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Cremophor-free intravenous microemulsions for paclitaxel II. Stability, in vitro release and pharmacokinetics

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Abstract

Two cremophor-free microemulsion systems LBMW (lecithin:butanol:myvacet:water) and CMW (capmul:myvacet:water), for intravenous (IV) administration of paclitaxel (PAC) were previously developed and characterized. Their chemical stability, in vitro release and pharmacokinetics of PAC were assessed using Taxol® (cremophor:ethanol 1:1, 6 mg/ml) as a reference. The shelf-lives of PAC at 25 °C in Taxol®, LBMW and CMW, in an accelerated stability study, were 71, 57 and 31 days, respectively. The activation energy (E_a) for PAC in Taxol®, LBMW and CMW was 23, 16 and 14 kcal/mol, respectively. PAC released from LBMW and CMW using a dialysis technique was significantly slower than that from Taxol®. The extents of release of PAC from LBMW and CMW were 25 and 50% of that from Taxol®. In vivo pharmacokinetic studies in male Sprague–Dawley rats after IV administration revealed that PAC in LBMW and CMW remained in the systemic circulation five and two times longer and was eight and three times more widely distributed than PAC from Taxol®. LBMW and CMW offer a significant clinical advantage in terms of the prolonged half-life and wide tissue distribution, indicating that PAC delivered by these systems intravenously may result in prolonged exposure of PAC to the tumor and subsequently an improved clinical efficacy.

Keywords: Paclitaxel; Microemulsion; Intravenous; In vitro release; Stability; Pharmacokinetics

1. Introduction

Clinically, PAC (6 mg/ml) is supplied as Taxol® for IV infusion to patients as a clear to colorless cremophor EL (polyoxyethylated castor oil):ethanol, 1:1 v/v formulation in 5 ml vials. This formulation is chemically stable at room temperature for 27 days once diluted with IV fluids. However, precipitation of PAC is evident after 3 days; hence in-line filters with IV sets were used (Vyas, 1995). Extraction of diethylhexylphthalate plasticizers from conventional polyvinyl chloride IV administration and extension sets as well as solution containers continues to be a disadvantage of this diluted formulation (Vyas, 1995). This nonaqueous Taxol® vehicle is used because of the limited aqueous solubility (10.8 μg/ml) and lipophilicity ($K_{o/w} = 311$) of PAC (Trissel, 1997). This limited solubility can in part be explained by PAC's complex diterpenoid structure (Wani et al., 1971), having a bulky, extended fused ring system as well as a number of hydrophobic substituents. PAC does not contain any ionizable functional groups; hence altering the pH will not improve the solubility as the usual attempts to increase its aqueous solubility through salt formation or addition of charged complexing agents offers no benefit.

Microemulsions have evolved as feasible formulation options that use biocompatible ingredients with the potential to improve the solubilization of lipophilic drugs such as PAC. Microemulsions are thermodynamically stable, have the capacity for supersolvency, with a small droplet size and are easy to manufacture (Bagwe et al., 2001). The obvious benefits of the drug delivery system have led to the development of several systems for IV and oral administrations of PAC over the last 5 years (Gursoy et al., 2003; He et al., 2003; Kang et al., 2004; Yang et al., 2004; Zhang et al., 2005). These systems all contain cremophor, except one (Gursoy et al., 2003). Cremophor EL contained in Taxol® has been implicated in anaphylactic reactions in certain individuals (Taxol® Solution for Injection, Drug Information, 2007), making it necessary to pre-medicate patients with antihistamines

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and corticosteroids to reduce the frequency of the reactions to an acceptable level. Also, cremophor may affect red blood cells (RBC) because they penetrate biological membranes to cause an increase in permeability and cell damage (Bielawski, 1990). Rouleaux formation of RBC and a change in shape of white blood cells have been reported in blood smears in a study on the effect of Taxol® on human RBC (Shimomura et al., 1998).

The only cremophor-free microemulsion for IV administration of PAC reported to date (Gursoy et al., 2003) containing 3% w/w of PAC (self-emulsifying drug delivery system, SEDDS, diluted with water) was not stable for more than 24 h at 37 °C losing 20–25% of its chemical integrity during that period. The authors stated that a ready-to-use SEDDS formulation could not be anticipated in the future. Therefore, we developed two cremophor-free microemulsions ready for IV administration. The chemical stability, in vitro release characteristics, and in vivo pharmacokinetics of the two previously developed and characterized cremophor-free microemulsions were assessed using Taxol® as a reference. These microemulsions with an average droplet size of 110 nm contain as much as 12 mg/g PAC, cause insignificant hemolysis whilst retaining the cytotoxic activity of PAC (Nornoo et al., submitted).

2. Materials and methods

2.1. Materials

The reagents and supplies used included butanol (Fisher Scientific, Houston, TX); inulin ^{14}C-carboxylated 1.2 mCi/g (Sigma Chemical Co., St. Louis, MO) *n*-benzylbenzamide (Aldrich Chemical Co. Inc., Milwaukee, WI); methanol, acetonitrile HPLC grade (VWR Scientific Products, Sugarland, TX); paclitaxel (Hande Technology Development Co. USA Inc., Houston, TX); sodium dodecyl sulfate (MCB Manufacturing Chemists Inc., Cincinnati, OH); Taxol® Injection 30 mg (each ml contains 6 mg of paclitaxel, 527 mg Cremophor® EL (polyoxyethylated castor oil) and 49.7% v/v, dehydrated alcohol USP) in 5 ml (Mead-Johnson, Bristol-Myers Squibb Co., Princeton, NJ); Spectra/Por molecular porous membrane tubing, 32 mm, MWCO 12–14,000 (VWR Scientific, Spectrum Medical Instruments Inc., Houston, TX); bioSafe II biodegradable counting cocktail (Research Products International, Prospect, IL) and HEPES buffer pH 7 consisting of 140 mM NaCl, 10 mM HEPES, 5 mM KCl and 0.01% polyethylene glycol (Sigma–Aldrich, St. Louis, MO).

Surfactants included Capmul MCM® (mono/diglycerides of caprylic/capric acid in glycerol) (Abitec Corp., Janesville, WI); L-α-phosphatidylcholine (L-α-lecithin) from soybean (Sigma Chemical Co., St. Louis, MO) and oil was Myvacet 9-45® (distilled acetylated monoglycerides) (Eastman Chemical Co., Kingsport, TN).

2.2. Methods

2.2.1. Preparation of PAC containing microemulsions

Microemulsions of LBMW (lecithin:butanol:myvacet:water, 20:10:68:2 w/w) and CMW (capmul:myvacet:water, 24:74:2 w/w), containing PAC, were prepared by dissolving an appropriate amount of PAC in methanol. Myvacet oil was added to the drug solution and methanol was evaporated under vacuum. The surfactants and water were then added and shaken to form the microemulsion.

2.2.2. HPLC assay for PAC samples

Concentrations of PAC in the formulations and plasma were determined using a modified high-performance liquid chromatography (HPLC) method (Wang et al., 2003). The method was modified to include a different flow rate, quantity of organic phase in the mobile phase and column length. The chromatographic system consisted of a Waters 600 controller and pump, a Waters 717 plus autosampler, and a Waters 996 photodiode array detector (Waters Corp., Milford, MA). Chromatographic separations were achieved using a Synergi Hydro reversed-phase C18 (150 mm × 4.6 mm, 4 μm) column and a Synergi Hydro C18 guard column (4 mm *L* × 3.0 mm ID;) (Phenomenex, Torrance, CA). The mobile phase consisting of 48% acetonitrile in water was pumped through the column at a flow rate of 1.2 ml/min at room temperature. The UV detection wavelength for PAC was 228 nm using *N*-benzylbenzamide (*N*-BB) as an internal standard.

One-hundred microgram per milliliter stock solutions of PAC and *N*-BB were prepared in methanol. These stock solutions were diluted with methanol to obtain 0.45, 0.9, 1.8, 2.7, 4.5 and 9 μg/ml working standards for PAC and 1 μg/ml for *N*-BB. Linearity was determined by plotting the peak height ratios of PAC to *N*-BB versus PAC concentrations. Precision was determined using three PAC standards (0.9, 2.7 and 9 μg/ml). The mean, standard deviation and relative standard deviation (R.S.D.) or correlations of variation of the determined concentrations were recorded. Within- and between-day variations of PAC concentrations were determined by constructing calibration curves on the same day (within-day) and on different days over a 3-month period (between-day). The mean, standard deviation and relative standard deviation of the slopes were recorded. Accuracy of the method was determined by spiking plasma with PAC standards to produce PAC concentrations of 0.9, 2.7 and 9 μg/ml after extraction procedures (Section 2.2.5) and with addition of internal standard. The spiked samples were analyzed and the experimentally determined values were compared to the theoretical amount. The bias values were reported as well as the mean, standard deviation and relative standard deviation of the determined PAC concentrations.

2.2.3. Chemical stability of PAC in the formulations

Chemical stability of PAC in LBMW and CMW each containing PAC 6 mg/g compared to Taxol® was monitored at 25, 35, 45, 55 and 65 °C over a 3-month period. The microemulsions contained only 6 mg/g to be consistent with the drug loading in Taxol® (6 mg/ml). Aliquots of the formulations were placed in 2 ml vials and placed in ovens (Labline Instruments Inc., Melrose Park, IL) at the selected temperatures. Samples were removed in triplicate at selected time intervals and assayed for PAC by the HPLC assay. Plots of log (percentage PAC remaining in the formulation) versus the duration of storage

were constructed at the various storage temperatures for each formulation.

2.2.3.1. Data analysis. The first order degradation rate constants (k) for PAC at all five temperatures were determined from the slopes of the curves. The half-lives of degradation and the shelf lives of PAC in the formulations at different storage temperatures were then calculated. An Arrhenius plot was constructed by plotting the logarithm of the rate constant (k) versus the reciprocal of temperature in Kelvin. The activation energy (E_a) was determined from the slope of the Arrhenius plot.

2.2.4. In vitro release of PAC from the formulations

The rate and extent of PAC released from the w/o microemulsions of LBMW and CMW, each containing PAC 12 mg/g, compared to Taxol® were studied over a 7-day period. The drug release study was conducted using a dialysis technique. Approximately 2 g of the formulations was transferred to dialysis tubing (M.W. cut off 12,000–14,000 Da, 32 mm × 104 mm flat width by flat length, volume/length: 3.3 ml/cm) previously moistened with water. Both ends of the bag were clamped and immersed in a beaker containing 125 ml of a 0.5% sodium lauryl sulfate (SLS) aqueous solution. The beaker was placed in a water bath/shaker maintained at 37 °C. One milliliter samples were withdrawn at 4, 24, 48, 72, 120, 144 and 168 h and subsequently replenished with release media after each sampling. Samples were assayed for PAC by the HPLC assay. The recovery of PAC from the dialysis bag was determined by comparing the amount of PAC added into the dialysis bag at the start of experiment with the sum of the amount of PAC from the withdrawn samples, the contents of the dialysis bag and the dialysis media at the end of 168 h.

2.2.4.1. Data analysis. To characterize the release profiles of PAC from the microemulsions, the cumulative amount of PAC that was released into the medium at each time point was determined with a volume correction taken into account. ^{14}C-inulin (20 μl) was added to the release medium to correct for volume change inside the dialysis bag and was measured using liquid scintillation counting (LS 7500, Beckman, Fullerton, CA). The change in the volume in the dialysis bag was accounted for using a correction factor. A cumulative correction was also made for the removed samples. A plot of cumulative amount of PAC released versus time was then constructed. The extent of PAC released was calculated as the total amount released at 168 h. The initial release rate was obtained by a linear regression analysis of the first four datum points.

2.2.5. In vivo assessment of PAC in rat plasma after IV administrations of the formulations

The in vivo preclinical pharmacokinetic parameters of PAC, distribution half-life ($t_{1/2\alpha}$) elimination half-life ($t_{1/2\beta}$), total clearance (CL), area-under-the-curve (AUC), initial volume of distribution (V_p) and volume of distribution at steady-state (V_{ss}), were determined after a single IV dose of PAC (9 mg/kg) in LBMW, CMW and Taxol®, respectively in rats (180–200 g male Sprague–Dawley, Charles River Inc., Indianapolis, IN). All animal studies conducted were approved by the University of Houston's IACUC. The formulations were diluted to a total volume of 1.5 ml with normal saline and infused (Bioanalytical System Inc., West Lafayette, IN) through the jugular vein over 5–10 min at a rate of 250 μl/min. Blood samples (0.5 ml) were taken from the jugular vein at 0.25, 0.75, 2, 4, 6 and 8 h for Taxol® and 0.33, 1, 2, 4, 6 and 8 h for the LBMW and CMW groups.

2.2.5.1. PAC extraction from plasma. The blood samples were centrifuged and plasma collected for analysis of PAC by the HPLC assay. Four hundred microliter of acetonitrile:water (30:70) was added to 200 μl of rat plasma and PAC was extracted from plasma using a C_{18} solid phase extraction cartridge (Sep-Pak®, Vac, 1 ml, 100 mg). Each cartridge was preconditioned with 1 ml of acetonitrile followed by 1 ml of water. Samples were washed with 2 ml of water followed by 1 ml of methanol:water (50:50). PAC was collected with 2 ml of acetonitrile. After evaporation of the acetonitrile, the residue was reconstituted with methanol and spiked with the internal standard *N*-BB. An aliquot of the reconstituted sample was assayed for PAC by the HPLC assay. The recovery of PAC from plasma was measured by comparing the slope of the standard curves of PAC in plasma to that of the standard curve of PAC in methanol.

2.2.5.2. Data analysis. A plot of plasma PAC concentration versus time was constructed for each formulation and analyzed for one-compartment (CMW and Taxol®) and two-compartment (LBMW) pharmacokinetic parameters (WinNonlin, version 5.0).

2.2.6. Statistical analysis

The stability, release, and pharmacokinetic parameters for PAC from the microemulsions and Taxol® were compared statistically using ANOVA and the Student–Newman–Keuls test for individual differences (PRIMER of Biostatistics, ver. 6.0) at a $p < 0.05$ level of significance.

3. Results and discussion

3.1. HPLC assay for PAC samples

Baseline resolution between the PAC and *N*-BB peaks was achieved for PAC in both methanol and plasma, where PAC and *N*-BB retention times were approximately 13.0 and 5.8 min, respectively. There were no interfering peaks in the blank methanol or plasma chromatogram at the retention time of either PAC or *N*-BB. Linearity was demonstrated over a concentration range of 0.45–9 μg/ml ($R^2 = 0.9997$) for PAC in methanol and 0.9–9 μg/ml ($R^2 = 0.9989$) for PAC in plasma. The HPLC method for PAC in methanol was reproducible with within-day and between-day variations of 5.75 and 5.97%, respectively. The method was precise in the sample analysis with coefficient of variations of 1.33, 3.53 and 1.79% at 0.9, 2.7 and 9 μg/ml, respectively. The HPLC method used to quantify PAC in rat plasma samples was reproducible with within and between-day variations of 1.69 and 16.12%, respectively. The recovery of PAC from plasma was 93.93%. The method was precise in the plasma

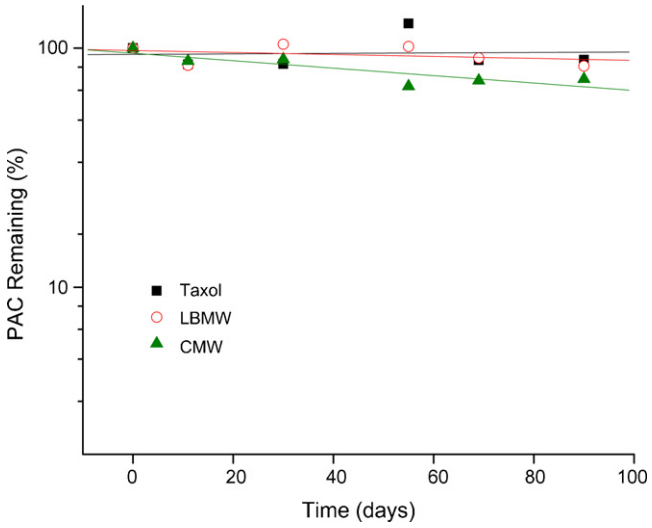

Fig. 1. PAC stability in Taxol®, LBMW and CMW at 25 °C. LBMW (20:10:68:2); CMW (24:74:2).

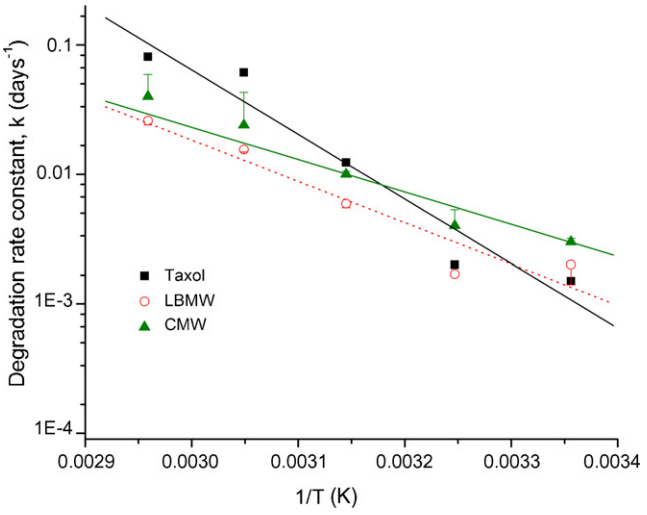

Fig. 2. Arrhenius plot of PAC stability in three formulations. Degradation rate constants were calculated in Table 1. Activation energies (E_a) were 22.7, 15.6 and 14.4 kcal/mol, for Taxol®, LBMW and CMW, respectively.

sample analysis with coefficient of variations of 7.97, 12.26 and 2.87% at 0.9, 2.7 and 9 μg/ml, respectively. The method was accurate with bias of 1.51, 0.77 and −2.52% at 0.9, 2.7 and 9 μg/ml, respectively.

3.2. Stability studies

The chemical stability of PAC in CMW and LBMW was evaluated and compared with that in Taxol®. The percentages of PAC remaining in these formulations decreased logarithmically with time at the different storage temperatures (Fig. 1). The linear decline in PAC indicates first-order degradation kinetics. First-order degradation rate constants (k) were determined from the slopes by linear regression. The k, half-life ($t_{1/2}$) and shelf-life (t_{90}) of PAC at the different temperatures in the three formulations are summarized in Table 1. In general, degradation rate constants (k) increased with increasing temperature for all three formulations. The degradation rate constant of PAC in LBMW was lower when compared to Taxol® at all temperatures, except at 25 °C where k was higher. However, they were higher from CMW at 25 and 35 °C, and then consistently lower at 45–65 °C. The half-lives of PAC in the two microemulsions were longer than that in Taxol® at 45–65 °C. At 25 and 35 °C, the half-life of PAC in LBMW (373.1 and 429.9 days, respectively) was comparable to that in Taxol® (470.17 and 354.01 days, respectively), whilst that in the CMW (206.5 and 186.6 days, respectively) was approximately half of that in Taxol®. The shelf-lives of PAC at 25 °C in the formulations were 71, 57 and 31 days in the Taxol®, LBMW and CMW systems, respectively. This indicates that the stability of PAC in LBMW was comparable to that in Taxol®, but PAC in CMW was less stable. The shelf-lives of PAC in LBMW and CMW at temperatures above 45 °C decreased to values of 18 and 11 days, respectively, which were longer than that of Taxol® (8.5 days).

An Arrhenius plot was constructed by plotting the logarithm of the first order degradation rate constants versus the reciprocal of the corresponding absolute temperatures ($1/T$ in kelvin, Fig. 2). Linear relationships between the degradation rate constant and the reciprocal absolute temperature ($1/T$) were established for the three formulations. Energy of activation (E_a) for the degradation of PAC in the three formulations, estimated from the plot was 22.7, 15.6 and 14.4 kcal/mol, respectively, for the Taxol®, LBMW and CMW. The difference in the acti-

Table 1
Degradation kinetics of PAC in three formulations over a 3-month period

Temperature (°C)	Formulation								
	Taxol ($n=1$)			LBMW ($n=3$)			CMW ($n=3$)		
	k (days^{-1})	$t_{1/2}$ (days)	t_{90} (days)	k (days^{-1})	$t_{1/2}$ (days)	t_{90} (days)	k (days^{-1})	$t_{1/2}$ (days)	t_{90} (days)
25	0.002	470.2	71.2	0.002 (0.0006)[a]	373.1 (123.2)	56.5 (18.7)	0.003 (0.0002)	206.5 (10.9)	31.3 (1.7)
35	0.002	354.0	53.6	0.002 (0.006)	429.9 (159.6)	65.1 (24.2)	0.004 (0.0013)	186.6 (67.9)	28.3 (10.3)
45	0.012	56.1	8.5	0.006 (0.0004)	116.1 (9.15)	17.6 (1.4)	0.010* (0.0005)	70.6* (3.8)	10.7* (0.58)
55	0.061	11.3	1.7	0.016 (0.0006)	44.7 (1.8)	6.8 (0.27)	0.024 (0.019)	41.3 (22.9)	6.3 (3.5)
65	0.081	8.5	1.3	0.026 (0.0018)	26.8 (1.8)	4.1 (0.27)	0.040 (0.0191)	20.8 (11.7)	3.2 (1.8)

LBMW (20:10:68:2); CMW (24:74:2).
[a] S.D.
* Significantly different from LBMW at $p<0.05$.

vation energies may indicate differences in the mechanisms of degradation.

Most solvolytic reactions of pharmaceuticals exhibit E_a's in the range of 8–20 kcal/mol (Wigent, 2005). The greater the energy requirement (E_a), the smaller the proportion of colliding molecules that will have the necessary energy to initiate a reaction, indicating a relatively slow reaction. In this study the E_a for PAC degradation in Taxol was 22.74 kcal/mol, which was larger than the value of the upper range stated, while the E_a for PAC degradation in CMW and LBMW, 14.4 and 15.61 kcal/mol, respectively, were within the range. The degradation profile of Taxol® was constructed only once due to the limited supply of the preparation for the overall project.

In literature, it appears that there are no reports on the stability of PAC in the undiluted Taxol® vehicle. The drug information insert reports that unopened vials for injection concentrate are stable until the date indicated on the package when stored under refrigeration at 2–8 °C in its original package (Taxol® Solution for Injection, Drug Information, 2007). Also, a diluted solution (0.3–1.2 mg/ml of PAC) is stable for 27 h at room temperature. In another report, the stability of 0.1 and 1 mg/ml PAC solutions in 5% dextrose and 0.9% sodium chloride were assessed at 4, 22 and 32 °C, stored for 31 days (Meerum Terwogt et al., 1997). No chemical degradation occurred, however, precipitation was observed after 3 days of storage. In our experiments, the time required for PAC to decrease its potency to 90% (t_{90}) in the undiluted Taxol® formulations at 25 °C, was 71 days, compared to PAC in the two microemulsions, 57 and 31 days for LBMW and CMW, respectively. These studies illustrate the stability of PAC in the two microemulsions as compared to other cremophor-free microemulsion reported. The only cremophor-free microemulsion for IV administration of PAC reported to date (Gursoy et al., 2003) containing 3% w/w of PAC (self-emulsifying drug delivery system, SEDDS, diluted with water) was not stable for more than 24 h at 37 °C losing 20–25% of its chemical integrity during that period.

3.3. Release studies

Release studies were conducted in order to predict the potential in vivo pharmacokinetic behavior of PAC from the three formulations. A dialysis technique was used to determine the in vitro release behavior of PAC from Taxol®, LBMW and CMW, respectively. The release media contained 0.5% sodium lauryl sulphate (Vinod et al., 1989) in order to maintain the sink condition. Micellar solubilization of PAC occurred when the dissolved surfactant was present at concentrations exceeding its critical micelle concentration thereby enhancing the aqueous solubility of PAC. Also, ^{14}C-inulin was used as a marker in the medium, to monitor the influx of the release medium into the dialysis bag. The volume correction study showed that appreciable volumes of release medium were drawn into the dialysis bags within the first 4 h, 9.3, 12.5 and 10.8 ml for Taxol®, LBMW and CMW, respectively (Table 2). This could be accounted for by the osmotic pressure differences between the formulation and the surrounding release medium. Based on these observations, a correction factor was used to calculate the amount of PAC released.

Table 2
Release characteristics of PAC from three formulations

Formulations	Taxol®	LBMW	CMW
Extent of release (168 h)	78.03 ± 2.49	21.23 ± 8.4*,†	40.31 ± 12.09*
Rate of release (%/h)	1.22 ± 0.18	0.05 ± 0.02*	0.29 ± 0.14*
Volume in bag (ml)	9.33 ± 3.09	12.5 ± 1.99	10.77 ± 2.31
Recovery**	108.79 ± 16.58	93.5 ± 2 2.08	82.35 ± 7.87

Taxol®; LBMW (20:10:68:2); CMW (24:74:2).
* Significantly different from Taxol® at $p < 0.05$.
** Percent PAC recovered after experiment.
† Significantly different from CMW $p < 0.05$.

The initial release rates of PAC from CMW and LBMW were significantly slower (0.29 and 0.05 %/h) compared to that from Taxol® of 1.22%/h (Fig. 3 and Table 2). The extent of release for PAC from Taxol® (78%) was 1.9 and 3.7 times of those from CMW (40.3%) and LBMW (21.2%), respectively. The release of PAC plateaus by approximately 125 h for both Taxol® and CMW; however, that from LBMW did not occur at the end of 168 h. The recoveries of PAC from Taxol®, LBMW and CMW were 108.8, 93.5 and 82.4, respectively (Table 2).

PAC in CMW and LBMW exhibited sustained release characteristics when compared to that in Taxol® (Fig. 3) and this may be attributed to the fact that PAC is solubilized in the oil component of the microemulsion. This sustained release characteristic would be advantageous in cancer treatment since PAC levels in the systemic circulation would be sustained and prolonged exposure of PAC to cancer cells is desirable.

3.4. Pharmacokinetic studies

The dispositions of PAC from the three formulations were characterized after a single IV infusion (9 mg/kg) dose to male

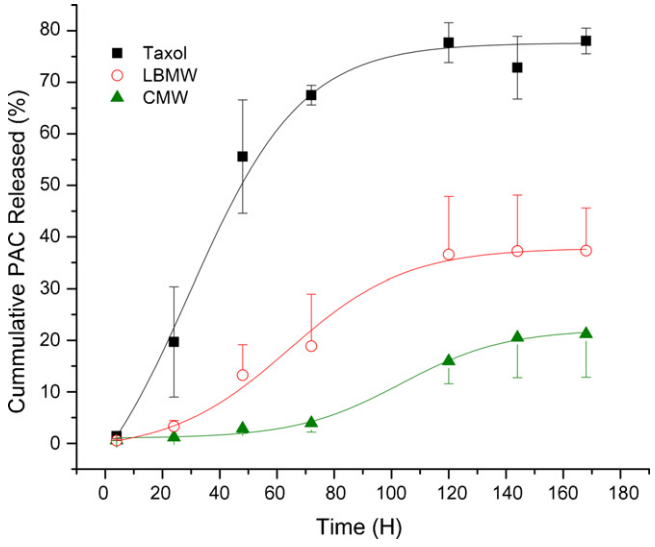

Fig. 3. Release profiles of PAC from three formulations. Dialysis bag contained 12 mg/g of PAC which was released into 0.5% sodium lauryl sulphate in water over 168 h, $n = 3$–5. The formulations were Taxol®, LBMW (20:10:68:2) and CMW (24:74:2).

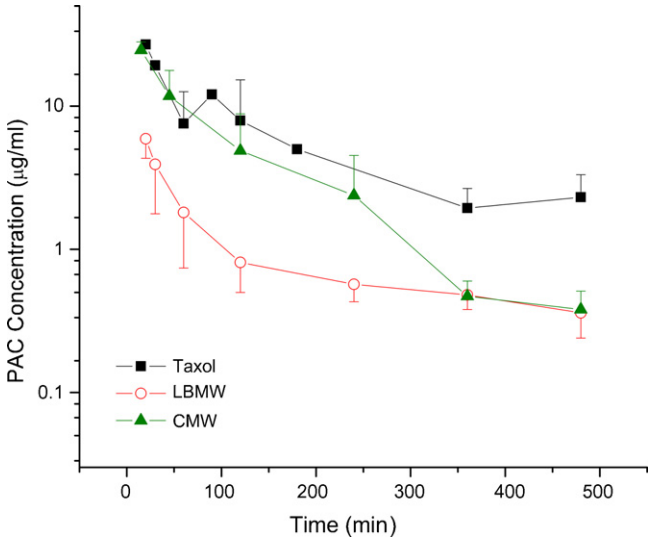

Fig. 4. Mean pharmacokinetic profiles of PAC from three different formulations in rats (n = 4–5). Taxol®; CMW (15:83:2); LBMW (10:5:83:2). Dose 9 mg/kg of PAC as a 5–10 min infusion of diluted formulation in 1.5 ml of normal saline.

Sprague–Dawley rats. PAC concentrations appeared to decline in a biexponential manner for LBMW and monoexponentially for Taxol® and CMW (Fig. 4). Two-compartment modeling was therefore used to obtain the pharmacokinetic parameters of PAC in LBMW and one-compartment modeling for Taxol® and CMW (WinNonlin ver. 5.0). There is a distinct rapid distribution phase ($t_{1/2\alpha}$ = 15.87 min) in the PAC profile from the LBMW system followed by a slow elimination phase as compared to that in either Taxol® or CMW. LBMW and CMW yielded sustained levels of PAC in the blood circulation from 2 to 8 h post-dose.

The pharmacokinetic parameters of PAC from Taxol® were consistent with that in literature (Gaver et al., 1993). In that study male and female Sprague–Dawley rats were given an 8–9 mg/kg ^{14}C-PAC dose as a 5 min IV infusion. The terminals half-lives were 72 and 126 min in female and male rats, respectively, compared to the value of 79 min in male rats of this study. The total body clearances were 4.8 and 2.75 ml/(min kg), for females and males, respectively, which was comparable to our value of 3.8 ml/(min kg) (0.76 ml/min for rats of 0.2 kg). The volume of distribution was 0.5 l/kg for both female and male rats. The AUC values are 1.9 and 3.3 mg/(min ml) in female and male rats, respectively. Similar values of 0.43 l/kg and 2.6 mg/(min ml), for V_{ss} and AUC, respectively, were derived in this study with rats of 0.2 kg.

The pharmacokinetic parameters of PAC from LBMW formulation were significantly different from those of Taxol® or CMW (Table 3). Half-life was fivefold and twofold longer in the LBMW (399.4 min) and CMW (155.5 min) as compared to that in the Taxol® (79.1 min). The clearance of PAC from the circulation in the LBMW (2.34 ml/min) was two and three times of that in CMW (1.15 ml/min) or Taxol® (0.76 ml/min), respectively. PAC from LBMW was eight and three times more widely distributed (709 ml) in the rat body than that from Taxol® or CMW (86.2 and 270.5 ml, respectively). The area under the curve or the extent of exposure (AUC) after dosing of PAC in LBMW was approximately 35% of that from either Taxol® or CMW (0.9 mg/(min ml)) versus 2.6 mg/(min ml) and 2.4 mg/(min ml), respectively).

PAC in LBMW was eight and three times more widely distributed than that from Taxol® or CMW, respectively. Reports in literature suggest that a large volume of distribution at steady state (even though plasma protein binding is high, 93–98%) is due to extensive distribution, binding to tissues and extravascular protein binding including tubulin in humans and mice (Beijnen et al., 1994). PAC may remain solubilized in the oil phase of the microemulsions leading to an enhanced ability of PAC to cross biological membranes and become more widely distributed. This could explain the sustained levels of PAC from both the LBMW and CMW formulations. The fivefold longer half-life seen with PAC in LBMW compared to Taxol® could be accounted for by the eightfold wider volume of distribution offsetting the threefold faster clearance. The same is true for PAC in CMW compared to Taxol® where a twofold increase in half-life is accounted for by a threefold wider volume of distribution and a 1.5 times faster clearance. These results could therefore be related to the in vitro release studies where a sustained release of PAC was observed from both LBMW and CMW. This slower release of PAC from LBMW as compared to CMW may in part explain the significantly longer half-life of PAC in LBMW as compared to Taxol® or CMW. The three and twofold lower AUC seen with PAC in LBMW compared to that in Taxol® or CMW could be accounted for by the three and twofold higher clearance values of PAC in LBMW. These results may in part be explained by the fact that lecithin is an endogenous substance and capmul and cremophor are not, perhaps making lecithin a better substrate for clearance mechanisms.

Table 3
Comparison of the mean pharmacokinetic parameters of PAC from three formulations in rats

Parameter	Taxol®	LBMW	CMW
AUC (μg/(min ml))	2627.06 (567.83)	908.42 (349.28)*	2382.43 (1278.13)**
V_{ss} (ml)	86.18 (13.37)	708.98 (264.24)*	270.48 (216.80)**
V_p (ml)	n/a	131.19 (89.55)	n/a
Cl (ml/min)	0.76 (0.14)	2.34 (0.73)*	1.15 (0.83)**
$t_{1/2\alpha}$ (min)	n/a	15.87 (8.58)	n/a
$t_{1/2\beta}$ (min)	79.08 (6.40)	399.4 (243.50)*	155.50 (29.02)**

Taxol® (one-compartment model); LBMW (10:5:83:2), two-compartment model; CMW (15:83:2), one-compartment model.
* Significantly different from Taxol® at $p < 0.05$.
** Significantly different from LBMW at $p < 0.05$.

The benefits of these microemulsions to deliver PAC are obvious. The slower release of PAC from the microemulsion translates to a sustained release effect in vivo as observed by the significantly longer half-lives of PAC. The therapeutic implications could mean a more effective passive targeting of PAC to the tumor sites as a result of the wider distribution and longer half-life. Indeed, PAC microemulsions developed exhibit a significantly longer half-life than a previously developed cremophor containing microemulsion for PAC (He et al., 2003). He et al. (2003) administrated Taxol® or PAC microemulsion via the tail vein of five rats to study the hypersensitivity and pharmacokinetics of PAC in these formulations. They observed that the area under the curve (AUC) was significantly higher in the microemulsion group (2098.8 μg/(ml min)) than that in the Taxol® group (1318.8 μg/(ml min)). Also, half-life was longer in the microemulsion group (73.2 min) compared with the Taxol® group (32.4 min). The cremophor-free microemulsions developed in this study produced as much as a five (LBMW ∼ 400 min) and twofold (CMW ∼ 155 min) longer circulation time of PAC compared to the cremophor-containing microemulsion (73.2 min).

In conclusion, the pharmacokinetic parameters of PAC were significantly altered in LBMW and CMW. PAC in LBMW and CMW had a prolonged half-life (five and twofold, respectively) and larger volume of distribution (eight and threefold, respectively) as compared to Taxol®, indicating a potentially enhanced exposure of PAC to tumor sites. Efficacy studies in tumor-bearing rats are warranted to determine the in vivo efficacy of these PAC microemulsions.

4. Summary and conclusion

Two previously developed cremophor-free microemulsions for IV administration of PAC were evaluated and assessed for chemical stability, in vitro release characteristics, and in vivo pharmacokinetics of PAC as compared to the reference Taxol®. These microemulsions with an average droplet size of 110 nm contain as much as 12 mg/g PAC, cause insignificant hemolysis whilst retaining the cytotoxic activity of PAC (Nornoo et al, submitted). In this study, PAC was stable at room temperature (25 °C), in Taxol®, LBMW and CMW for 71, 57 and 31 days, respectively. PAC in LBMW and CMW exhibited significant sustained release characteristics as compared with that of the Taxol®. The pharmacokinetic parameters of PAC were significantly altered in LBMW and CMW. The half-life of PAC was prolonged five and twofold and was more eight and three times more widely distributed in the LBMW and CMW, respectively, compared to Taxol®. Taken together LBMW and CMW, with a droplet size in the 110 nm range are alternative stable formulations for PAC to be administered intravenously. They are safe with insignificant hemolytic potential, retaining the cytotoxic activity of PAC and releasing PAC in a sustained manner. PAC in LBMW and CMW may potentially be more clinically effective, since it is more widely distributed and results in sustained blood levels that may lead to a prolonged exposure of the tumor to the drug.

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