Formulation Development and Antitumor Activity of a Filter-Sterilizable Emulsion of Paclitaxel

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Purpose. Paclitaxel is currently administered i.v. as a slow infusion of a solution of the drug in an ethanol:surfactant:saline admixture. However, poor solubilization and toxicity are associated with this drug therapy. Alternative drug delivery systems, including parenteral emulsions, are under development in recent years to reduce drug toxicity, improve efficacy and eliminate premedication.

Methods. Paclitaxel emulsions were prepared by high-shear homogenization. The particle size of the emulsions was measured by dynamic light scattering. Drug concentration was quantified by HPLC and *in vitro* drug release was monitored by membrane dialysis. The physical stability of emulsions was monitored by particle size changes in both the mean droplet diameter and 99% cumulative distribution. Paclitaxel potency and changes in the concentration of known degradants were used as chemical stability indicators. Single dose acute toxicity studies were conducted in healthy mice and efficacy studies in B16 melanoma tumor-bearing mice.

Results. QW8184, a physically and chemically stable sub-micron oil-in-water (o/w) emulsion of paclitaxel, can be prepared at high drug loading (8–10 mg/mL) having a mean droplet diameter of $<$100 nm and 99% cumulative particle size distribution of $<$200 nm. *In vitro* release studies demonstrated low and sustained drug release both in the presence and absence of human serum albumin. Based on single dose acute toxicity studies, QW8184 is well tolerated both in mice and rats with about a 3-fold increase in the maximum-tolerated-dose (MTD) over the current marketed drug formulation. Using the B16 mouse melanoma model, a significant improvement in drug efficacy was observed with QW8184 over Taxol®.

Conclusions. QW8184, a stable sub-micron o/w emulsion of paclitaxel has been developed that can be filter-sterilized and administered i.v. as a bolus dose. When compared to Taxol®, this emulsion exhibited reduced toxicity and improved efficacy most likely due to the composition and dependent physicochemical characteristics of the emulsion.

KEY WORDS: paclitaxel; emulsions; filter-sterization; particle size; stability

INTRODUCTION

Paclitaxel is an important chemotherapeutic agent with a wide spectrum of activity against solid tumors primarily breast, ovarian, colon and non-small cell lung carcinomas (1). The drug exerts its antitumor activity by binding to tubulin and stabilizing microtubules and thus blocking cell mitosis (2). Paclitaxel is a natural product present in the bark of the Pacific Yew tree and like other natural products has limited aqueous solubility (3). The drug is only administered intravenously since it is orally inactive due to membrane transport and liver metabolism limitations (4).

The commercially available product, Taxol® (paclitaxel injection, Bristol-Myers Squibb Oncology), is currently formulated in a vehicle containing approximately a 1:1 v/v mixture of polyoxyethylated castor oil (Cremophor EL) and ethanol. Cremophor EL, a commonly used surfactant for lipophilic compounds, has been associated with bronchospasms, hypotension, and other manifestations of hypersensitivity particularly following rapid administration (5,6). Long infusion times upon a 10-fold dilution of the ethanol:Cremophor EL solution and premedication are therefore required to reduce these adverse effects. Furthermore, this formulation is associated with a number of issues such as stability with the possibility for drug precipitation upon dilution, filtering requirements and use of non-plasticized containers and administration sets (6). It is thus apparent that there is a need for new formulations of paclitaxel that are efficacious and less toxic than the commercial product and can alleviate drug administration issues.

In recent years considerable emphasis has been given to the development of new formulations of paclitaxel that are suitable for intravenous administration to address the aforementioned drug solubility and toxicity issues. These include dispersed systems such as emulsions (7–11), liposomes (4,12,13), mixed micelles (14), cyclodextrins (15) and microspheres (16). Water-soluble prodrugs such as polyethylene glycol- and polyglutamate-paclitaxel with promising antitumor activity have also been developed (17,18).

An o/w emulsion of paclitaxel using triacetin and ethyl oleate as the oil phase, lecithin and pluronic F-68 as the surfactant has been reported earlier by Tarr et al. (7). Although high levels of paclitaxel have been solubilized in this emulsion (10–15 mg/mL), no antitumor activity was reported. This is due to slow precipitation of the drug upon dilution with a dextrose solution and toxicity of triacetin at concentrations required for delivering therapeutic doses of paclitaxel, with a reported LD_{50} of 1.2 mL/kg (7).

Oil-in-water emulsions using safflower or soybean oil, lecithin and cholesterol and incorporating paclitaxel up to 5 mg/mL have been reported by Kaufman et al; (8). The mean droplet diameter of these emulsions was reported to remain between 0.2 to 0.4 μm upon a six-week storage at 40°C. An o/w emulsion of paclitaxel and Taxol® were evaluated for toxicity in rats upon dilution to 1 mg of paclitaxel per mL followed by i.v. infusion over 30 min. At 42 mg/kg (42 mL/kg of the diluted formulation) there were signs of severe toxicity in the animals treated with Taxol® whereas animals treated with the emulsion showed low toxicity that was manifested primarily as a body weight loss (8). When the emulsion and the Taxol® formulation were evaluated *in vitro* for efficacy against mouse lymphocytic leukemia (L1210) or rat mammary adenocarcinoma (NMU) cell lines, similar antitumor activity was observed (8).

Lundberg (9) reported on the preparation and evaluation of complex submicron paclitaxel emulsions incorporating triglycerides, phospholipids, polysorbate 80 and pegylated phospholipids to prolong the circulation of the emulsion particles

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in the blood. These emulsions, triolein:dipalmitoylphosphatidylcholine: polysorbate 80: PEG-phosphatidylethanolamine: paclitaxel (1:1:0.4:0.1:0.03, mass ratio) with a mean droplet diameter of about 40 nm (9) showed good physical and chemical stability at low temperature and/or as a lyophilized powder. Paclitaxel-loaded emulsions demonstrated good antitumor activity (*in vitro*) when compared to Taxol®. No *in vivo* studies were reported.

Recently, some prototype o/w emulsions using benzyl benzoate and tributyrin as the oil phase, Tween 80 and Arlacel 20 as surfactants and incorporating up to 5 mg/mL paclitaxel have been reported by Simamora et al. (10). Although these emulsions were found to be stable upon dilution with a variety of i.v. fluids with no evidence of local irritation, studies on vehicle toxicity and drug efficacy have not been reported.

The objective of this study was to develop an injectable emulsion formulation of paclitaxel using vitamin E as the oil phase and having the following characteristics: a) incorporate high levels of paclitaxel (8–10 mg/mL) and is physically and chemically stable; b) has mean droplet diameter and 99% cumulative distribution of less than 0.2 μm and thus can be filter-sterilized, and c) less toxic and at least as efficacious as the commercial formulation Taxol®. The use of vitamin E as the oil phase in the present emulsions may have beneficial effects in reducing drug toxicity since vitamin E has been reported to be therapeutic of mucositis (19), one of the principal side effects of Taxol® therapy. Vitamin E is widely reported to reduce the generalized toxicity of other cytotoxics (20).

MATERIALS AND METHODS

Materials

Vitamin E (DL-α-tocopherol) USP/FCC grade was purchased from Roche (Belvidere, NJ). Eastman's vitamin E-TPGS (α-tocopherylpolyethyleneglycol-1000 succinate) was supplied by B&D Nutritional Ingredients, Inc. (Carlsbad, CA). Polyethylene glycol 400 N.F. (PEG 400) and Poloxamer 407 (Pluronic F-127) were purchased from Spectrum Quality Products, Inc. (Gardena, CA) and BASF Corporation (Mount Olive, NJ), respectively. Paclitaxel (>99% purity) was supplied by Hauser Laboratories (Boulder, CO) and Hande Tech. Development Co. (Houston, TX) and stored desiccated. Taxol®, 5 mL vial at 6 mg/mL, was purchased from a pharmacy and stored refrigerated.

Particle Size

Mean droplet diameter and particle size distribution were determined with a Nicomp 370 Submicron Particle Sizer using a 5 mW laser beam at 632.8 nm (Particle Sizing Systems, Santa Barbara, CA). Polystyrene bead standards were used to verify the calibration of the instrument. Data was analyzed in terms of intensity, volume and number distributions and reported here as volume weighted distribution.

Emulsion Preparation

A pre-emulsion was first prepared by adding the oil phase (α-tocopherol) in PEG 400 containing the surfactants (TPGS and Poloxamer 407) with or without paclitaxel to degassed water with vigorous mixing at 45°C followed by homogenization in the Avestin Emulsiflex C-5 (20–200 mL batches) or Emulsiflex C-50 (0.5–3.0 L batches) high-pressure homogenizers (Ottawa, Canada). Following homogenization, the finished product was terminally sterilized by filtration through a 0.2 μm Posidyne filter (Pall Corp., East Hills, NY). The resulting emulsions contained (%, w/w): a-tocopherol, TPGS (1–10%), Poloxamer 407 (1–5%), PEG 400 (1–10%), paclitaxel (0.5–1.0%) and water for injection (60–90%).

Physical and Chemical Stability

Emulsions were analyzed for paclitaxel content by high performance liquid chromatography (21) on a Phenosphere CN column (5 microns, 150 × 4.6 mm). The mobile phase consisted of a methanol/water linear gradient beginning with 40:60 mixture (v/v) at a flow rate of 1.0 mL/min and reaching 100% methanol after 15 minutes. A UV detector at 227 nm was used to detect and quantitate paclitaxel. A single peak was detected which had a retention time and mass spectrogram consistent with reference paclitaxel obtained from Hauser Laboratories (Boulder, CO).

Emulsions were stored at 4°C or 25°C and samples were removed at predetermined time points and assayed for physical and chemical stability. The mean droplet diameter and 99% cumulative distribution of the particles were used as indicators of physical stability. Paclitaxel potency and the change in concentration of the known degradants were monitored for chemical stability. The following degradants were monitored during the stability program: 7-epi-paclitaxel, 10-deacetyl-paclitaxel and baccatin III.

Drug Release

In vitro release of paclitaxel from different formulations in the presence and absence of human serum albumin was monitored by membrane dialysis at 37°C (22) using phosphate-buffered saline as a sink solution at pH 7.0. The concentration of paclitaxel in Taxol® and QW8184, injectable paclitaxel emulsion, was 6.0 and 8.8 mg/mL, respectively, and the drug to albumin molar ratio was 1000 to 1. The sample volume in the dialysis bag (cassette) was 1 mL and the sink volume 500 mL. Assuming 100% drug release, the concentration of paclitaxel in the sink solution will be <20 μg/mL which is within the reported aqueous paclitaxel solubility range of 1–40 μg/mL (9). Pierce Slide-A-Lyzer® dialysis cassettes with a MW cutoff of 10K, and thus freely permeable to paclitaxel (MW = 853), were used and temperature control was maintained with jacketed beakers via circulating bath. Concentrations of the drug in pre-/post-dialysis samples and aliquots at various time intervals were determined and drug release profiles (% drug released vs time) were generated.

Single Dose Acute Toxicity

The maximum tolerated dose (MTD) of paclitaxel emulsions was determined in mice following a tail vein injection using 3–6 animals per group. Emulsions were administered i.v at bolus doses ranging from 20–90 mg/kg or 2–10 mL/kg of vehicle. Taxol® was first diluted with saline and then administered as 1–2 minute i.v infusion at doses ranging from 5–25 mg/kg. A saline control group was also included. The determination of the MTD was based on body weight loss (>15%) and mortality, i.e. number of deaths per group (23,24).

Efficacy: B16 Melanoma

Female B6D2F mice (8 mice per group) were subcutaneously (sc) implanted with 10^7 B16 melanoma tumor cells (23,24). Four days after implantation, mice were randomly sorted into treatment groups and were administered i.v with saline, Taxol®, or QW8184 emulsion with and without incorporated paclitaxel on a schedule of either q3d × 5 or q4d × 5, that is, once every three or four days, respectively, for a total of 5 doses. QW8184 was administered as a bolus injection and Taxol® was infused over 2 minutes following 10-fold dilution with saline. The administered volume of saline and drug-free emulsion (vehicle) were 7 and 7 or 8 mL/kg, respectively. For specific dosages and schedules see the Figs. 4 and 5 legends. Antitumor activity was assessed according to the guidelines established by the National Cancer Institute (24).

RESULTS AND DISCUSSION

Development of Filter-Sterilizable Emulsions

The formulation development strategy of using α-tocopherol as the oil phase and TPGS as the primary surfactant was followed to develop a stable and efficacious emulsion of paclitaxel at high drug loading that can be filter-sterilized. Ensuring product sterility of parenteral emulsions is crucial and terminal heat sterilization has been generally used for this purpose. Alternatively, certain emulsions can be lyophilized and reconstituted prior to administration without loss of viability and drug potency (25). If, however, the components of a particular drug-emulsion are heat labile, filter sterilization of the product may be a viable option, provided that the emulsion droplets pass through 0.2 micron pore. However, filter sterilization of emulsions is quite challenging and use of this non-invasive method of sterilization has been limited (26).

Several prototype formulations were developed which, upon further optimization, yielded the lead development candidate QW8184. Formulation optimization was based on the use of an alternate co-surfactant or co-solvent, and the resulting emulsions were evaluated for acute and cumulative toxicity, filterability, drug loading, and where appropriate, stability and efficacy. Several of the prototype formulations were found to be stable and efficacious; however, only data with the most advanced formulation tested to date, QW8184, is presented.

Physical and Chemical Stability

Physical stability of an emulsion is one of the most important desired product characteristics. Emulsions are heterogeneous systems and thermodynamically unstable and, therefore, have a significant tendency to lose physical stability on storage. The extent of this process is dependent on the characteristics of each formulation and storage conditions. Some of the factors that affect the physical stability of emulsions include the type and levels of surfactant(s) used to stabilize the droplets, the phase volume ratio, droplet size, compatibility of drug and excipients with the emulsion, and storage conditions of the emulsion (25,27). In general, the smaller the particle size of the emulsion, the better its physical stability (25,27). The assessment of the physical stability of emulsions and of dispersed systems in general, in addition to being extremely useful during formulation development and optimization, also allows setting up of product specifications and expiration dates.

The aforementioned factors were considered during formulation development and optimization of the paclitaxel emulsion in order to develop a stable and filter-sterilizable emulsion. The mean droplet diameter and 99% cumulative distribution of a typical stability lot of QW8184, lot A, are shown in Fig. 1. There were no significant changes in particle size either at 4°C or 25°C over twelve months. The actual volume-weighted particle size distribution of QW8184 incorporating 9 mg/mL of paclitaxel is shown in the insert with a mean droplet diameter of 62 nm. No precipitation or other gross changes were observed during storage. Essentially the same particle size was obtained with a small (50 mL) and a large (3 L) batch of QW8184. When the two batches were compared after one month storage at 4°C, the mean droplet/99% cumulative distribution were 67/150 nm and 64/149 nm for the small and large batches, respectively. An independently manufactured lot of QW8184, lot B, produced the following physical stability data in terms of the mean droplet diameter and 99% cumulative distribution (mean ± SD, n = 3): 63 ± 2.1 nm (initial), 62.9 ± 3.5 nm (4°C, 9 months), 59.1 ± 0.7 nm (25°C, 9 months) and 147 ± 1.9 nm (initial), 146.1 ± 2.8 nm (4°C, 9 months), 144.8 ± 1.3 nm (25 °C, 9 months), respectively.

The chemical stability of the two independently manufactured lots of QW8184, A and B, in terms of paclitaxel potency and levels of known degradants at 4°C and 25°C and at time zero, nine and twelve months is shown in Table 1. Intermediate stability data was very similar to that shown in Table 1, for clarity however, is not shown. There is no major change in the concentration of the active ingredient at least during a 12-month storage at either temperature. No significant changes in the levels of any of the degradants were observed under these storage conditions. Long-term stability is ongoing.

In Vitro Drug Release

One of the desired characteristics of a drug delivery vehicle is to provide sustained release of the incorporated drug, a characteristic quite often correlated with improved pharmacokinetics and efficacy (28,29). In particular, long-circulating emulsions

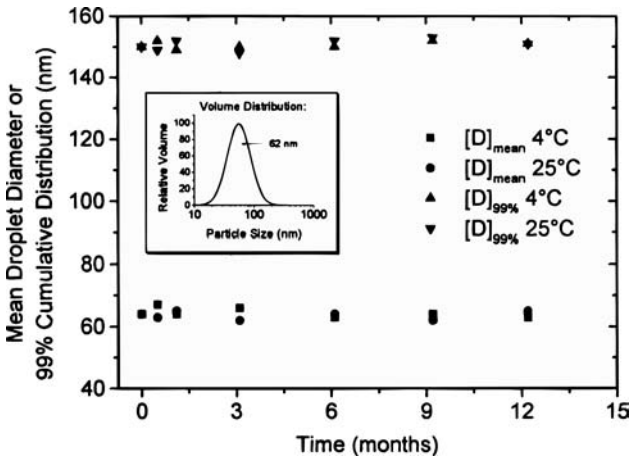

Fig. 1. Mean droplet diameter, $[D]_{mean}$, and 99% cumulative distribution, $[D]_{99\%}$, of QW8184 as a function of storage time and temperature. This particular lot of QW8184 (lot A) incorporated 9.0 mg/mL paclitaxel and was stored at 4°C or 25°C. The actual volume-weighted particle size distribution of QW8184 is shown in the insert with a mean droplet diameter of 62 nm.

Table 1. QW8184 Chemical Stability: Paclitaxel Potency and Degradants

Storage time, months (Lot, temp.)	Paclitaxel potency, mg/mL (mean ± SD)[a]	Degradants (%, mean ± SD)[a]		
		7-Epi-paclitaxel	Baccatin-3	10-Deacetyl-paclitaxel
0.0 (A, 4 °C)	8.4 ± 0.6	0.61 ± 0.29	0.16 ± 0.01	0.15 ± 0.02
9.2 (A, 4 °C)	9.2 ± 0.1	0.36 ± 0.02	0.17 ± 0.00	0.18 ± 0.01
12.2 (A, 4 °C)	8.8 ± 0.3	0.30 ± 0.04	0.21 ± 0.04	0.20 ± 0.03
9.2 (A, 25 °C)	9.0 ± 0.7	0.40 ± 0.02	0.18 ± 0.01	0.18 ± 0.01
12.2 (A, 25 °C)	8.3 ± 0.8	0.35 ± 0.07	0.21 ± 0.04	0.21 ± 0.04
0.0 (B, 4 °C)	9.1 ± 0.0	0.50 ± 0.07	0.07 ± 0.01	0.11 ± 0.01
9.2 (B, 4 °C)	9.4 ± 0.0	0.43 ± 0.03	0.08 ± 0.01	0.13 ± 0.02
9.2 (B, 25 °C)	9.2 ± 0.1	0.41 ± 0.04	0.08 ± 0.01	0.13 ± 0.01

[a] n = 10 (lot A); n = 3 (lot B).

of paclitaxel can improve the delivery of the drug to cancer sites in the body. The release kinetics of paclitaxel from QW8184, both in the absence and presence of human serum albumin (HSA) at a drug to albumin molar ratio of 1000:1 and from the commercial formulation Taxol® are shown in Fig. 2. The concentration of paclitaxel in Taxol® and QW8184 was 6.0 and 8.8 mg/mL, respectively. The release of paclitaxel from the emulsion was slow both in the absence and presence of serum albumin with less than 5% drug being released over 24 hours, whereas about 12% was released from the commercial formulation.

Acute Single Dose Toxicity

Single dose toxicity studies of paclitaxel emulsions in healthy mice were carried out and representative data with QW8184 is shown in Fig. 3. The paclitaxel emulsion was well tolerated and the maximum tolerated dose (MTD) was determined to be approximately 70 mg/kg for QW8184 as compared to approximately 20 mg/kg for Taxol®. The marketed drug product Taxol® is primarily supplied in 5 mL vials at a drug concentration of 6 mg/mL and prior to administration it requires dilution with commonly used i.v. fluids to a final concentration of 0.3 to 1.2 mg/mL that corresponds to a 20-fold and 5-fold dilution, respectively. When Taxol® was administered i.v. at 24 mg/kg, either as a bolus dose or 2 min infusion, after a 10-fold dilution with saline, severe prostration and catatonia were observed. When Taxol® was first diluted 1:4 with saline and then administered i.v. at 24 mg/kg as a 2 or 5 min infusion, 100% mortality was observed as compared to 50% mortality when the drug was administered as i.v. bolus. Weight loss was less than 15% even in the group receiving the highest dose of QW8184 and the animals recovered or gained weight over a period of 10 days post injection. Vehicle toxicity was also evaluated. Animals receiving drug-free emulsion at 7 or 8 mL/kg (the equivalent paclitaxel dose of 63–72 mg/kg at drug loading of 9.0 mg/mL) grew rapidly and gained slightly more weight than animals receiving saline. This may be attributed to the vitamin and calorie content of the formulation. The clinical dose of paclitaxel is 175 mg/m^2 (300 mg total per person) or 4.3 mg/kg for a 70 kg average human body weight (1,23). The equivalent dose/volume of QW8184 is about 0.5 mL/kg at a drug loading of 9.0 mg/mL which is about an order of magnitude below the dose where the emulsion vehicle may show signs of toxicity. The high drug loading in QW8184

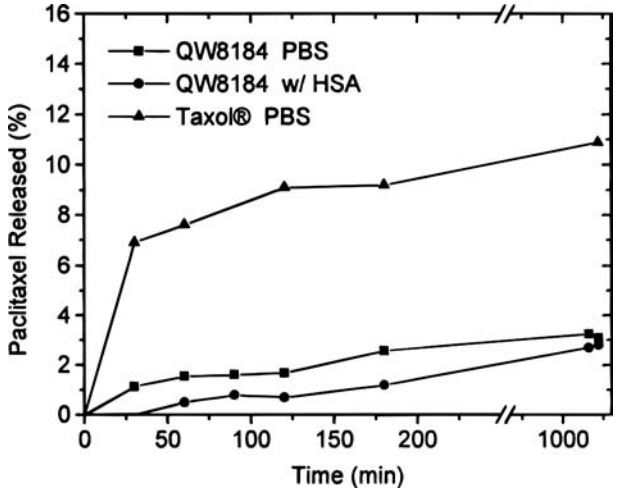

Fig. 2. Percent paclitaxel released as a function of time at 37°C. Drug release from the lipid emulsion, QW8184, in the presence and absence of HSA (human serum albumin) and the commercial formulation Taxol® was monitored by membrane dialysis as described in Methods using PBS (phosphate-buffered saline) as a sink solution at pH 7.0. The drug to albumin molar ratio was 1000:1.

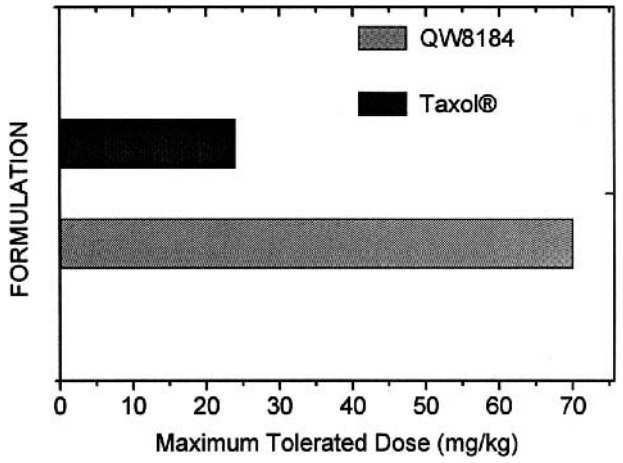

Fig. 3. Maximum tolerated dose of paclitaxel in mice. The determination of the MTD was based on body weight loss (>15% is considered toxic) and mortality (number of deaths per group of 6 animals).

without prior dilution enables one to administer much lower volumes, therefore reducing or potentially eliminating issues of vehicle toxicity.

Efficacy Against B16 Melanoma

B16 Melanoma is a fast growing solid murine tumor that has been commonly used in early screening of different anticancer agents (23,24). Figures 4, A and B present tumor growth regression and percent survival, respectively, for the dosing regimen of q3d × 5. QW8184 was evaluated at different doses and compared to the reference Taxol® formulation at its MTD (20 mg/kg). The corresponding data for the dosing schedule of q4d × 5 are shown in Figure 5, A and B, respectively. QW8184 illustrated a definitive dose response in both schedules. The q3d × 5 schedule, however, seems to be more effective. Administration of QW8184 at dosages of 20 mg/kg (63 mg/m^2) and 40 mg/kg (125 mg/m^2) on a schedule of q3d × 5 resulted in mean increases in survival times of 65% and 94%, respectively, as compared to control group (Table 2).

Log-cell kill values of 1.8 and 3.0 were observed with QW8184 at dosages of 20 and 40 mg/kg, respectively (Table 2), as compared to a value of 0.5 obtained with Taxol® at 20 mg/kg (q3d × 5). In addition, there was a significant reduction in tumor growth in these animals as depicted in Fig. 4A. A significant reduction in tumor growth was also observed in animals administered QW8184 on a schedule of q4d × 5 as illustrated in Fig. 5A. A dosage of 60 mg/kg with QW8184 was toxic in the q3d × 5 regimen with only 1 survivor at the end of the dosing schedule (Fig. 4B). In the q4d × 5 dosing schedule, 70 mg/kg of QW8184 was toxic with only 2 survivors at the end of the dosing schedule (Fig. 5B). Statistical analysis (t-test) of the survival time between groups was performed. The results indicate that Taxol® was not statistically different from the saline control at the q3d × 5 ($p = 0.25$) or q4d × 5 ($p = 0.09$) schedule. QW8184 resulted in a statistically significant increase in survival time in the q3d × 5 groups at dosages of 20 mg/kg ($p = 0.005$) and 40 mg/kg ($p < 0.005$) as compared

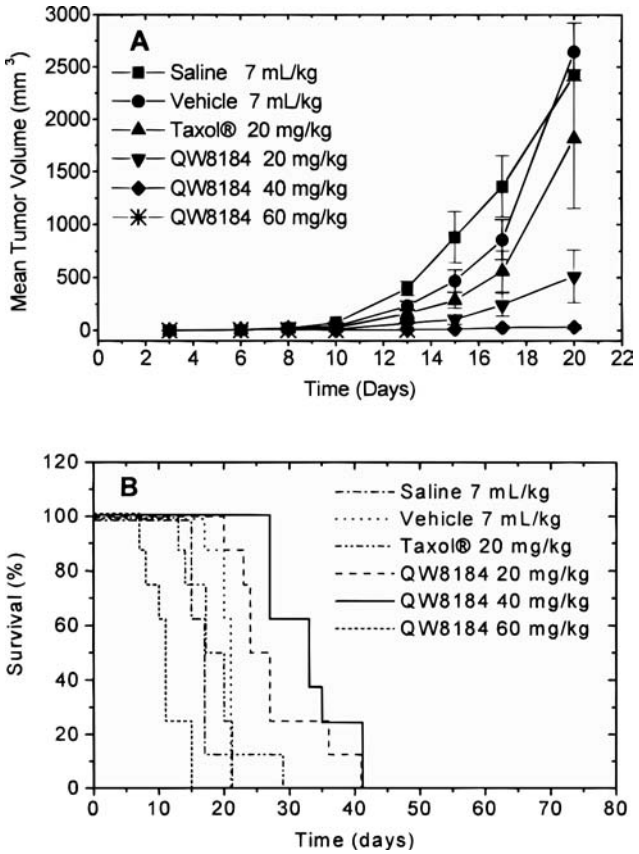

Fig. 4. A) B16 melanoma mean tumor regression to QW8184 and Taxol® as a function of time on a q3d × 5 schedule. Error bars represent SEM (n = 8). B) Percent B16 melanoma survival in response to QW8184 and Taxol® as a function of time on a q3d × 5 schedule.

Table 2. Antitumor Activity of QW8184 vs Taxol® in the B16 Melanoma Model

Test article	Dose mg/kg n = 8	Schedule days	Median tumor weight on day 3	15	20	23	34	Survival (mean ± SD) days	% Mortality day 20	% T/C[a] day 20	% TGI[b] day 20	T-C[c] days	Log cell kill[d]
Saline	Control	q3d x 5	0	699	2422	—	—	17 ± 2	87.5	—	—	—	—
Vehicle	Control	q3d x 5	0	388	2358	—	—	20 ± 1	0[e]	93	3	3	—
Taxol®	20	q3d x 5	0	306	1871	—	—	19 ± 5	50	77	23	3	0.5
QW8184	20	q3d x 5	0	50	270	903	—	28 ± 7	0	11	89	10	1.8
QW8184	40	q3d x 5	0	7	6	139	1340	33 ± 5	0[f]	0	100	17	3.0
QW8184	60	q3d x 5	0	—	—	—	—	11 ± 3	100	—	—	—	—
Vehicle	Control	q4d x 5	0	400	2658	—	—	16 ± 6	62.5	100	0	0	0
Taxol®	20	q4d x 5	0	184	1679	—	—	12 ± 7	75	69	31	3	0.5
QW8184	20	q4d x 5	0	223	855	1079	—	20 ± 3	50	35	65	3	0.5
QW8184	50	q4d x 5	0	49	246	407	—	31 ± 4	0	10	90	7	1.2
QW8184	70	q4d x 5	0	2	2	15	1577	17 ± 16	62.5	0	100	17	3.0

[a] % T/C = (Median Tumor Wt of treated / Median Tumor Wt of control) × 100.
[b] %TGI = 100 − (%T/C).
[c] T-C = Tumor Growth Delay Value (median time for the treatment group (T) and control (C) to reach a predetermined size (> 750 mg).
[d] Log Cell Kill = (T-C value)/(3.32 × tumor doubling time).
[e] All animals were sacrificed due to excessive tumor size.
[f] All animals but three survived to day 27 when they were sacrificed due to tumor size (>10% of body weight).

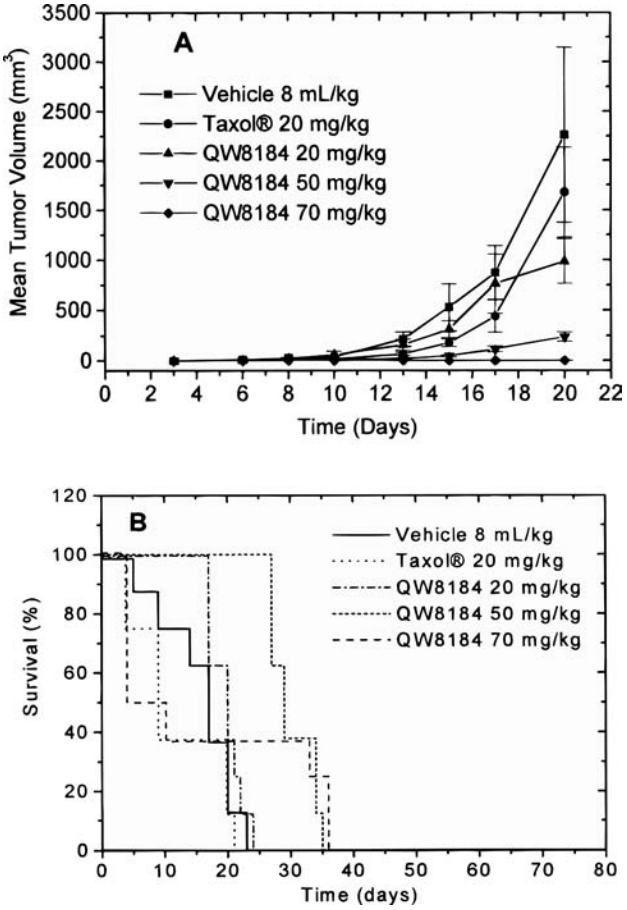

Fig. 5. A) B16 melanoma mean tumor regression to QW8184 and Taxol® as a function of time on a q4d × 5 schedule. Error bars represent SEM (n = 8). B) Percent B16 melanoma survival in response to QW8184 and Taxol® as a function of time on a q4d × 5 schedule.

to the saline control. The animals administered QW8184 at q4d × 5 resulted in a statistically significant increase in survival time at dosages of 20 mg/kg ($p = 0.05$) and 50 mg/kg ($p < 0.005$) as compared to the saline control.

Table 2 summarizes the overall results of the efficacy study including mean survival times, median tumor weights and log cell kills for each group and treatment schedule. By all end points of efficacy (24), QW8184 exhibited superior antitumor activity in mice at doses that included or well exceeded the MTD of Taxol® but which were well tolerated. In our laboratory, the MTD for Taxol® was approximately 20 mg/kg and it is supported by other literature reports. Bissery et. al; (30) reported that the highest dose of Taxol® that could be administered to mice without causing death or undue toxicity was 21.7 mg/kg/injection. Furthermore, recent reports on the MTD of Taxol® upon i.v. administration in mice indicate that it is 20 mg/kg on a q1d × 3 (31) or 25 mg/kg on a q1d × 5 (32). From Table 2, it is evident that Taxol® did result in a slight reduction in tumor growth at both the 15 and 20 days post tumor implant as compared to the controls. It should also be noted that Taxol® has been shown to be minimally effective in the B16 melanoma tumor model (33). In addition, drug related mortality is evident at day 20 in the Taxol® group thus providing further evidence that doses higher than 20 mg/kg could not have been tolerated. Therefore, the observed minimal therapeutic effect from Taxol® in the B16 melanoma model is not believed to be due to sub-optimal dosing.

The effects observed in the present study have not been reported with previous injectable emulsions of paclitaxel (7–10). The improved efficacy of QW8184 may be related to its preferential uptake by tumor cells as a result of its physicochemical characteristics, particularly lipid composition and particle size. The *in vitro* drug release data suggest that emulsion droplets may serve as long-circulating drug reservoirs, thus improving the delivery of paclitaxel to tumor sites, due to increased cellular/droplet interactions (34). To penetrate tumors in tissue, particles must be small enough to pass through endothelial fenestrae, i.e. < 70 nm. This mechanism is consistent with literature reports on long-circulating emulsions and liposomes (29,34) where lipid composition, particularly the inclusion of pegylated surfactants and small droplet size, direct lipophilic drugs away from RES in the liver and spleen to other targeting tissues, such as inflammatory tissues.

In addition to the studies reported with drug emulsions, toxicity and antitumor activity was reported with other lipid-based carriers of paclitaxel, such as liposomes and mixed micelles. The most extensive work with liposomal formulations of paclitaxel has been reported by Straubinger *et al*; (3,12,13). They developed over 300 liposome formulations of various lipid compositions and evaluated them for stability and antitumor activity. Both *in vitro* and *in vivo* activity using Colon-26, a Taxol-resistant murine tumor, were demonstrated (12). Paclitaxel liposomes, however, were both unstable and toxic at high drug loading.

Paclitaxel was solubilized in mixed micelles formed by a mixture of bile salts and phospholipids followed by a spontaneous transformation into drug-loaded liposomes, thus avoiding drug precipitation (14). These formulations, incorporating less than 1.0 mg of paclitaxel per mL, produced significant antitumor activity *in vitro* and appeared to be less toxic than the Cremophor EL vehicle (14). It is anticipated however, that at high drug loading these formulations can be both unstable and toxic with limited clinical use.

Emulsion and liposomal formulations of paclitaxel are more biocompatible and thus less toxic and at least as efficacious as the marketed Taxol®. Emulsions have higher drug solubilization capacity than liposomes and are easier to process and manufacture in a sterile form. The injectable paclitaxel emulsion QW8184 described in the present studies exhibits these advantages. Due to the high paclitaxel loading (8–10 mg/mL), QW8184 can be administered clinically undiluted at high dose without drug precipitation. As a result of its small droplet size (<200 nm for both the mean droplet and cumulative distribution), QW8184 can be filter-sterilized, exhibits improved shelf-life, efficacy and targeting to solid tumors. Furthermore, QW8184 can result in better quality of life, since it can be administered to patients as a bolus dose and has low toxicity. Emulsion formulations offer an appealing alternative for the bolus administration of paclitaxel and other poorly soluble drugs due to their effectiveness for drug solubilization, improved efficacy and patient quality of life arising from reduced side effects. A real therapeutic gain with a particular new formulation of paclitaxel, including QW8184, can be realized through its

progression to the clinic. As new and safer drug delivery methods of paclitaxel emerge, it is likely that the clinical use of the drug will expand.

CONCLUSIONS

QW8184, a stable, injectable and filter sterilizable o/w emulsion of paclitaxel has been developed at high drug loading (8–10 mg/mL) with a mean droplet diameter and 99% cumulative particle size distribution of < 0.2 μm. Compared to the *in vitro* drug release from Taxol®, drug release from QW8184 at 37°C is slow both in the presence and absence of human serum albumin with less than 5% drug being released within 24 hours. In the B16 melanoma tumor model in mice the drug emulsion was better tolerated and was more efficacious than Taxol®. The composition and dependent physicochemical characteristics of the emulsion may be related to reduced toxicity and improved efficacy.

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