in PC-3 cells; all numbers within the range reported by the National Cancer Institute: IC_{50} of 12.6 nM for MCF-7 and 50.1 nM for PC-3, respectively30. In comparison, our drug-encapsulated micelles had IC_{50} of 160 ± 57 nM in MCF-7 and 536 ± 13 nM in PC-3 cells, and the cremophor formulation had IC_{50} of 120 ± 32 nM in MCF-7 and 330 ± 48 nM in PC-3 cells (Table 2). This may be due to prolonged release of the drugs from both types of formulation, as well the eventual equilibrium reached between release and drug partitioning back into the micellar core in a closed system. PEO-b-PDLLA micelles alone showed no apparent toxicity in the cell lines investigated (IC_{50} > 10 000 nM). Although the encapsulated drugs appear less active than 17-AAG for both formulation, nanoencapsulation may increase the overall efficacy of 17-AAG delivery *in vivo* due to enhanced tumor accumulation. Similarly, Li *et al.* reported a poly-(L-glutamic acid)-paclitaxel conjugate was significantly less potent than paclitaxel *in vitro* but demonstrated superior activity *in vivo* due to enhanced accumulation in tumors.31

Pharmacokinetic studies

There were some differences in the pharmacokinetic profiles of PEO-b-PDLLA (12:6 kDa) micelle formulation as compared to the standard formulation with CrEL-EtOH-PEG400. The micellar formulation demonstrated increased serum AUC 24-hrs after injection (Figure 3a) as observed by a measurable quantity of 17-AAG from the micellar formulation up to 24 hours compared to the control formulation (measurable quantity only up to 12 hours before going below the limit of quantification). The micellar formulation also exhibited increased presence of 17-AAG in the urine after 24-hrs (Figure 3b), greater rate of renal clearance over 36-hrs (Figure 3c), and higher levels of drug in the urine 48-hrs post-injection (Figure 3d). Specifically, at 10 mg/kg (Table 3), the serum area under the curve (AUC) of PEO-b-PDLLA micelles was 1.3-fold that of the standard formulation. We observed a 1.7 fold increase in the volume of distribution (V_d) with the micelle formulation due to its prolonged presence in the blood as observed by a lower total clearance (1.3-fold decrease), and higher half-life in serum (2.7-fold increase) and in urine (1.2-fold increase) compared to the control standard vehicle formulation. The renal clearance of the drug (CL renal) increased (4.2 fold) with the micelle formulation as compared to the standard vehicle, which demonstrated a higher (1.5 fold) hepatic clearance (CL hepatic). Finally, we found that there was no significant difference in mean residence time (MRT) between the two formulations.

It is important to re-emphasize that although, overall, the pharmacokinetic parameters between the two formulations are not dramatically different, we can expect a drastic difference in side effects in our nanocarrier formulation. The standard formulation uses CrEL, which is a harmful surfactant known to cause anaphylaxis in patients and requires pretreatment with anti-histamines and steroids. 17-AAG in PEO-b-PDLLA micelles was well tolerated by the rats with no observable acute signs of toxicity throughout the length of the study, and no mortality was observed with the nanocarrier formulation compared to 35% mortality within 24 hours observed with the standard formulation of CrEL-EtOH-PEG400. The lack of toxicity of PEO-b-PDLLA micelles as nanocarriers for paclitaxel compared to its standard CrEL formulation was also previously demonstrated in tumor-bearing animals, 32 where all animals dosed with the CrEL formulation died within 1 day of injection. Overall, this indicates that our nanocarrier formulation can retain the pharmacokinetic

disposition of 17-AAG without the need for toxic agents such as EtOH and CrEL, and is an important step towards solubilizing this promising chemotherapeutic agent in cancer therapy.

Biodistribution Studies

Quantifiable amounts of 17-AAG were observed in all assayed tissues (Figure 4 upper panel). The tissue collection was performed at 3 hours post i.v. administration of the different formulations at 10 mg/kg. The order of 17-AAG concentrations from highest to lowest for the control formulation was: lungs > kidney > liver > spleen > heart > brain > urinary bladder > bone > muscle > serum > whole blood. For the micellar formulation, the order from highest concentration to lowest was: lungs > kidney > liver > spleen > brain > bone > urinary bladder > heart > muscle > serum > whole blood. The micellar system provided some differences in the tissue distribution of 17-AAG into brain and heart, while similar concentrations to the standard were found in other tissues analyzed. The tissue to serum ratio (K_p) values in all tissues (Figure 4 lower panel) for the micellar formulation was lower than the control. The Kp values following administration of the standard formulation (CrEL-EtOH-PEG400) and for 17-AAG encapsulated in the micellar formulation (Figure 4 lower panel) were statistically different for brain, heart, bladder and whole blood; this might be attributed to differences in disposition between the carriers, such as partitioning preferences into tissues, clearance, and volume of distribution.

CONCLUSIONS

The lack of non-toxic 17-AAG formulations has hindered its progression into clinical trials. Newer derivatives, e.g. 17-DMAG, have overcome some problems with water solubility; however, the preferential and rapid clearance of these derivatives by the liver may limit drug distribution into tumors, thereby severely limiting the efficacy of the drug. We have reported a formulation of 17-AAG that does not require organic co-solvents or harsh surfactants, solubilizing up to 1.5 mg/mL of 17-AAG in PEO-b-PDLLA (12:6 kDa) micelles. Although PEO-b-PDLLA micelles reported herein did not demonstrate substantial sustained release in comparison to a standard formulation composed of CrEL-EtOH-PEG400, similar work with paclitaxel encapsulation into PEO-b-PDLLA micelles has demonstrated that this safer micellar formulation may minimize adverse side effects associated with CrEL following administration of the drug to patients. In addition, the nanoscale dimensions and stability of polymeric micelles may further benefit tumor specificity of the drug through the enhanced permeability and retention effect (EPR) effect 33 in the absence of targeting ligands. These results may be of interest for the clinical treatment of solid tumors and in the formulation of other large, lipophilic chemotherapeutics requiring harsh co-solvents like CrEL for systemic delivery.

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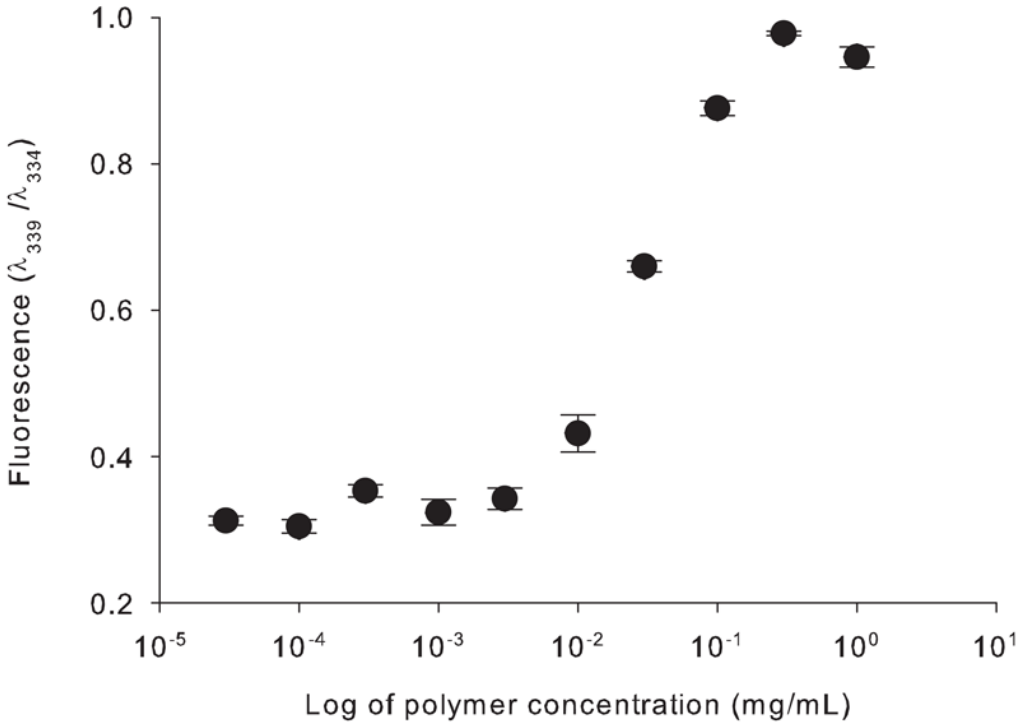

FIGURE 1.
Characterization of micelles revealing the critical micelle concentration of PEO-b-PDLLA (12:6 kDa) which was determined to be 350 nM.

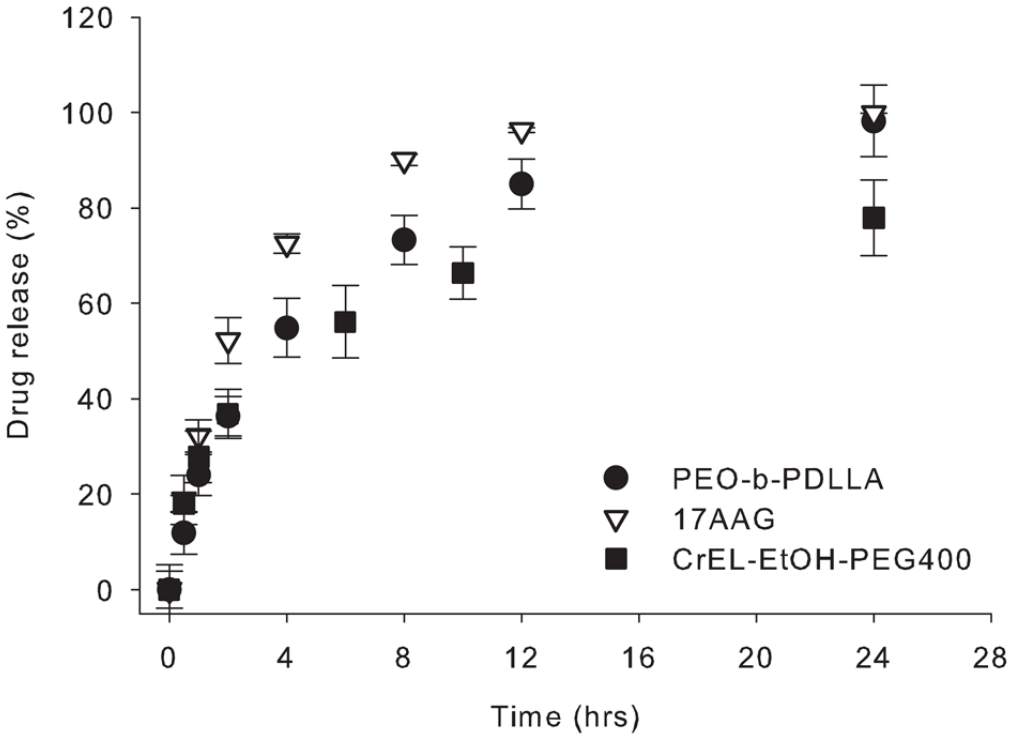

FIGURE 2.

In vitro release of 17-AAG from 0.3-mM PEO-b-PDLLA micelles in ddH_2O at 37ºC and pH 7.4. Micelles were prepared with a targeted loading of 10% w/w as noted in Table 1; PEO-b-PDLLA drug-loaded micelles (●), drug alone (△), and 17-AAG formulated in CrEL-EtOH-PEG400 (■).

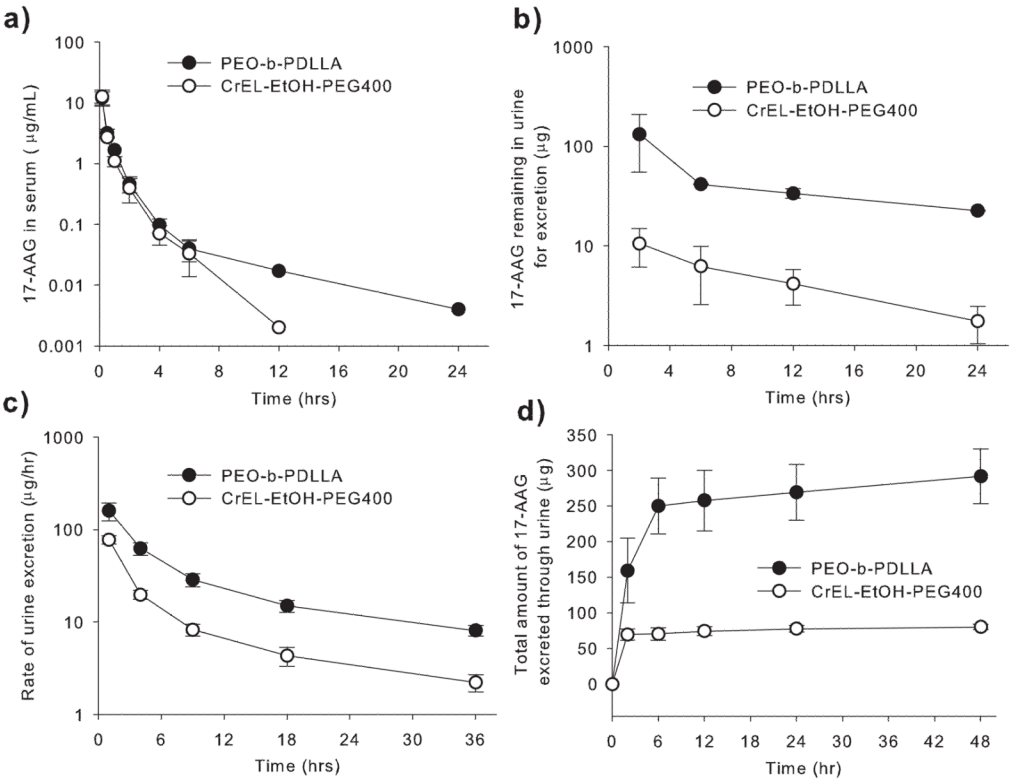

FIGURE 3.

Pharmacokinetic profiles in rats. Shown are (a) serum, (b) amount of 17-AAG remaining in the urine, (c) rate of urine excretion of 17-AAG, and (d) total amount of 17-AAG excreted through the urine.

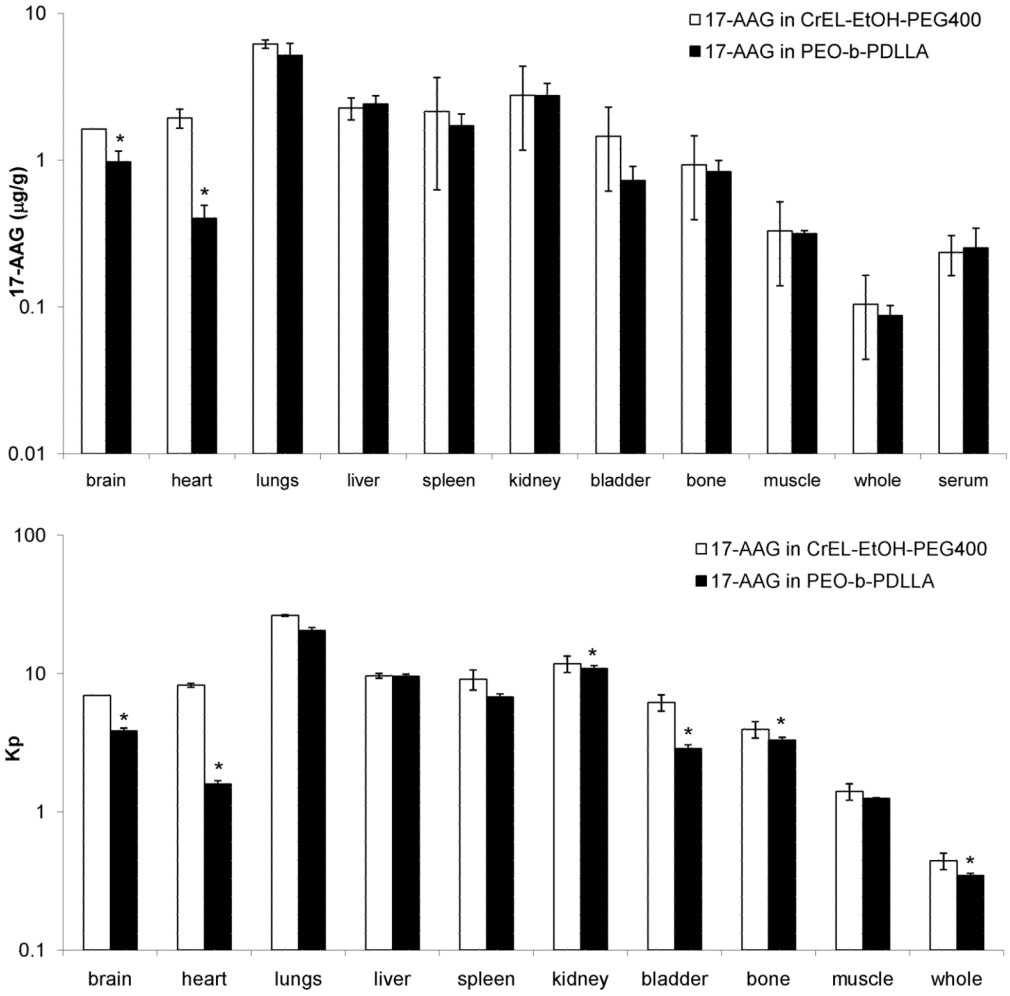

FIGURE 4.

Tissue distribution (upper panel) and K_p or Tissue to Serum Ratio (lower panel) following intravenous administration of 17-AAG (10 mg/kg) to rats 3 hours post dose (n = 5, mean ± SEM). * denotes statistically significant differences ($p<0.05$) between the standard formulation of CrEL-EtOH-PEG400 and 17-AAG encapsulated in PEO-b-PDLLA micelles.

TABLE 1

Summary of 17-AAG loading for a 0.3 mM PEO-b-PDLLA (5.4 mg/mL) micelle solution

Properties	
Actual 17-AAG added (mg/mL)	0.62
17-AAG solubilized (mg/mL)	0.083 ± 0.012
17-AAG loaded (mg/mg) [1]	0.014 ± 0.0020
17-AAG : PEO-b-PDLLA (mol:mol)	0.47 ± 0.068
Loading efficiency (%) [2]	14 ± 2.0
Final 17-AAG solubilized after concentrating (mg/mL) [3]	1.5 ± 0.20

[1] Loading based on weight 17-AAG solubilized versus total weight of carrier, including 17-AAG and PEO-b-PDLLA

[2] Loading efficiency based on 17-AAG loaded, 1.4%, divided by % loading attempted of 10%

[3] Loading of 17-AAG after micelles were concentrated by rotary evaporation.

TABLE 2

Cytotoxicity summaries for 17-AAG, 17-AAG in CrEL formulation, 17-AAG drug-loaded micelles, and micelles alone

Test agent	MCF-7 IC_{50} (nm) [a]	PC-3 IC_{50} (nm) [a]
17-AAG	22 ± 14	74 ± 14
CrEL-EtOH-PEG400	120 ± 32	330 ± 48
PEO-b-PDLLA drug-loaded micelles	160 ± 57 [b]	536 ± 13 [b]
PEO-b-PDLLA micelles	> 10 000	> 10 000

[a] Cells were incubated with test agents for 72 h (n = 6), and growth inhibition was measured by monitoring metabolic rates using the resazurin dye.

[b] Denotes statistically significant differences ($p<0.05$) between 17-AAG alone and 17-AAG in PEO-b-PDLLA micelles.

TABLE 3

Pharmacokinetic parameters determined by modeling.

Parameter Mean (SEM)	PEO-b-PDLLA 10 mg/kg(n = 5)	CrEL-EtOH-PEG400 10 mg/kg(n = 5)
$AUC_{0\rightarrow\infty}$ (μg h mL^{-1})	6.42 (1.42)	4.85 (1.06)
V_d (L kg^{-1})	8.93 (2.34) [a]	5.17 (2.24)
CL_{total} (L h^{-1} kg^{-1})	1.64 (0.36) [a]	2.17 (0.47)
$t_{1/2}$ serum (h)	4.21 (1.92) [a]	1.58 (0.38)
KE (h^{-1})	0.21 (0.095) [a]	0.47 (0.11)
MRT (h)	0.90 (0.16)	0.93 (0.19)
$t_{1/2}$ urine (h)	13.4 (4.44)	10.9 (0.73)
KE urine (h^{-1})	0.058 (0.019) [a]	0.064 (0.0043)
CL renal (L h^{-1} kg^{-1})	0.18 (0.017) [a]	0.043 (0.041)
CL hepatic (L h^{-1} kg^{-1})	1.45 (0.34) [a]	2.12 (0.43)

[a] Denotes statistically significant differences ($p<0.05$) between standard formulation with CrEL-EtOH-PEG400 and 17-AAG in PEO-b-PDLLA micelles