A High-Throughput Combinatorial Approach for the Discovery of a Cremophor EL-Free Paclitaxel Formulation

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Purpose. The purpose of this work was to replace Cremophor-EL in the commercial paclitaxel intravenous formulation, Taxol®, using a novel high-throughput combinatorial formulation approach.

Methods. Full factorial combinations of 12 generally regarded as safe excipients at three different concentrations were screened using an automated liquid dispenser. The hit formulations were further optimized to give the final optimized formulation TPI-1. TPI-1 was then tested in rats to compare its pharmacokinetic profile to Taxol®.

Results. Of the 9,880 combinations tested in the initial screen, 19 were identified as hit combinations. These were further optimized to give the final formulation TPI-1. When tested in rats, TPI-1 was well tolerated at both the low and high doses of 5 mg/kg and 10 mg/kg, whereas Taxol® killed all the rats at the high dose. TPI-1 experienced slower elimination compared to Taxol®. Similar to Taxol®, TPI-1 also exhibited nonlinear pharmacokinetics.

Conclusions. This study demonstrated the power of a high-throughput combinatorial approach for alternative paclitaxel formulations. We believe that this approach can be applied to drug formulation in general and it can improve the speed and efficiency of drug formulation design.

KEY WORDS: high throughput; combinatorial; formulation; paclitaxel; cremophor EL-free.

INTRODUCTION

Paclitaxel is a natural chemotherapeutic agent first extracted from the bark of the Pacific Yew tree in the early 1960s (1). It is effective against several types of cancers, such as ovarian and breast cancer. It works by stabilizing cellular microtubules through polymerization (2,3). The drug has very limited aqueous solubility and is currently formulated in the commercial product (Taxol®, Bristol-Myers Squibb, New York, NY, USA) as a nonaqueous concentrate containing 6 mg/mL paclitaxel in 1:1 v/v mixture of Cremophor EL (BASF, Mount Olive, NJ, USA) and ethanol. Before intravenous administration, Taxol® must be diluted 5- to 20-fold in normal saline or 5% dextrose solution. Once diluted, the formulation only has limited physical stability because drug particles tend to precipitate out over 12–24 h (2,3). An in-line filter is typically used for the infusion line to remove any precipitated particulates (2,3).

Cremophor EL is a mixture of hydrogenated castor oils that can cause severe anaphylactic reactions in patients (2,3). To avoid these side effects, a pretreatment of corticosteroids and an antihistamine is required before the administration of Taxol®. Over the past few decades, there has been considerable effort in the development of non-Cremophor EL formulations for paclitaxel using traditional approaches, such as the use of cosolvents, cyclodextrins, liposomes, and oil-in-water emulsions. (2–7). The main challenge in all of these approaches is the difficulty of maintaining paclitaxel in solution after diluting the concentrate into intravenous infusion fluids for at least 24 h and preferably 48 h (2,3).

In this article, we describe the identification of Cremophor EL-free paclitaxel formulations by replacing Cremophor EL in Taxol® with other excipients or excipient combinations, while retaining ethanol as a co-solvent. In contrast to traditional methods, we describe here a high throughput combinatorial approach to the discovery and optimization of new Cremophor EL-free formulations. High-throughput screening approaches have been widely used in drug discovery programs in the past decade and have revolutionized the way pharmaceutical discovery is conducted. In this study, we demonstrate that a similar approach can be applied to formulation discovery and optimization.

The screens in this study were designed to find formulations with the following characteristics: 1) they can dissolve at least 6 mg/mL paclitaxel in their concentrated states; 2) they do not contain Cremophor EL but like Taxol contain ethanol as a cosolvent; and 3) they will be able to maintain paclitaxel in solution for 48 h upon dilution of the concentrates into infusion fluids to a final paclitaxel concentration of 1.2–0.3 mg/mL (representing 5- to 20-fold dilution).

The study began with the optimization of the third characteristic, by finding non-Cremophor EL excipient components that can keep paclitaxel in solution for 48 h in diluted aqueous conditions. This was achieved using a high throughput combinatorial approach, through examining the time-dependent solubility of paclitaxel in the diluted solutions at the concentration to be infused into the patients. The optimized excipient compositions were then used, together with the cosolvent ethanol, to form the final concentrate(s) of 6 mg/ml paclitaxel.

MATERIALS AND METHODS

Materials

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) except for the following: γ-cyclodextrin was purchased from Fluka (Ronkonkoma, NY, USA); Poloxamer® 188 was obtained from Spectrum Chemicals (Gardena, CA, USA); USP absolute alcohol was obtained from AAPER alcohol (Shelbyville, KY, USA); and paclitaxel was supplied by Samyang Corporation (Seoul, Korea).

Instrumentation for Combinatorial Excipient Preparation

A TECAN Genesis liquid dispenser (Tecan-US, RTP, NC; Fig. 1A) with a source deck was used to prepare the excipient combinations. The commercial source deck was

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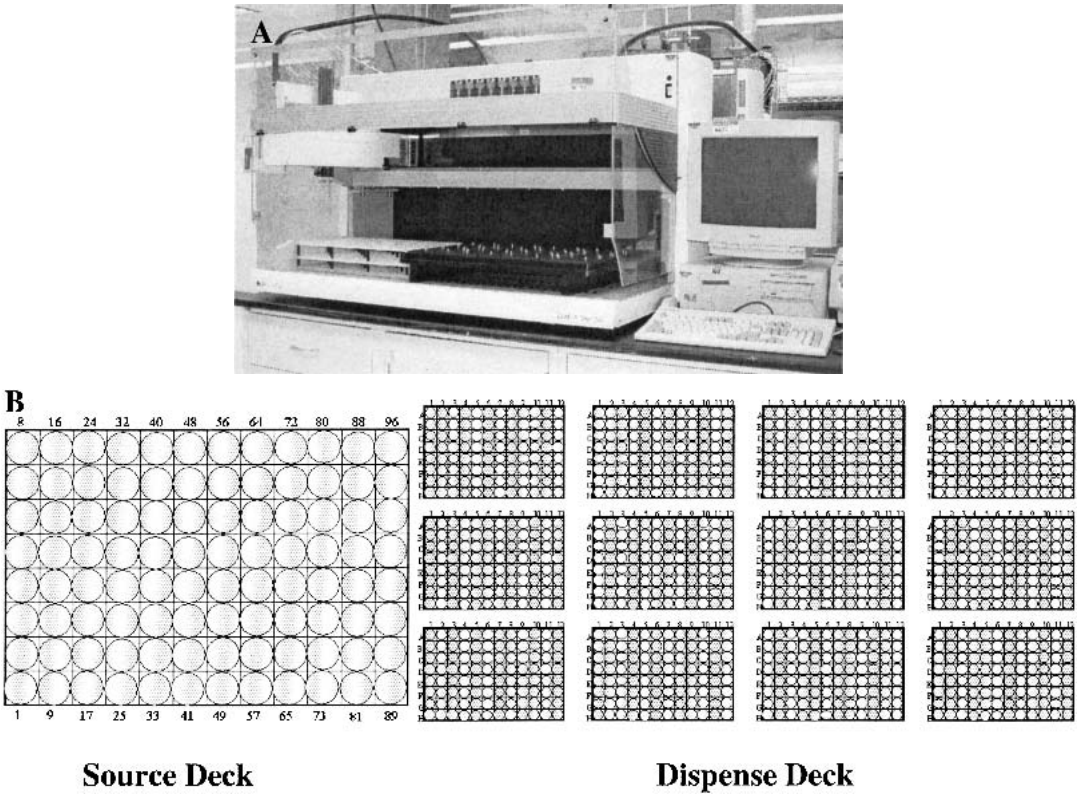

Fig. 1. (A) TECAN instrument; (B) layout of source deck and dispense deck on TECAN.

modified to hold 96 excipient solutions in 50-mL Falcon centrifuge tubes, and the source deck was configured as shown in Fig. 1B. The numbers on the source deck represent the physical locations on the deck. Twelve 96-well microtiter plates were positioned on the dispense deck next to the source deck (Fig. 1B). The TECAN dispenser has eight separate tips that can travel in the x, y, and z directions, and they were used to aspirate solutions from the selected positions on the source deck and dispense them to desired well locations on the dispense deck.

Methodology of Combinatorial Excipient Preparation

Twelve generally regarded as safe excipients (Table I) that have been previously used in approved intravenous products were selected from the FDA inactive ingredient list and the *Handbook of Pharmaceutical Excipients*. They were dissolved in phosphate-buffered saline (PBS, pH 6.0) at three different concentrations, 90 mg/mL, 60 mg/mL, and 30 mg/mL, and were filtered through 0.22-μm filters (Millipore, Bedford, MA, USA) before the experiment. Each resulting excipient solution was assigned a number between 1 and 36, and was loaded onto the corresponding physical location on the Genesis source deck (Fig. 1B).

Table I. Excipients Selected for Combinatorial Screen

Excipients for initial screen
Polysorbate 80
Polysorbate 20
Propylene glycol
Polyethylene glycol (PEG) 300
Polyethylene glycol (PEG) 400
Polyethylene glycol (PEG) 600
Docusate sodium
Glycerin
Deoxycholate
γ-Cyclodextrin
Providone
Poloxamer® 188

The excipient combinations were prepared in 96-well polystyrene plates (Millipore) as full factorial combinations of the 36 excipient solutions (12 excipients at three concentrations each), with each combination containing three of the 36 excipient solutions. A list of all possible combinations of the 36 excipient solutions was generated randomly with the MatLab program from Mathworks (Natick, MA, USA) according to the general formula for M excipient solutions choosing N (M>N):

$$\frac{(M+N-1)!}{N!\,(M-1)!}.$$

In our initial experiment, M was 36 and N was 3. The total number of unique excipient combinations was therefore:

$$\frac{(36+3-1)!}{3!\,(36-1)!} = 9{,}880.$$

Each combination was prepared in triplicate, giving a total of 29,640 samples and therefore 309 plates. Each plate also contained a negative control (PBS only without any excipients) in triplicate in the last three wells.

A worklist was then generated by combining the MatLab list of 9,880 combinations with Genesis-appropriate commands. Once the worklist was generated, it controlled the liquid dispenser to create the desired combinations in 96-well

plates. For this study, each excipient solution was dispensed at a volume of 30 μL into the wells, resulting in a final excipient volume of 90 μL per well.

After excipient dispense, the 96-well plates were agitated for 5 min at 400 rpm on a Titer Plate Shaker (Lab-line Instruments, Melrose Park, IL, USA) to allow for complete mixing of the excipients. Baseline turbidity of the excipient combinations was recorded with a UV plate reader at 500 nm (SpectraMax Plus, Molecular Devices, Sunnyvale, CA, USA).

A stock solution of paclitaxel in USP absolute ethanol was then prepared at 10 mg/mL. Twelve microliters of the paclitaxel–ethanol solution was added to each well containing predispensed excipients, including the negative control wells. This gave a paclitaxel concentration of 1.2 mg/mL in each combination, therefore simulating the paclitaxel concentration in the diluted solutions to be infused into the patients. The plates were immediately sealed using aluminum sealing tapes with pressure sensitive adhesives (VWR Scientific, West Chester, PA, USA) and were agitated again for 5 min on the Titer Plate Shaker before they were incubated at 25°C for 48 h.

Solubility of Paclitaxel in Excipient Combinations without Cremophor EL

After 48 h of incubation, the plates were unsealed and the turbidity measured again with the UV plate reader at 500 nm to detect paclitaxel precipitation. All data were exported to Excel and the %Transmittance was calculated for each combination by comparing the readings after 48 h of incubation with those before paclitaxel addition (see Results and Discussion for details on the calculation). The final results were imported into a data visualization program, Spotfire (Spotfire, Cambridge, MA, USA) for analysis. Excipient combinations that demonstrated greater than 90% Transmittance were identified as hit combinations.

Based on the frequency of appearance in the hit combinations, a subset of the 12 excipients were selected and rescreened at different concentrations for further optimization. The optimized excipient combination was combined with ethanol to make a Cremophor EL-free paclitaxel formulation concentrate (TPI-1). The dilution stability of TPI-1 was confirmed by diluting the concentrate 5- to 20-fold in normal saline solution.

Animal Pharmacokinetic Study of Paclitaxel Formulations without Cremophor EL

A pharmacokinetic study was conducted at MDS Pharma Services, Montreal, in male Sprague–Dawley rats (7 weeks old, average weight 300 g, from Charles River Canada) to compare the Cremophor-free formulation (TPI-1) against the commercial formulation Taxol®. All animals were handled according to established guidelines and principles. After an overnight fast, four groups of six rats each were dosed over the period of ca 1 min (slow push) via jugular venipuncture: 1) formulation TPI-1 at 5 mg/kg; 2) formulation TPI-1 at 10 mg/kg; 3) Taxol® at 5 mg/kg; and 4) Taxol® at 10 mg/kg.

After dose administration, blood samples (0.5 mL) were collected by jugular venipuncture from three animals/group at 5 min, 1 and 6 h postdose, and from the three remaining animals of each group at 20 min and at 2 and 12 h post-dose. Blood samples were collected into heparinized tubes and placed on wet ice. A 0.2-mL aliquot of each blood sample was separated and stored at −20°C for analysis of paclitaxel by LC-MS/MS. The remaining blood was centrifuged at ca 3200*g* at 4°C for 10 min. The resulting plasma samples were harvested by gentle aspiration and stored at −20°C for analysis of paclitaxel by LC-MS/MS. No significant degradation of paclitaxel occurred under the storage conditions.

All animals were observed constantly during dose administration and blood sampling period. Any adverse observations were recorded. At the end of the sampling, rats were humanely sacrificed.

Concentrations of paclitaxel in rat plasma and whole blood were determined using an ESI-LC/MS/MS method developed at MDS Pharma Services. Briefly, 200 μL of internal standard working solution (100 ng/mL of etoposide in acetonitrile) was added to 50 μL of each sample. The samples were vortexed and centrifuged at 3,200 × g for 15 min at 4°C, and 150 μL of their supernatants were aliquoted into injection vials. The samples were then analyzed using an online column switching setup consisting of a Perkin Elmer Series 200 autosampler (Perkin–Elmer Instruments, Shelton, CT, USA), HP 1090 Series II quaternary piston pumps (Agilent Technologies, Palo Alto, CA, USA) for sample loading and washing, Perkin Elmer Series 200 pumps (Perkin–Elmer Instruments) for sample elution, and a VICI model A-60-S six-port switch-valve (Valco Instruments Co. Inc., Houston, TX, USA) for switching between sample loading and sample elution. Each sample was injected (30 μL) and trapped on a Zorbax XDB-C18 guard column (4.6 × 1.25 mm, 5 μ d_p, Agilent Technologies) with a flow rate of 1.0 mL/min and a loading phase consisting of acetonitrile in type one water (10/90, v/v)/methanol (100/0, v/v). Initial conditions for gradient loading were held for 1.0 min. The flow rate was increased to 2.0 mL/min at 1.1 min. Methanol was increased to 100% at 2.0 min and held for 0.5 min. Methanol was then decreased to 0% at 2.6 min. Original conditions were resumed at 3.1 min. After 1.0 min the guard column was switched online and the sample was eluted on a Zorbax SB-C18 analytical column (4.6 × 30 mm, 3.5 μ d_p, Agilent Technologies) with a flow rate of 0.5 mL/min and an elution phase consisting of 1 mM ammonium acetate with 0.05% formic acid/acetonitrile (90/10, v/v). Initial conditions for gradient elution were held for 1.0 min. Acetonitrile was increased to 90% at 2.0 min and held for 1.2 min. Original conditions were resumed at 3.4 min. The column effluent was analyzed using a triple quadruple mass spectrometer (Thermo Finnigan TSQ 7000, Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ion source operating in positive ion mode. The retention times for paclitaxel and etoposide were 2.62 and 2.30 min, respectively.

Pharmacokinetic analysis of mean paclitaxel concentrations in plasma and blood was performed using the PhAST Software Program (Version 2.3, Phoenix International Life Sciences Inc.). C_0 was extrapolated using the y-axis intercept function in Excel spreadsheet. The first two measured concentrations (0.08 and 0.33 h post-dose) were used for this calculation. The area under the concentration vs. time curve to the last measurable concentration ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal method (8). The observed terminal phase constant (Kel) was calculated as the slope of the terminal portion of the log concentration vs. linear time curve by linear regression. The number of time-points included in the calculation of Kel was selected such to maximize the r^2

value of the regression analysis. The area under the concentration vs. time curve from zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and the ratio of the last measurable concentration by Kel. The terminal phase half-life ($t1/2$) was calculated by dividing 0.693 by Kel. In addition, the plasma or blood clearance (CL) and the apparent volume of distribution (V_{dss}) were also calculated.

RESULTS AND DISCUSSION

Combinatorial Excipient Screen for Paclitaxel Formulations without Cremophor EL

A successful replacement of Cremophor EL in Taxol® must prevent precipitation of paclitaxel from solution for at least 24 h, preferably 48 h, after diluting the formulation concentrate with intravenous infusion fluids. Figure 2 illustrates the nature of this challenge for paclitaxel. Most paclitaxel formulations exhibit a steep drop-off of paclitaxel solubility (curve A) as soon as any water is introduced. As a result, paclitaxel solubility in the diluted condition drops below the nominal concentration of the drug (curve B), leading to drug precipitation. The aim for our study therefore, is to find excipient combinations that can maintain paclitaxel solubility at or above the nominal drug concentration upon dilution.

The initial screen of 9,880 combinations was completed within one week using the TECAN liquid dispenser and the plate-based UV spectrophotometer. Two measurements of turbidity were conducted in the screen, one immediately before paclitaxel was added, and the other after 48 h incubation at room temperature. Readings from the triplicates on the same combination were averaged and the averages were used to calculate the %Transmittance using the following formula:

$$\%\text{Transmittance} = \left(\frac{A_{PBS} - A_{48}}{A_{PBS} - A_0}\right) \times 100\%,$$

where A_{48} is the average reading of a specific combination after 48 h of incubation, A_0 is the average reading of the same combination before paclitaxel was added, and A_{PBS} is the average reading of the negative controls plus paclitaxel after 48 h of incubation. When no precipitation occurs, %Transmittance should be 100%.

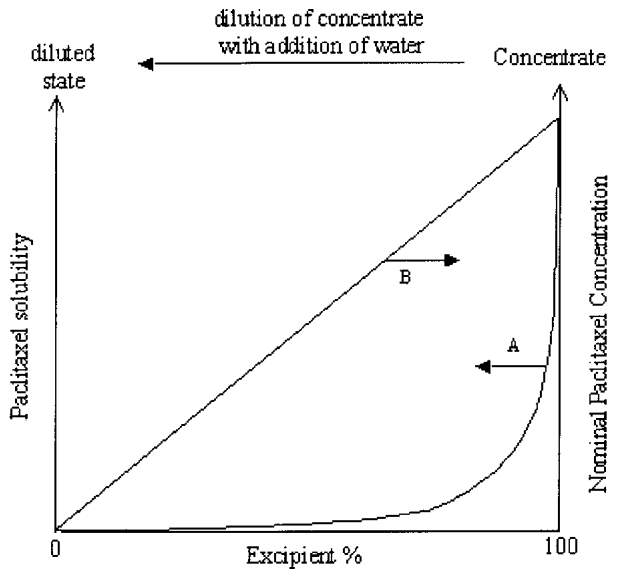

Fig. 2. Illustration of paclitaxel solubility behavior upon dilution of formulation concentrates.

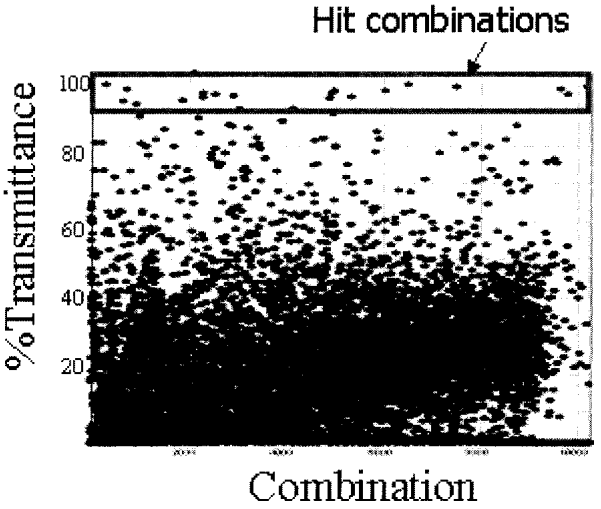

Fig. 3. Screen results plotted in Spotfire in %Transmittance (calculated as described in the text). The combinations that exhibited >90% Transmittance are highlighted in the box as hit combinations.

The final calculated results were imported to Spotfire and are shown in a scatter plot in Fig. 3. The majority of the combinations tested showed increased turbidity as indicated by low %Transmittance, indicating that paclitaxel precipitated out from those combinations during the 48-h incubation. Only a small number of combinations (19 of the 9,880 screened) showed no significant increase in turbidity as indicated by their high %Transmittance (>90% Transmittance). These were identified as hit combinations.

The hit combinations and their excipient compositions were analyzed to reveal the key excipients contributing to paclitaxel solubility in the diluted state. Most of the excipients tested, such as Poloxamer® 188 and γ-cyclodextrin, had little impact on keeping paclitaxel in solution. Two key excipients appearing in all of the hit combinations identified were PEG 400 and polysorbate 80.

It is interesting to point out that PEG 400 alone can dissolve paclitaxel at greater than 200 mg/mL in the concentrated state, whereas polysorbate 80 can only dissolve paclitaxel at about 25 mg/mL. However, a paclitaxel-PEG 400 concentrate precipitates immediately upon dilution. In other words, the presence of polysorbate 80 is essential for keeping paclitaxel in solution in the diluted state.

These two excipients were rescreened at different ratios to minimize the amount of excipients needed to keep paclitaxel in solution at 1.2 mg/mL. The final optimized composition is listed in Table II. Based on this composition, an

Table II. Composition of Optimal Formulation TPI-1

Optimal composition in diluted state	Optimal composition of concentrate (TPI-1)
1.20 mg/mL paclitaxel	6.00 mg/mL paclitaxel
5.88% PEG 400	29.40% PEG 400
9.70% polysorbate 80	48.50% polysorbate 80
84.42% normal saline	22.10% ethanol

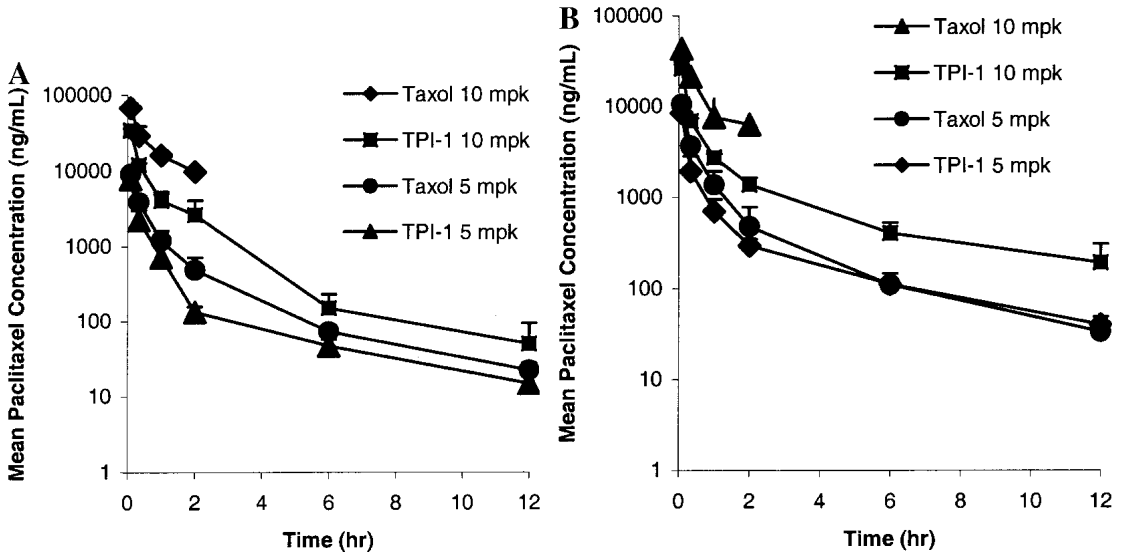

Fig. 4. Paclitaxel plasma (A) and blood (B) concentration vs. time curves for TPI-1 and Taxol® in rats. All data plotted as mean ± SD, n = 3.

optimal formulation concentrate (TPI-1) was assembled (Table II).

Pharmacokinetic Study

The plasma and blood concentration-time profiles of paclitaxel for the different groups are shown in Fig. 4A and B. In general, TPI-1 showed lower plasma and blood concentrations of paclitaxel compared to Taxol® at the same dose. All rats tolerated the 5 mg/kg doses well. In the 10 mg/kg Taxol® group, two of the six rats were dead by the 1-h sample time. Three more were dead by the 3-h sampling point, and the last rat died after the 6-h sample time. In contrast, no death occurred in the group dosed with formulation TPI-1 at the high dose of 10mg/kg, allowing the generation of a complete pharmacokinetic profile at this dose. These observations were consistent with a previous pharmacokinetics study conducted at Samyang Corporation on TPI-1 and Taxol® (unpublished data).

The pharmacokinetic parameters are listed in Table III. Generally, a larger steady-state volume of distribution was observed for TPI-1, indicating that paclitaxel distributed more widely when given in the TPI-1 formulation. At the 5 mg/kg dose, TPI-1's lower AUC lead to a higher calculated clearance (996 mL/h/kg vs. 702 mL/h/kg in blood and 1234 mL/h/kg vs. 775 mL/hr/kg in plasma; Table III), indicating that paclitaxel is also cleared differently when it is formulated in TPI-1. Because both Cremophor EL and polysorbate 80 possess p-glycoprotein inhibition activity *in vitro* (9,10), it has been suggested that these excipients might interfere with p-glycoprotein mediated biliary secretion *in vivo*, thereby reducing paclitaxel elimination (9,10). Compared to Taxol® at the same dose (5 mg/kg), TPI-1 also appeared to be eliminated less rapidly since it exhibited an increased terminal half-life (Table III). These differences could help to explain the lower plasma and blood concentrations observed for TPI-1 as compared to Taxol®. It is not known how these differences would ultimately affect the tissue distribution, tar-

Table III. Mean Pharmacokinetic Parameters for Paclitaxel in Plasma and Blood of Rat after a Single Intravenous (5 and 10 mg/kg) Dose of Taxol® or TPI-1

Group	Test article	Matrix	Dose (mg/kg)	C_0 (ng/mL)	AUC(0-t) (ng · h/mL)	AUC(I) (ng · h/mL)	$t½$ (h)	V_{dss} (mL/kg)	CL (mL/h · kg)
1	Taxol®	Plasma	5	11897	6377	6452	2.3	992	775
1	Taxol®	Blood	5	14641	6999	7125	2.6	1056	702
2[a]	Taxol®	Plasma	10	–	–	–	–	–	–
2[a]	Taxol®	Blood	10	–	–	–	–	–	–
3	TPI-1	Plasma	5	11642	3985	4053	3.2	1455	1234
3	TPI-1	Blood	5	13476	4820	5019	3.5	2114	996
4	TPI-1	Plasma	10	47813	23842	24111	3.8	510	415
4	TPI-1	Blood	10	40414	17447	18444	3.6	1375	542

[a] PK parameters not determined due to animal death.
C_0: Extrapolated initial concentration (at time = 0).
AUC(0-t): The area under the concentration vs. time-curve from time zero to last measurable concentration.
AUC(I): The area under the concentration vs. time curve from time zero to infinity.
$t½$: Terminal phase half-life.
V_{dss}: Apparent volume of distribution.
CL: Plasma or blood clearance.

get tissue concentration, and the pharmacodynamics of paclitaxel. Further studies examining tissue distribution and efficacy need to be conducted.

At the 5 mg/kg dose, both Taxol® and TPI-1 showed slightly lower AUCs in plasma compared with the whole blood, indicating that paclitaxel is preferentially distributed in the red blood cells. The opposite was observed at the 10 mg/kg dose (only TPI-1 data available), where paclitaxel remained preferentially in the plasma compared to the red blood cells. This change in paclitaxel plasma/RBC partition as a function of dose is probably related to previous observations reported in the literature that higher amounts of surfactants such as Cremophor EL resulted from an increased dose tend to sequester paclitaxel in micelles in plasma and therefore decrease the drug's binding to the red blood cells (9, 11–15).

Cremophor EL-containing Taxol® is known to exhibit concentration-dependent non-linear plasma phamacokinetics in human and other animal species (9,11–18). Our data indicate that TPI-1 also showed non-linearity in AUC when the dose was increased from 5 mg/kg to 10 mg/kg, as evidenced by a decrease in plasma clearance from 1234 mL/h/kg to 415 mL/hr/kg. This nonlinearity is still present when the blood clearance was compared, but the non-linearity is less pronounced (996 mL/h/kg at 5 mg/kg dose and 542 mL/h/kg at 10 mg/kg dose; Table III).

The nonlinear behavior of Taxol® is dose- and infusion duration-dependent (9,11–18). Generally, the higher the dose and the shorter the infusion, the more severe the nonlinearity becomes (9,11–18). For a short infusion (i.e., <6 h), the nonlinearity is observed starting at approximately 4 mg/kg dose (16,17). At lower doses (<4 mg/kg), no such deviation from linearity was seen (16,17). In our experiment, the animals were dosed with a bolus injection. This could have exaggerated the nonlinear behavior observed for TPI-1.

The death of the rats in the Taxol® group at the 10 mg/kg dose is probably the result of the toxicity exhibited by Cremophor EL at this high dose. Extensive literature information now indicates that Cremophor EL is the primary cause of the anaphylactic reactions observed in animals and humans (16,17) after Taxol® administration. In addition, a study performed in rats with Cremophor EL free-paclitaxel suggested that Cremophor EL might also be responsible for the neurotoxicity commonly observed (16,17).

Nevertheless, the lower plasma and blood concentrations exhibited by TPI-1 (Fig. 4A and B), and the intrinsic toxicity of paclitaxel itself makes it difficult to directly compare the toxicities of the two formulations based on this initial study. Given the differences in the plasma and blood concentrations observed for the two formulations, future studies comparing TPI-1 and Taxol® toxicity should be conducted at comparable plasma and blood paclitaxel levels. The formulation vehicles should be included to help determine any side effects caused by the excipients alone.

It is worth pointing out that formulation TPI-1 contains a significant amount of polysorbate 80 in the concentrate. As described above, polysorbate 80 is required in the formulation to support paclitaxel solubility upon dilution. Polysorbate 80 has been used as a solubilizer in several other injectables, including Taxotere® (Aventis Pharmaceuticals, Bridgewater, NJ, USA) (19). It has been shown that after administration, polysorbate 80 breaks down rapidly in both mouse and human plasma due to the presence of esterases (20). This may, in part, help to explain the improved tolerance of TPI-1 compared with Taxol®.

CONCLUSIONS

In this article, we demonstrated the feasibility and the power of using a high-throughput combinatorial approach for formulation optimization. Specifically, we used paclitaxel as an example and successfully replaced Cremphor EL from the commercial formulation of Taxol®. The optimized formulation can dissolve at least 6 mg/ml paclitaxel in its concentrated state, and is able to keep paclitaxel in solution for 48 h upon dilution of the concentrate into normal saline. The high-throughput technology permits rapid screening of many conditions in a very short time period. The combinatorial approach also allows the examination of excipient interactions critical for formulation optimization. Specifically in the paclitaxel example, around 10,000 combinations were screened within a week in the initial experiment to give rise to about 20 hit combinations, which in turn served as the basis for further optimization for the final formulation. This represents a 0.2% hit rate and the hits are likely not identifiable if traditional low throughput trial-and error methods or a smaller numbers of combinations were used. The two key excipients identified from the initial screen, PEG 400 and polysorbate, are both critical to the final optimal formulation. PEG 400 helps to keep paclitaxel in solution in the formulation concentrate, and polysorbate 80 plays a key role in paclitaxel solubilization in the diluted state. We believe that a high-throughput combinatorial approach such as the one described in this article can significantly improve the efficiency and quality of drug formulation development in general.

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