Published in final edited form as:

J Control Release. 2008 July 2; 129(1): 33–40.

Formulation of a Geldanamycin Prodrug in mPEG-b-PCL Micelles Greatly Enhances Tolerability and Pharmacokinetics in Rats

May P. Xiong[†], Jaime A. Yáñez[‡], Connie M. Remsberg[‡], Yusuke Ohgami[‡], Glen S. Kwon[§], Neal M. Davies[‡], and M. Laird Forrest^{†,*}

[†] Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66047-3729, USA

[‡] Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164, USA

[§] Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin 777 Highland Avenue, Madison, WI 53705-2222, USA

Abstract

Geldanamycin (GA) and its analogues inhibit heat shock protein 90 (Hsp90) and have shown significant antitumor activity *in vivo*; however, clinical development of GA has been hampered by its poor solubility and severe hepatotoxicity. More soluble analogues, such as 17-DMAG and 17-AAG, are easier to formulate, and have progressed through early clinical trials. However the large volume of distribution and systemic toxicity associated with these analogues may limit their distribution into tumors, thereby severely reducing efficacy and increasing nonspecific toxicities. We have evaluated a formulation of a lipophilic GA prodrug, 17'GAC₁₆Br encapsulated in methoxy-capped poly(ethylene glycol)-block-poly(ε-caprolactone) (mPEG-b-PCL) micelles, by comparing it to free 17-DMAG at 10 mg/kg in rats. mPEG-b-PCL micelles reported herein demonstrated substantial sustained release and conversion of 17'GAC₁₆Br into 17'GAOH at significantly greater levels in all tissues analyzed compared to the free drug, allowing for a 72-fold enhancement in the AUC, a 21-fold decrease in V_d, an 11-fold decrease in CL_{tot}, and a 2-fold and 7-fold enhancement in the overall MRT of 17'GAC₁₆Br and 17'GAOH, respectively. Importantly, the micellar formulation exhibited lower systemic toxicity than 17-DMAG, with a MTD > 200 mg/kg and a 2000-fold enhancement in the AUC. 17'GAC₁₆Br in micelles were poorly cleared renally, in contrast to 17-DMAG and 17'GAOH, but showed preferential accumulation and prodrug conversion in reticuloendothelial organs of normal animals. Overall, the data indicates that this nanocarrier system is a promising alternative to the current 17-DMAG formulation and offers excellent potential for further pre-clinical and clinical cancer studies.

Keywords

Geldanamycin prodrug; Poly(ethylene glycol)-b-poly(ε-caprolactone); Pharmacokinetics; Polymeric micelle; free 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin)

* To whom correspondence should be addressed. Phone: +1-785-864-4388; Fax: +1-785-864-5736; E-mail: mforrest@ku.edu.

[†]The University of Kansas.

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.

INTRODUCTION

Geldanamycin (GA) (Figure 1a) binds strongly to the ATP/ADP binding pocket of Heat shock protein 90 (Hsp90), interfering with the survival and proliferation of a diverse family of tumors [1–4]. However, clinical development of GA has been hampered by its poor solubility and severe hepatotoxicity [4, 5]. Several analogues have been developed to alleviate these issues: the allylamino analogue 17-AAG, and the dimethylaminoethylamino analogue 17-DMAG (Figure 1b). Nonetheless, to improve aqueous solubility, 17-AAG requires Cremophor EL (CrEL), DMSO or ethanol (EtOH) in parenteral formulations [6]. This is undesirable from a patient tolerability standpoint since CrEL is known to induce hypersensitivity reactions and anaphylaxis in patients, and requires pre-treatment with antihistamines and steroids before administration [7]. In addition, although considerably much more water soluble than 17-AAG (ca. 0.01 mg/mL) [8], 17-DMAG (>1 mg/mL) has demonstrated a greater volume of distribution (V_d) and considerable systemic toxicity at low doses in male Fisher 344 rats (<20 mg/kg), although no apparent toxicity in female CD2F1 mice were observed (<75 mg/kg) [9–11]. The volume of distribution is an apparent volume which assesses the distribution of a drug through the body after administration, and is dependent on the lipid or water solubility of the drug and its particular affinity for a given structure or tissue. A large volume of distribution indicates significant removal of the drug from the bloodstream into peripheral organs and a small volume of distribution indicates lower distribution to tissues and greater levels of the drug in the plasma for longer periods of time. Because 17-DMAG possesses superior aqueous solubility, potency, and greater oral bioavailability compared to 17-AAG [12, 13], several of the more promising leads toward clinical translation have been directed at developing 17-DMAG as the more pharmaceutically practical formulation [6, 14]. To minimize the non-specific tissue toxicity associated with the larger volume of distribution associated with 17-DMAG, safer and more effective delivery of GA relies on the development of biocompatible delivery systems capable of solubilizing the drug and improving its pharmacokinetic properties.

As such, micellar drug delivery systems are fast becoming one of the most versatile types of carriers currently investigated for formulating a variety of hydrophobic drugs; mostly because of their nanometer-sized dimensions, stealth properties arising from the hydrophilic shell present on the micellar surface, and the ease by which they can be chemically modified to be compatible with the drug of interest [15–21]. The main disadvantage with micellar systems is that unstable micelles can fall apart rapidly in plasma leading to excessive drug loss [22]. However, the utilization of self-assembled diblock micelles of type AB, where A represents the methoxy-capped polyethylene glycol (mPEG) block and B represent the poly(ϵ -caprolactone) (PCL) block, termed mPEG-b-PCL, has been effective at encapsulating different hydrophobic drug molecules without the inclusion of potentially harmful surfactants and excipients such as CrEL or EtOH [23–25]. PCL is an extremely attractive polymer for drug delivery due to the biocompatible nature of the degradation products [26] and PCL is currently approved by the FDA for use in humans. The advantage with mPEG-b-PCL micelles is that they are usually characterized by low critical micelle concentrations (CMCs) which are indicative of high stability leading to sustained drug release in the plasma [27,28], and are kinetically stable *in vivo* following i.v. injections into animals [29,30]. Recently, we reported on the use of micelles composed of mPEG-b-PCL (MW 5000:10000 g/mol) as biocompatible nanocarriers for a series of lipophilic GA prodrugs [15]. This system was highly efficient at solubilizing the lipophilic prodrug 17'GAC₁₆Br (Figure 1c) and providing sustained drug release ($t_{1/2} = 9.6 \pm 1.6$ days) from micelles, followed by its rapid hydrolysis ($t_{1/2} < 4$ h) into potent 17'GAOH (Figure 1d, IC₅₀ = 240 ± 30 nM in MCF-7 cells) [15]. Such mPEG-b-PCL micelles were characterized by a low critical micelle concentration (CMC) of 3.69 ± 0.57 mg·L⁻¹, increased prodrug loading capacity (25% w/w), and diameters averaging 119 ± 55 nm [15]. Herein, we report on the tolerability, pharmacokinetic properties, and tissue distribution

of 17'GAC₁₆Br encapsulated in mPEG-b-PCL micelles. Since it was impossible to encapsulate 17-DMAG in mPEG-b-PCL micelles or to directly administer 17'GAC₁₆Br to rodents due to its insolubility in aqueous media [15], we compared data from our micellar formulation to free 17-DMAG administered in a 0.9% saline solution. The results suggest that mPEG-b-PCL micelles can dramatically increase the tolerability of 17'GAC₁₆Br by modifying its pharmacokinetics and biodistribution compared to free 17-DMAG.

EXPERIMENTAL PROCEDURES

Synthesis and preparation of 17'GAC₁₆Br

The lipophilic prodrug 17'GAC₁₆Br was synthesized according to our previously published procedures [15]. Briefly, 17-β-hydroxyethylamino-17-demethoxygeldanamycin (17'GAOH) was synthesized by Michael's addition of ethanolamine to the 17-C position of GA (LCLabs, Woburn, MA), followed by N,N'-diisopropylcarbodiimide/4-dimethylaminopyridine (DIC/DMAP) conjugation of 2-bromohexadecanoic acid to the newly formed hydroxyl, and subsequently purified by prep-scale reverse phase high performance liquid chromatography (RP-HPLC, >99.8% purity) [15,31]. mPEG-b-PCL (5000:9200 g/mol) was synthesized through acid-catalyzed ring opening polymerization of ε-caprolactone initiated by hydroxyl-terminated poly(ethylene glycol) (5000 g/mol, Polymer Source, Dorval Quebec) [32]. Next, the prodrug and polymer (1:3 w/w prodrug:polymer) were dissolved in acetone (150 mg polymer/mL) and added dropwise to vigorously stirred ddH₂O (final 33% v/v acetone). The organic solvent was then removed by stirring overnight under N₂ purge, and the remaining aqueous solution containing drug-loaded micelles was filtered through a 0.22-μm polyestersulfone filter to remove insoluble material and un-incorporated drug. Using 0.5-mM mPEG-b-PCL micelles, we had reported a 2.7 mg/mL solubility of the prodrug [15], however solubility can be increased by respectively loading the prodrug in more concentrated micelle solutions. In this manner, the final concentration of prodrug solubilized in micelles was 14.4 mg/mL for this study. Drug solubility was measured by RP-HPLC, and drug incorporation into micelles was verified by size exclusion chromatography as previously described [15,16].

Reverse-phase HPLC quantitative assay

An internal standard, 17-β-hydroxyhexanolamino-17-demethoxygeldanamycin (IS, 17'GA6OH) was prepared using similar procedures for synthesis of 17'GAOH, as reported earlier [15], by the addition of aminohexanol to GA. Tissue and serum samples were prepared by mixing 100 mg (or μL) of the tissue or serum, and 100 μL of the IS (25 μg/mL) in a microcentrifuge tube and precipitating with 1 mL of cold acetonitrile. Next, samples were centrifuged (3000 g's, 5 min), the organic layer was extracted and dried by vacuum centrifugation, and the residue was reconstituted in 400 μL of the initial mobile phase before analysis. Urine samples (400 μL) and 100 μL IS were mixed, spun down to remove insoluble material, dried by vacuum centrifugation, and the residue was reconstituted in 400 μL of initial mobile phase. Typically, a 150 μL sample of reconstituted serum, urine or tissue (with IS) was analyzed by RP-HPLC (Genesis 33 × 4.6-mm 3-μm C18 column, 332-nm detection, flow rate 1 mL/min at room temperature). The chromatography conditions were as follows, using a mobile phase A of 50 mM acetic acid+10 mM triethylamine (TEA) (pH 3.0) and B of methanol +10 mM TEA (17'GAC₁₆Br samples: 0–3 min 75% B, 3–11 min 99% B, 11–18 min 75% B; peak elution of 17'GAOH occurred at 6.5 min, IS at 7.3 min, and 17'GAC₁₆Br at 11.1 min; free 17-DMAG samples: 0–3 min 40% B, 3–8 min 75% B, 8–14 min 40% B; peak elution of free 17-DMAG occurred at 5.3 min and IS at 7.1 min). Inter and intra-day variances were <10% at all concentrations measured. The lowest detection limit for all compounds was 25 ng/mL per 100 μL sample. Recovery of 17'GAC₁₆Br, 17'GAOH, and 17-DMAG from serum and urine was >95%. The recovery of 17'GAC₁₆Br, 17'GAOH, and 17-DMAG from the different tissues was 95.5–97.2%, 96.2–98.3%, and 95.1–98.1% respectively.

Surgical procedures

Healthy male Sprague-Dawley rats (200–240 g) were obtained from Simonsen Labs (Gilroy, CA, USA) and given food (Purina Rat Chow 5001) and water *ad libitum* for at least 3 days before use. Rats were housed in temperature controlled rooms with a 12 h light/dark cycle. The day before the pharmacokinetic experiment, rats were put under isoflurane anesthesia and their right jugular veins were catheterized with a sterile silastic cannula (Dow Corning, Midland, MI, USA). Animals were similarly cannulated for the biodistribution studies since it facilitates intravenous administration of the formulations, parallels the injection route utilized in the pharmacokinetic study, and permits ease of blood sample collection before termination of the biodistribution study. Following each cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) connected to the cannula was exteriorized through the dorsal skin and flushed with 0.9% saline. Animals were subsequently transferred to metabolic cages and fasted overnight before all experiments. 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. Final injection volumes administered to rodents ranged between 1 mL (10 mg/kg dosing) and 3 mL (200 mg/kg dosing).

Tolerability and pharmacokinetic studies

On the days of the experiment, animals were intravenously administered a single bolus injection of test compounds. The maximum tolerated dose (MTD) was determined through dose escalation studies (n=3 animals/dose): free 17-DMAG doses were 10, 20, 40 mg/kg and 17' GAC₁₆Br in micelle doses were 10, 20, 40, 200 mg/kg. Subsequently, for the pharmacokinetic studies, free 17-DMAG was administered at the MTD of 10 mg/kg (n=5 for each treatment group). The prodrug formulation in mPEG-b-PCL micelles was administered at 10 mg/kg for comparison to free 17-DMAG and at 200 mg/kg, corresponding to the MTD tested in tolerability studies.

Animals were fed 2 h following intravenous administration of all test agents. Blood and urine samples were collected over 48-h and 72-h, respectively. At each particular time point, blood samples (~0.30 mL) were drawn from the cannula, and the cannula was subsequently flushed with 0.3 mL 0.9% saline to replenish the blood volume that was withdrawn. Blinded-observers were asked to evaluate all animals for signs of acute toxicity (visible behavior changes, bleeding, and overall appearance). Blood samples were collected into regular polypropylene microcentrifuge tubes. Tubes were spun down at 5000 rpm for 5 min, and the supernatant containing serum was collected and stored in separate microcentrifuge tubes at –70°C until further analysis. Similarly, urine samples were collected at appropriate times following i.v. administration and stored at –70°C until further analysis.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using data from individual rats. The mean and standard error of the mean (mean ± sem) were calculated for each group. The elimination rate constant (KE) was estimated by linear regression of the blood or plasma concentrations in the log-linear terminal phase. In order to estimate the immediate initial serum concentration (C₀) following injection of the nanocarriers and standard formula, a two-compartmental model was utilized to fit the raw serum concentration versus time data (WinNonlin® software, Ver. 5.1). The estimated C₀ and raw measured serum concentrations were then utilized to determine the area under the concentration-time curve (AUC). The total AUC_{0–∞} was calculated by means of the combined log-linear trapezoidal rule, from time of dosing to the last measured concentration, plus the quotient (the remainder area under the curve) of the last measured concentration divided by KE. Following, non-compartmental pharmacokinetic methods were used to calculate the mean residence time (MRT = AUMC_{0–∞}/AUC_{0–∞}), total clearance

($CL_{tot} = \text{Dose}/AUC_{0-\infty}$) and volume of distribution ($V_d = CL_{tot}/KE$). After obtaining the cumulative urinary excretion of the drug (ΣXu), the fraction excreted in urine ($F_e = \Sigma Xu/C_0$), renal clearance ($CL_{renal} = F_e \times CL$), and hepatic clearance ($CL_{hepatic} = CL_{tot} - CL_{renal}$) with extraction ratio ($ER = CL_{hepatic}/Q$, where Q = mean hepatic flow) were determined. Note that the mean hepatic blood flow (Q) is approximately 3.22 L/h/kg in rats [33], and since the serum was analyzed, the hematocrit value of 0.48 in rats [33] was employed to lead to a mean hepatic plasma flow (Q) of 1.74 L/h/kg in the pharmacokinetic analysis.

Biodistribution studies

To assess the effect of formulation on the tissue distribution, healthy rats ($n=5$ for each group, 200–240 g) were cannulated and intravenously administered with either free 17-DMAG given with 0.9% NaCl or 17'GAC₁₆Br in mPEG-b-PCL micelles at a single bolus injection of 10 mg/kg per rat. Due to the rapid clearance of free 17-DMAG following i.v. administration, limit of detection of the instrument for all the test compounds (25 ng/mL), and rapid hydrolysis rate of 17'GAC₁₆Br into 17'GAOH ($t_{1/2} < 4$ h), animals were sacrificed 3-h post-i.v. injection to quantifiably assess biodistribution of all the drugs in the various tissues. At the appropriate time, each animal was anaesthetized and ex-sanguinated by cardiac puncture. Brain, heart, lungs, liver, spleen, kidneys, urinary bladder, bone, muscle and serum samples were collected. Tissue samples were blotted with paper towels, washed in ice-cold saline, bottled to remove excess fluid before weighing, rapidly frozen in liquid nitrogen, and pulverized to a fine powder using mortar and pestle before storing at -70°C for HPLC drug analysis.

Data Analysis

Compiled data were presented as mean and standard error of the mean (mean $\pm$ sem). 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

RP-HPLC quantitative tissue assay

The internal standard 17'GA6OH demonstrated excellent linearity ($R^2=0.99$) when utilized as a calibration curve over the range of concentrations studied in various tissues (0.1–500 $\mu\text{g/mL}$). Inter- and intra- day variances were within International Harmonization criteria for assay validation and were at $<10\%$ for all concentrations measured. The lowest detection limit for all compounds tested was 25 ng/mL per 100 μL sample. Chromatograms were free of interference from endogenous components and individual compounds eluted as distinct peaks under appropriately optimized gradient conditions (Figure 2). Tissue processing was conducted under low temperature conditions, and analysis was performed within 24 h of tissue collection when possible to minimize hydrolysis of 17'GAC₁₆Br into 17'GAOH. No hydrolysis or degradation was observed in tissue standards processed as described above, and also when stored up to one week at -70°C .

Tolerability and pharmacokinetic studies

Rodents were initially escalated from 10 to 40 mg/kg free 17-DMAG. At 20 mg/kg, one of three rodents died. Similarly, at 40 mg/kg one of three rodents also died immediately. In both cases the cause of death was undetermined. All animals at 10 mg/kg of free 17-DMAG ($n=3$) survived. For 17'GAC₁₆Br in mPEG-b-PCL micelles, rodents were escalated starting from 10 mg/kg. At 40 mg/kg, all rodents survived through 72 h with normal urine output and no outward signs of acute toxicity. Following, the dose was escalated to 200 mg/kg 17'GAC₁₆Br in mPEG-b-PCL micelles ($n=4$). This corresponds to an i.v. dose averaging 44 mg prodrug per rodent

(average body weight of 220 g) or an injection volume of approximately 3-mL (14.4 mg drug/mL in micelles). Of the four animals, one died within 24 h with greatly reduced urine output. The remaining rodents survived through 72 h and demonstrated no visible signs of acute toxicity. Observations performed by blinded observers reported that 12 hours post-i.v. dosing of free 17-DMAG at concentrations above 10 mg/kg, the rats presented nose bleeding, disorientation, heavy breathing, and slight decrease in response to sound. The animals that received 17'GAC₁₆Br in the mPEG-b-PCL micelle formulation did not display adverse effects for the first 24 hours at 40 mg/kg dosage, but did demonstrate mild diarrhea and nose bleeding 48 hours post-administration of the dose.

In the pharmacokinetic studies, five animals were dosed at 10 mg/kg of 17'GAC₁₆Br in mPEG-b-PCL micelles for comparison to free 17-DMAG, and at the 200 mg/kg 17'GAC₁₆Br formulation for comparison to its own 10 mg/kg dosage. In Figure 3, the serum levels of free 17-DMAG and 17'GAC₁₆Br concentration vs. time profiles at 10 mg/kg differed, with the micellar formulation demonstrating prolonged circulation in the blood compared to the more rapidly eliminated free 17-DMAG. It was also observed that 17'GAC₁₆Br was rapidly converted to 17'GAOH following administration, as evidenced by its early presence in serum (Figure 3). This rapid release of the prodrug from micelles at the onset of the pharmacokinetic profile is most likely a result of prodrug molecules that had not been fully encapsulated within the semi-crystalline PCL core, which rapidly diffuses out into the blood following injection. This is also observed to correlate with a rapid 17'GAOH distribution phase and a much slower elimination phase following sustained release of prodrugs from micelles over 48 h.

At 200 mg/kg 17'GAC₁₆Br, we observed greater initial concentrations of the micelles in serum as well as a greater level of hydrolyzed prodrug (17'GAOH) due to initial rapid release of the drug. However at 12 h, the serum levels of the 200 mg/kg micellar dose were similar to 10 mg/kg levels but the hydrolyzed product was eliminated from serum at a faster rate than the 10 mg/kg dose. There was a 1.8-fold greater hepatic clearance of 17'GAOH by the liver at 200 mg/kg compared to the same 10 mg/kg dose (Table 1). The un-hydrolyzed lipophilic prodrug is protected in the micelles, and therefore its rate of elimination is in proportion to the rate of clearance of the micelle as well as release of lipophilic prodrug molecules from the micelles at both dosages. Specifically, we observe that at 10 mg/kg, the AUC of 17'GAC₁₆Br in micelles is 72-fold greater than free 17-DMAG administered at the same dose (Table 1). Furthermore, at 200 mg/kg of 17'GAC₁₆Br in micelles, the AUC jumps to a dramatic 2000-fold improvement and the volume of distribution (V_d) decreased 21-fold compared to free 17-DMAG.

The largest contribution to total clearance occurred in the liver since it is the principal organ for drug metabolism (Table 1). Although the 200 mg/kg dose of 17'GAC₁₆Br in micelles resulted in greater initial concentrations of 17'GAOH in serum, it was also characterized by an extraction ratio 2.7-fold greater than free 17-DMAG at 10 mg/kg (Table 1). Because a greater portion of the prodrug was lost during its passage through the liver, the half-life of the prodrug was only 1.4-fold greater than that of free 17-DMAG at 10 mg/kg in spite of its higher serum concentration. In Figure 4a, the data revealed that free 17-DMAG at 10 mg/kg was cleared through the urine at similar levels to 17'GAOH at 200 mg/kg. Interestingly, their rates of urinary excretion were also similar despite the dosage differences (Figure 4b). In contrast to free 17-DMAG (10 mg/kg) and 17'GAOH (200 mg/kg), the micelles were cleared slowly through the urine (Figure 4b). The total renal clearance (CL_{renal}) of free 17-DMAG is ca. 52 000-fold and 27 000-fold greater than the micelle formulation at 10 and 200 mg/kg respectively (Table 1). Taken together, at 10 mg/kg the total clearance (CL_{tot}) for 17'GAC₁₆Br in mPEG-b-PCL micelles decreased 11-fold over free 17-DMAG, leading to a significant improvement in mean residence time for the lipophilic prodrug encapsulated in micelles (2-fold) and its hydrolyzed product 17'GAOH (7-fold). Taken together, the data suggest that the micellar formulation decreases non-specific systemic exposure through sustained release of 17'GAOH.

Biodistribution studies

Quantifiable amounts of prodrugs were observed in all tissues assayed (Figure 5a). The tissue collection was performed 3-h post i.v. at the 10 mg/kg dosage for the two formulations: free 17-DMAG in 0.9% NaCl and 17'GAC₁₆Br in mPEG-b-PCL micelles. The tissue distribution timepoint was chosen based on serum pharmacokinetic data for free 17-DMAG (which exhibited rapid clearance), that would still allow for accurate HPLC quantification of drug concentrations in all tissues. The order of prodrug concentrations from highest to lowest for free 17-DMAG were: urinary bladder > spleen > lungs > kidneys > serum > liver > bone > heart > muscle > brain. For 17'GAC₁₆Br in mPEG-b-PCL micelles, the order from highest concentration to lowest was: spleen > serum > liver > lungs > muscle > heart > bone > kidney > brain > urinary bladder. For 17'GAOH, the order from highest concentration to lowest was: spleen > urinary bladder > liver > kidney > lungs > heart > bone > muscle > serum > brain. The tissue to serum ratio (K_p) values in all tissues (Figure 5b), except for spleen and brain, for the micellar formulation was lower than free 17-DMAG and is consistent with the much larger volume of distribution usually attributed to 17-DMAG [10]. These differences in K_p values might be ascribed to the differences in partitioning and clearance between free 17-DMAG and the micelles. This can be observed based on the significantly higher concentrations of the micelles in serum 3-h post administration (Figure 5a). Of interest also is the presence of 17'GAOH which was detected at significantly greater levels than either 17'GAC₁₆Br or free 17-DMAG in all tissues assayed, except for spleen, muscle, serum and brain. The highest ratio of 17'GAC₁₆Br to 17'GAOH in tissues occurred in the following decreasing order: urinary bladder > kidney > liver > lungs > bone > heart > muscle > spleen > brain > serum (Figure 6). This may indicate that prodrug conversion occurs much more rapidly in the organs or that 17'GAOH quickly partitions into internal organs following release/conversion from mPEG-b-PCL micelles.

DISCUSSION

17-DMAG (solubility >1 mg/mL) has demonstrated a high volume of distribution (V_d) and considerable systemic toxicity at low doses in rats (<20 mg/kg) [10,11]. To minimize systemic toxicity due to the large volume of distribution associated with 17-DMAG, safer and more effective delivery of GA relies on the development of biocompatible delivery systems capable of solubilizing the drug and improving its pharmacokinetic properties. The utilization of self-assembled mPEG-b-PCL micelles has been effective at encapsulating other hydrophobic drug molecules for modifying pharmacokinetics and biodistributions [24,25,34]. In addition, there is literature precedence for synthesizing lipophilic prodrugs, such as daunorubicin or 5-fluorouracil, for increasing drug hydrophobicity and enhancing encapsulation into liposomal delivery systems [35–37]. Nanoemulsions of a lipophilic paclitaxel oleate prodrug into cholesterol-rich nanoparticles have also shown increased solubilization and improved pharmacokinetic properties compared to the parent compound alone [38].

We found that mPEG-b-PCL could not encapsulate GA (Figure 1a) or 17-DMAG (Figure 1b), however the system was highly efficient at solubilizing the lipophilic prodrug 17'GAC₁₆Br (Figure 1c) and dramatically increased its loading capacity (25% w/w) into micelles. Prodrug-loaded micelles are characterized by diameters averaging 119 ± 55 nm, and exhibit sustained release from micelles ($t_{1/2} = 9.6 \pm 1.6$ days) followed by rapid hydrolysis of the prodrug into potent 17'GAOH (Figure 1d) ($IC_{50} = 240 \pm 30$ nM in MCF-7 cells). The hydrolysis rate of 17'GAC₁₆Br to 17'GAOH was <4 hrs, as determined from a 70% v/v mixture of DMSO/propylene glycol (1:1 v/v) and 20-mM phosphate buffer at pH 7.4 and 37°C [15]. At aqueous mixtures above 70% v/v, the lipophilic 17'GAC₁₆Br precipitated out of solution and made it impossible to measure hydrolysis rates. Based on these promising data, 17'GAC₁₆Br encapsulated in mPEG-b-PCL micelles was evaluated in rats to investigate the potential of the micellar

formulation to modify the pharmacokinetics and biodistribution of the prodrug in relation to free 17-DMAG.

Overall, there were dramatic differences in the pharmacokinetic properties of 17'GAC₁₆Br in micelles compared to free 17-DMAG. The AUC of 17'GAC₁₆Br in micelles improved 72-fold compared to the standard at 10 mg/kg. When the dose for 17'GAC₁₆Br in micelles was raised to 200 mg/kg, the AUC dramatically increased 2000-fold compared to free 17-DMAG at 10 mg/kg. This indicates that mPEG-b-PCL micelles were significantly stable in blood, allowing for sustained release and conversion of 17'GAC₁₆Br over 48-h without leading to significant systemic toxicities, especially evident at the high dosage of 200 mg/kg. mPEG-b-PCL micelle stability in blood is further justified by recent work which has shown that a significant portion of these block-copolymers do indeed remain intact as micelles *in vivo* [29,30]. There was evidence of rapid release in serum for 17'GAOH at 10 and 200 mg/kg 17'GAC₁₆Br loaded-micelles, which was not apparent during *in vitro* characterizations in ddH₂O at 37°C and pH 7.4 [15]. This might be because *in vivo*, lipophilic prodrug molecules not fully solubilized within the semi-crystalline micellar core, in contrast to prodrugs that are fully encapsulated, are more favorably displaced by serum proteins and may result in the rapid apparent burst release observed. Despite some drug loss, a substantial portion of the micellar formulation demonstrates evidence of long circulating nanoparticles capable of providing sustained prodrug release (Figure 3). At 10 mg/kg, the increase in AUC for mPEG-b-PCL micelles was therefore a result of an 11-fold reduction in CL_{tot}, a 21-fold decrease in V_d for the encapsulated prodrug and a 2-fold increase in MRT (Table 1).

At 200 mg/kg, 17'GAOH apparent burst release is greater than at 10 mg/kg, and both 17-DMAG and 17'GAOH (200 mg/kg dose) are preferentially cleared through the urine (Figure 4a) at similar excretion rates (Figure 4b). At 10 mg/kg, 17'GAOH levels are much lower in the urine (Figure 4a, insert) and its excretion rate in urine is also an order of magnitude lower (Figure 4b). In Figure 5a, serum data reveals that 17'GAC₁₆Br (10 mg/kg) is present at greater levels than 17'GAOH (10 mg/kg), and possibly indicates slow rates of prodrug release from micelles and/or rapid partitioning of hydrolyzed 17'GAOH into tissues. For the two doses administered, CL_{hepatic} and extraction ratio are significantly different from each other (Table 1), indicative of possible saturation mechanisms at the higher dose. Although serum levels are expected to increase linearly in proportion to a dose given (in the absence of saturation in clearing organs), nonlinearity between doses might also arise due to drug-carrier release properties, low dissolution/hydrolysis of the prodrug, or partitioning preferences of individual prodrugs for specific tissues [39]. Without a more thorough investigation of all possible mechanisms, the exact cause of non-linearity between these parameters remains undetermined.

In contrast to serum level, 17'GAOH presence in all organs, except for spleen, muscle, serum and brain, is much higher than 17'GAC₁₆Br at 10 mg/kg (Figure 5a). This reinforces either that prodrug conversion occurred rapidly once in the organs or that 17'GAOH partitioned quickly to internal organs following release and hydrolysis of the prodrug from mPEG-b-PCL micelles. The biodistribution data also revealed that 17'GAC₁₆Br at 10 mg/kg in micelles exhibited the lowest total accumulation (not including brain) and K_p in the urinary bladder. This data corresponds well with the pharmacokinetic data which supported that micelles were poorly cleared through the urine compared to free 17-DMAG or 17'GAOH (Figure 4b). On the other hand, 17'GAOH was detected at much greater levels in the urinary bladder and kidneys 3-h post administration (Figure 5), and as explained before, this is most likely due to the rapid release effect and/or rapid conversion of 17'GAC₁₆Br to 17'GAOH in serum, resulting in high levels of renal clearance. Similarly, free 17-DMAG also demonstrated greater accumulation in the urinary bladder based on K_p values. Hence, the biodistribution data confirms that in the absence of the nanocarrier, 17'GAOH and free 17-DMAG undergo preferential renal clearance. For the micelles, the accumulation and K_p value for 17'GAC₁₆Br

were highest in spleen, followed by liver, and suggest preferential uptake of the micelles for clearance by the reticuloendothelial system (RES) (Figure 5b). Subsequently, this may also explain the high K_p values observed for 17'GAOH in spleen and liver, attributed to micelle degradations and prodrug conversions in those organs. Overall, sustained prodrug release or conversion from mPEG-b-PCL micelles resulted in significantly greater K_p values in all tissues collected for 17'GAOH in relation to free 17-DMAG.

These are the first sets of promising results available in the literature for improving delivery of a GA prodrug via a micellar nanocarrier. In addition to exhibiting favorably lower systemic toxicities, the stealth properties of the micelle and nanometer-sized dimensions may further impart dramatic improvements in drug localization for passive targeting to solid tumors due to the enhanced permeability and retention (EPR) effect [40]. Overall the data indicates that this nanocarrier system is a promising alternative to free 17-DMAG and offers excellent potential for further pre-clinical and clinical cancer studies.

CONCLUSIONS

17-DMAG is a GA derivative which has overcome some problems associated with water solubility; however its large volume of distribution and systemic toxicity may limit distribution into tumors, thereby severely reducing the efficacy of the drug. We have evaluated a formulation of a lipophilic GA prodrug, 17'GAC₁₆Br, encapsulated in mPEG-b-PCL micelles. mPEG-b-PCL micelles reported herein demonstrated substantial sustained release and conversion of 17'GAC₁₆Br into 17'GAOH in all tissues analyzed, at significantly greater levels than free 17-DMAG, allowing for a 72-fold enhancement in the AUC, a 21-fold decrease in V_d , an 11-fold decrease in CL_{tot} , and a 2-fold and 7-fold enhancement in the overall MRT of 17'GAC₁₆Br and 17'GAOH, respectively at 10 mg/kg dose. In addition, the nanoscale dimensions may further benefit tumor specificity of the drug through the EPR effect even 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 surfactants like CrEL for systemic delivery.

Acknowledgements

This research was supported by NIH grant AI-43346-08, NIH-COBRE P20 RR015563, generous grants from Hoffman-La Roche, Wisconsin Alumni Research Foundation, Shimadzu Scientific, PhRMA Foundation, and an American Foundation for Pharmaceutical Education (AFPE) Gateway to Research Scholarship to CMR.

References

1. Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J Med Chem* 1999;42(2):260–266. [PubMed: 9925731]
2. Goetz MP, Toft DO, Ames MM, Erlichman C. The Hsp90 chaperone complex as a novel target for cancer therapy. *Ann Oncol* 2003;14(8):1169–1176. [PubMed: 12881371]
3. Citri A, Kochupurakkal BS, Yarden Y. The achilles heel of ErbB-2/HER2: regulation by the Hsp90 chaperone machine and potential for pharmacological intervention. *Cell Cycle* 2004;3(1):51–60. [PubMed: 14657666]
4. Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* 1995;36(4):305–315. [PubMed: 7628050]
5. Ge J, Normant E, Porter JR, Ali JA, Dembski MS, Gao Y, Georges AT, Grenier L, Pak RH, Patterson J, Sydor JR, Tibbitts TT, Tong JK, Adams J, Palombella VJ. Design, synthesis, and biological evaluation of hydroquinone derivatives of 17-amino-17-demethoxygeldanamycin as potent, water-soluble inhibitors of Hsp90. *J Med Chem* 2006;49(15):4606–4615. [PubMed: 16854066]

6. Sausville EA, Tomaszewski JE, Ivy P. Clinical development of 17-allylamino, 17-demethoxygeldanamycin. *Curr Cancer Drug Targets* 2003;3(5):377–383. [PubMed: 14529389]
7. Dye D, Watkins J. Suspected anaphylactic reaction to Cremophor EL. *British medical journal* 1980;280(6228):1353. [PubMed: 7388538]
8. Geldanamycin Analogs. Development Therapeutics Program of the National Cancer Institute; Bethesda, MD: 2007.
9. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L, Pak RH, Ali JA, Dembski MS, Hudak J, Patterson J, Penders C, Pink M, Read MA, Sang J, Woodward C, Zhang Y, Grayzel DS, Wright J, Barrett JA, Palombella VJ, Adams J, Tong JK. Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. *Proceedings of the National Academy of Sciences of the United States of America* 2006;103(46):17408–17413. [PubMed: 17090671]
10. Egorin MJ, Lagattuta TF, Hamburger DR, Covey JM, White KD, Musser SM, Eiseman JL. Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. *Cancer Chemother Pharmacol* 2002;49(1):7–19. [PubMed: 11855755]
11. Sausville EA, Tomaszewski JE, Ivy P. Clinical development of 17-allylamino, 17-demethoxygeldanamycin. *Curr Cancer Drug Targets* 2003;3(5):377–383. [PubMed: 14529389]
12. Smith V, Sausville EA, Camalier RF, Fiebig HH, Burger AM. Comparison of 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17DMAG) and 17-allylamino-17-demethoxygeldanamycin (17AAG) in vitro: effects on Hsp90 and client proteins in melanoma models. *Cancer Chemother Pharmacol* 2005;56(2):126–137. [PubMed: 15841378]
13. Kaur G, Belotti D, Burger AM, Fisher-Nielson K, Borsotti P, Riccardi E, Thillainathan J, Hollingshead M, Sausville EA, Giavazzi R. Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin Cancer Res* 2004;10(14):4813–4821. [PubMed: 15269157]
14. Glaze ER, Lambert AL, Smith AC, Page JG, Johnson WD, McCormick DL, Brown AP, Levine BS, Covey JM, Egorin MJ, Eiseman JL, Holleran JL, Sausville EA, Tomaszewski JE. Preclinical toxicity of a geldanamycin analog, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), in rats and dogs: potential clinical relevance. *Cancer Chemother Pharmacol* 2005;56(6):637–647. [PubMed: 15986212]
15. Forrest ML, Zhao A, Won CY, Malick AW, Kwon GS. Lipophilic prodrugs of Hsp90 inhibitor geldanamycin for nanoencapsulation in poly(ethylene glycol)-b-poly(epsilon-caprolactone) micelles. *J Control Release*. 2006
16. Forrest ML, Won CY, Malick AW, Kwon GS. In vitro release of the mTOR inhibitor rapamycin from poly(ethylene glycol)-b-poly(epsilon-caprolactone) micelles. *J Control Release* 2006;110(2):370–377. [PubMed: 16298448]
17. Yamamoto T, Yokoyama M, Opanasopit P, Hayama A, Kawano K, Maitani Y. What are determining factors for stable drug incorporation into polymeric micelle carriers? Consideration on physical and chemical characters of the micelle inner core. *J Control Release* 2007;123(1):11–18. [PubMed: 17716772]
18. Kwon GS. Diblock copolymer nanoparticles for drug delivery. *Crit Rev Ther Drug Carrier Syst* 1998;15(5):481–512. [PubMed: 9822868]
19. Nishiyama N, Kataoka K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacology & therapeutics* 2006;112(3):630–648. [PubMed: 16815554]
20. Torchilin VP. Structure and design of polymeric surfactant-based drug delivery systems. *J Control Release* 2001;73(2–3):137–172. [PubMed: 11516494]
21. Gaucher G, Dufresne MH, Sant VP, Kang N, Maysinger D, Leroux JC. Block copolymer micelles: preparation, characterization and application in drug delivery. *J Control Release* 2005;109(1–3):169–188. [PubMed: 16289422]
22. Kwon GS, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Biodistribution of micelle-forming polymer-drug conjugates. *Pharmaceutical research* 1993;10(7):970–974. [PubMed: 8378259]

23. Jette KK, Law D, Schmitt EA, Kwon GS. Preparation and drug loading of poly(ethylene glycol)-block-poly(epsilon-caprolactone) micelles through the evaporation of a cosolvent azeotrope. *Pharm Res* 2004;21(7):1184–1191. [PubMed: 15290858]
24. Aliabadi HM, Mahmud A, Sharifabadi AD, Lavasanifar A. Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A. *J Control Release* 2005;104(2):301–311. [PubMed: 15907581]
25. Allen C, Han J, Yu Y, Maysinger D, Eisenberg A. Polycaprolactone-b-poly(ethylene oxide) copolymer micelles as a delivery vehicle for dihydrotestosterone. *J Control Release* 2000;63(3):275–286. [PubMed: 10601723]
26. Woodward SC, Brewer PS, Moatamed F, Schindler A, Pitt CG. The intracellular degradation of poly(epsilon-caprolactone). *J Biomed Mater Res* 1985;19(4):437–444. [PubMed: 4055826]
27. Soo P, Luo L, Maysinger D, Eisenberg A. Incorporation and release of hydrophobic probes in biocompatible polycaprolactone-block-poly(ethylene oxide) micelles: implications for drug delivery. *Langmuir* 2002;18:9996–10004.
28. Park Y, Lee J, Chang Y, Jeong J, Chung J, Lee M, Park K, Lee S. Radioisotope carrying polyethylene oxide-caprolactone copolymer micelles for targetable bone imaging. *Biomaterials* 2003;23:873–879. [PubMed: 11771706]
29. Liu J, Zeng F, Allen C. In vivo fate of unimers and micelles of a poly(ethylene glycol)-block-poly(caprolactone) copolymer in mice following intravenous administration. *Eur J Pharm Biopharm* 2007;65(3):309–319. [PubMed: 17257817]
30. Savic R, Azzam T, Eisenberg A, Maysinger D. Assessment of the integrity of poly(caprolactone)-b-poly(ethylene oxide) micelles under biological conditions: a fluorogenic-based approach. *Langmuir* 2006;22(8):3570–3578. [PubMed: 16584228]
31. Sasaki, K.; Inoue, Y. Geldanamycin derivatives and antitumor drug. US 4,261,989. April 14, 1981. 1980
32. Shibasaki Y, Sanada H, Yokoi M, Sanda F, Endo T. Activated monomer cationic polymerization of lactones and the application to well-defined block copolymer synthesis with seven-membered ring cyclic carbonate. *Macromolecules* 2000;33(12):4316–4320.
33. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharmaceutical research* 1993;10(7):1093–1095. [PubMed: 8378254]
34. Aliabadi HM, Brocks DR, Lavasanifar A. Polymeric micelles for the solubilization and delivery of cyclosporine A: pharmacokinetics and biodistribution. *Biomaterials* 2005;26(35):7251–7259. [PubMed: 16005061]
35. Versluis AJ, Rump ET, Rensen PC, Van Berkel TJ, Bijsterbosch MK. Synthesis of a lipophilic daunorubicin derivative and its incorporation into lipidic carriers developed for LDL receptor-mediated tumor therapy. *Pharm Res* 1998;15(4):531–537. [PubMed: 9587947]
36. Versluis AJ, Rump ET, Rensen PC, van Berkel TJ, Bijsterbosch MK. Stable incorporation of a lipophilic daunorubicin prodrug into apolipoprotein E-exposing liposomes induces uptake of prodrug via low-density lipoprotein receptor in vivo. *J Pharmacol Exp Ther* 1999;289(1):1–7. [PubMed: 10086980]
37. Hashida M, Sato K, Takakura Y, Sezaki H. Characterization of a lipophilic prodrug of 5-fluorouracil with a cholesterol moiety and its application to liposomes. *Chem Pharm Bull (Tokyo)* 1988;36(8):3186–3189. [PubMed: 3240523]
38. Rodrigues DG, Maria DA, Fernandes DC, Valduga CJ, Couto RD, Ibanez OC, Maranhao RC. Improvement of paclitaxel therapeutic index by derivatization and association to a cholesterol-rich microemulsion: in vitro and in vivo studies. *Cancer chemotherapy and pharmacology* 2005;55(6):565–576. [PubMed: 15726368]
39. Ritschel, WA.; Kearns, GL. Handbook of Basic Pharmacokinetics... Including Clinical Applications. APhA Publications; 2004.
40. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986;46(12):6387–6392. [PubMed: 2946403]

Figure 1. Chemical structure of GA (a), free 17-DMAG (b), the lipophilic 17'GAC₁₆Br (c), and its hydrolyzed product 17'GAOH (d).

Figure 2.

Chromatograms following extraction of drugs from tissues are free of interference from endogenous components. Individual drug compounds elute as distinct peaks under appropriately optimized gradient conditions: free 17-DMAG (10 $\mu\text{g/mL}$), 17'GAC₁₆Br (10 $\mu\text{g/mL}$), and 17'GA6OH (IS). Distinct peak separations were obtained for 17'GAOH, the IS and 17'GAC₁₆Br (left), and free 17-DMAG (right) when analyzed by RP-HPLC on a Genesis $33 \times 4.6\text{-mm } 3\text{-}\mu\text{m } \text{C}_{18}$ column, 332-nm detection, flow rate 1 mL/min at RT, injection volume of 150 μL (please see methods for details on the gradients). Mobile phase employed were A: 50mM acetic acid+10mM triethylamine (TEA) and B: Methanol+10mM TEA.

Figure 3. Concentration-time profile for the various compounds in serum following intravenous administration of formulations (10 and 200 mg/kg) to rats ($n=5$, mean $\pm$ sem) as detected by RP-HPLC.

Figure 4.

(a) Cumulative urinary excretion following intravenous administration of free 17-DMAG (10 mg/kg) and 17'GAC₁₆Br formulations to rats (10 and 200 mg/kg) (n=5, mean±sem). Insert is a magnification of the cumulative urinary excretion data between 0 and 25 μg ; (b) urinary rate plot following intravenous administration of GA formulations to rats (10 and 200 mg/kg) (n=5, mean±sem).

Figure 5. Drug quantification in tissues (a) and tissue to serum ratio (b) 3-h post intravenous administration of GA formulations to rats (10 mg/kg) (n=10, mean±sem). *denotes statistically significant differences (p<0.05) between 17'GAC₁₆Br and its hydrolyzed product 17'GAOH within the various organs.

Figure 6. Ratio of 17'GAC₁₆Br to 17'GAOH detected in tissues following intravenous administration of GA formulations (10 mg/kg) to rats 3-h post intravenous administration (n=5, mean±sem).

Pharmacokinetics of 17'GAC₁₆Br in mPEG-b-PCL micelles and the standard formulation free 17-DMAG in 0.9% NaCl after IV administration. Levels of prodrug concentrations in serum were monitored for 17'GAC₁₆Br in micelles and its hydrolyzed product 17'GAOH, as well as the standard free 17-DMAG. The intravenous dose for all the formulations was 10 mg/kg or 200 mg/kg to rats, as indicated (mean±sem, n=5 per group).

Table 1

Parameter	free 17-DMAG (Alvespimycin) 10 mg/ kg	17'GAC ₁₆ Br in mPEG-b- PCL 10 mg/kg	17'GAOH Conversion from 17'GAC ₁₆ Br 10 mg/ kg	17'GAC ₁₆ Br in mPEG-b- PCL 200 mg/kg	17'GAOH Conversion from 17'GAC ₁₆ Br 200 mg/kg
AUC _{0-∞} (μg h mL ⁻¹)	6.76 ± 0.98	489 ± 150 ^d	22.6 ± 3.3 ^a	13588 ± 710 ^d	253 ± 10 ^d
V _d (L kg ⁻¹)	6.81 ± 1.7	0.328 ± 0.12 ^a	6.29 ± 1.3	0.0770 ± 0.020 ^d	3.85 ± 1.9 ^d
CL _{renal} (L hr ⁻¹ kg ⁻¹)	0.0419 ± 0.0031	7.97E-07 ± 2.3E-07 ^a	0.00770 ± 0.0038 ^a	1.56E-06 ± 1.0E-06 ^d	0.00990 ± 0.0023 ^d
CL _{hepatic} (L hr ⁻¹ kg ⁻¹)	0.291 ± 0.0085	0.0425 ± 0.0040 ^a	0.443 ± 0.069 ^d	0.0148 ± 0.00080 ^d	0.782 ± 0.035 ^d
CL _{tot} (L hr ⁻¹ kg ⁻¹)	0.333 ± 0.0068	0.0301 ± 0.0079 ^a	0.451 ± 0.065 ^d	0.0148 ± 0.00080 ^d	0.792 ± 0.032 ^d
t _{1/2} (h) serum	2.96 ± 0.27	7.17 ± 1.3 ^a	9.57 ± 0.65 ^d	3.68 ± 1.1	3.31 ± 1.5
t _{1/2} (h) urine	16.5 ± 7.7	2.00 ± 0.50 ^a	2.12 ± 0.29 ^d	16.7 ± 0.36	11.0 ± 0.52
MRT (h)	2.02 ± 0.21	3.83 ± 1.5	14.1 ± 1.3 ^a	2.27 ± 0.18	2.82 ± 0.22 ^d
Extraction Ratio	0.167 ± 0.0049	0.0244 ± 0.0023 ^a	0.255 ± 0.039 ^d	0.00850 ± 0.00040 ^d	0.449 ± 0.020 ^d

^a Denotes statistically significant difference (p<0.05) between the standard (free 17-DMAG at 10 mg/kg) and other formulations AUC=area under the curve, V_d=volume of distribution, CL_{renal} = renal clearance, CL_{hepatic}= hepatic clearance, CL_{tot}=total clearance, t_{1/2}serum = half-life in serum, t_{1/2}urine = half-life in urine, MRT=mean residence time