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

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Received 11 December 2007; revised 21 April 2008; accepted 22 June 2008

Published online 27 August 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21509

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. J Pharm Sci 98:1577–1586, 2009

Keywords: geldanamycin; poly(ethylene oxide)-b-poly(D,L-lactide); Cremophor EL; polymeric micelle; pharmacokinetics; 17-AAG; 17-allylamino-17-demethoxygeldanamycin

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

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Journal of Pharmaceutical Sciences, Vol. 98, 1577–1586 (2009)

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 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),[5,11] 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 pretreatment 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 (Pittsburgh, PA) or Sigma–Aldrich (St. Louis, MO) 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 (3×), centrifuged at 2000*g* for 15 min, and evaporated to dryness. Yield: 95 mg, 95%; MS m/z 584 (M^-); ^{1}H NMR ($CDCl_3$) δ 0.99 (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 (12000 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 et al.[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* 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 λ_{332}). Micelle solutions were concentrated by rotary evaporation at decreased pressure and room temperature, followed by centrifugation (5000*g* 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,