European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 310–317

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Oral microemulsions of paclitaxel: In situ and pharmacokinetic studies

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ARTICLE INFO

Article history:
Received 17 December 2007
Accepted in revised form 15 August 2008
Available online 29 August 2008

Keywords:
Microemulsion
Paclitaxel
Permeability
In situ
Oral
Pharmacokinetics

ABSTRACT

The overall goal of this study was to develop cremophor-free oral microemulsions of paclitaxel (PAC) to enhance its permeability and oral absorption. The mechanism of this enhancement, as well as characteristics of the microemulsions relevant to the increase in permeability and absorption of the low solubility, low permeability PAC was investigated. Phase diagrams were used to determine the macroscopic phase behavior of the microemulsions and to compare the efficiency of different surfactant-oil mixtures to incorporate water. The microemulsion region on the phase diagrams utilizing surfactant-myvacet oil combinations was in decreasing order: lecithin: butanol: myvacet oil (LBM, 48.5%) > centromix CPS: 1-butanol: myvacet oil (CPS, 45.15%) > capmul MCM: polysorbate 80: myvacet oil (CPM, 27.6%) > capryol 90: polysorbate 80: myvacet oil (CP-P80, 23.9%) > capmul: myvacet oil (CM, 20%). Oil-in-water (o/w) microemulsions had larger droplet sizes (687–1010 nm) than the water-in-oil (w/o) microemulsions (272–363 nm) when measured using a Zetasizer nano series particle size analyzer. Utilizing nuclear magnetic resonance spectroscopy (NMR), the self-diffusion coefficient (D) of PAC in CM, LBM and CPM containing 10% of deuterium oxide (D_2O) was 2.24×10^{-11}, 1.97×10^{-11} and 0.51×10^{-11} m^2/s, respectively. These values indicate the faster molecular mobility of PAC in the two w/o microemulsions (CM and LBM) than the o/w microemulsion – CPM. The in situ permeability of PAC through male CD-IGS rat intestine was 3- and 11-fold higher from LBM and CM, respectively, than that from the control clinical formulation, Taxol® (CE, cremophor: ethanol) in a single pass perfusion study. PAC permeability was significantly increased in the presence of the pgp/CYP3A4 inhibitor cyclosporine A (CsA). This enhancement may be attributed to the pgp inhibitory effect of the surfactants, oil and/or the membrane perturbation effect of the surfactants. The oral disposition of PAC in CM, LBM and CPM compared to CE was studied in male CD-IGS rats after a single oral dose (20 mg/kg). The area-under-the-curve of PAC in CM was significantly larger than LBM, CPM and CE. Oral microemulsions of PAC were developed that increased both the permeability and AUC of PAC as compared to CE.

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1. Introduction

Microemulsions possess characteristics that make them ideal formulation candidates for poorly aqueous soluble- low permeability drugs, to be administered orally [1]. These unique characteristics include thermodynamic stability, supersolvency, small droplet size and the use of food grade, pharmacologically inactive excipients which are absorption enhancers. They are composed of a surfactant and/or surfactant mixture, co-surfactant, an oil and water with a dispersed phase of less than 100 nm in diameter [2,3]. Microemulsions have been used to deliver paclitaxel (PAC) both intravenously and orally. PAC, the model cytotoxic compound continues to demonstrate impressive clinical activity against many common types of solid malignancies including ovarian, breast and non-small cell lung carcinomas. Clinically, PAC (6 mg/ml) is administered by intravenous (IV) infusion as Taxol® (referred to as CE in this paper) to patients as a clear to colorless [cremophor EL (polyoxyethylated castor oil): ethanol, 1:1 v/v] formulation in 5 ml vials. PAC is administered IV because of its low aqueous solubility (10.8 μg/ml) and limited bioavailability (<2%) when administered orally [4].

Possible factors that contribute to PAC's limited bioavailability include: (i) instability in the gastrointestinal fluids, (ii) limited aqueous solubility and dissolution, (iii) affinity for intestinal and liver cytochrome P450 metabolic enzymes (CYP450), and (iv) the multidrug efflux pump, p-glycoprotein (pgp), which serves to protect the body from xenotoxins [5]. Microemulsions could potentially address some of these limitations. Firstly, a combination of surfactants with oils offers an advantage over a micellar or cosolvent system in terms of drug solubilization capacities for lipophilic compounds because of the extra locus for solubilization provided

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0939-6411/$ - see front matter Published by Elsevier B.V.
doi:10.1016/j.ejpb.2008.08.015

by the oil phase [2]. Indeed the solubility of PAC in a myvacet oil containing microemulsion was increased 1389-fold compared to its aqueous solubility [6]. PAC was stable in these microemulsions at room temperature (25 °C) for 31–57 days. Secondly, several reports [7–9] indicate that non-ionic surfactants such as polysorbate 20, polysorbate 80, acacia, solutol HL 15, vitamin E TPGS, and cremophor EL can act as pgp inhibitors in vitro, while an increase in oral absorption as a result of pgp inhibition by surfactants was only moderately observed in vivo [10]. Hence, microemulsions are not only useful drug delivery systems in terms of drug solubility and stability, but also contain components such as the surfactants and oils which could act as pgp/CYP450 inhibitors.

To date two in vivo studies have shown the potential of microemulsions to enhance the oral absorption of PAC [11,12]. Pharmacokinetic studies conducted in rats showed that the bioavailability of PAC in a supersaturable self-emulsifying drug delivery systems (S-SEDDS) was 9.5% compared to 2% in the orally dosed CE, a further increase in bioavailability to 22.6% was noted in the presence of cyclosporine A (CsA) [11]. In the other study [12] a novel self-emulsifying drug delivery system (SMEDDS) was developed for the oral delivery of PAC. Co-administration of CsA in these formulations was needed to attain the required therapeutic levels in rats. Both of these formulations include the surfactant cremophor EL which has been known to cause hypersensitivity reactions, requiring premedication with antihistamines and corticosteroids [13]. Orally administered CE has been shown to produce cremophor EL plasma levels in knockout mice [14]. Hence, the need for further exploration of these systems as delivery agents for low solubility, low permeability drugs. The overall goal of this study was to develop cremophor-free oral microemulsions of paclitaxel (PAC) to enhance its permeability and oral absorption. The mechanism of this enhancement, as well characteristics of the microemulsions relevant to the increase in permeability and oral absorption of the low solubility, low permeability PAC was investigated.

Oral microemulsions for PAC were developed empirically and characterized. Phase diagrams were used to delineate the microemulsion region without drug to be able to select an optimal formulation based on solubility (of PAC in the surfactant/oil combination) and dilutability of the surfactant/oil combination without precipitation of drug. The droplet size of the dispersed phase in the microemulsion was subsequently determined using photon correlation spectroscopy, and the self-diffusion coefficient (D) of PAC in the microemulsions was determined by nuclear magnetic resonance spectroscopy (NMR). D determined by NMR is a very useful parameter relating drug diffusion to permeability and physical stability and has been used extensively to study microemulsions [15–18].

In order to determine the potential of surfactants and/or oils to act as pgp/CYP3A4 inhibitors in the microemulsions compared to the control clinical formulation CE, in situ single pass perfusion studies were performed in the presence and absence of a pgp/CYP3A4 inhibitor CsA. The oral disposition of PAC was subsequently determined in the microemulsions compared to CE. The relationship between the physical characteristics of PAC in the microemulsions (droplet size and diffusion coefficient) and the permeability and bioavailability of PAC was investigated.

2. Materials and methods

2.1. Materials

Drugs and chemicals. Paclitaxel [Polymed Therapeutics, Inc., Houston, TX]; cyclosporine A [Alexis biochemicals, San Diego, CA], *N*-benzylbenzamine [Acros Organics USA, Morris Plains, New Jersey], deuterium oxide [Sigma–Aldrich, Milwaukee, WI], glacial acetic acid, ammonium acetate [J.T. Baker, Phillipsburg, NJ] HPLC grade methanol and acetonitrile [VWR Scientific Products, Sugarland, TX]; 1-butanol [Fisher Scientific, Houston, TX], ^{14}C polyethylene glycol 4000 (^{14}C-PEG), 10 – 20 mCi/g [Amersham Biosciences, Piscataway, NJ], bioSafe II biodegradable counting cocktail [Research Products International, Prospect, IL], Hepes buffer, pH 7, consisting of 140 mM NaCl, 10 mM Hepes, 5 mM KCl, 0.01% polyethylene glycol [Sigma–Aldrich, St. Louis, MO].

Surfactants and oil. 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], polyoxyethylene [20] sorbitan monooleate (polysorbate 80) [Croda, Inc., Parisppany, NJ], Centromix® CPS (soy lecithin and polysorbate 80) [Central Soya Company, Inc., Fort Wayne, IN], Caproyl 90 (propylene glycol monocaprylate) [Gattefosse Corporation, Westwood, NJ] and Myvacet 9-45® (distilled acetylated monoglycerides) [Eastman Chemical Co., Kingsport, TN].

2.2. Methods

2.2.1. Formulation development

2.2.1.1. Component screening and phase diagram construction. Oral microemulsions for PAC were developed empirically. Initially this involved combining digestible oils with food-grade non-ionic emulsifiers to form concentrates that can incorporate large quantities of water and remain a single phase liquid. Non-ionic surfactants were chosen as determined by their average hydrophile–lipophile balance (HLB) requirement for the proposed microemulsion. Other criteria included the potential toxicity and known application in drug delivery systems. Surfactants with a HLB value in the range of 3–6 are used for formulating w/o microemulsions, while those in the 8–18 range are used to formulate o/w microemulsions [19]. In this project surfactant and surfactant mixtures had HLB values that ranged from 5 to 14 that made both w/o and o/w microemulsions. Non-ionic surfactants used in this project included polysorbates, e.g. poly sorbate 80; medium chain mono- and diglycerides, e.g. capmul; and phospholipids, e.g. lecithin. Previous studies have reported a 15 and 1.2 mg/ml PAC solubility in myvacet and miglyol oils [6] as compared to that in water (10.8 μg/ml). Hence, myvacet oil (medium chain triglyceride) was selected as the only oil to be used because of its high PAC solubility and also to reduce the number of variables in this study.

Phase diagrams were constructed to determine regions of microemulsion formation, from which a large number of potential microemulsions could be identified. Various surfactants were combined with myvacet oil at ratios from 95:5 to 5:95 with 5% increments of the surfactant. The maximum amount of water that could be incorporated was determined for each surfactant: oil combination. Phase diagrams were then constructed by connecting the ratios of surfactant: oil: water at each transition point on a ternary phase diagram. Phase diagrams were constructed for the following systems: lecithin: 1-butanol: myvacet oil (LBM), capmul: myvacet oil (CM), capryol 90: polysorbate 80: myvacet oil (CPM), capmul: polysorbate 80: myvacet oil (CP-P80), centromix CPS: 1-butanol: myvacet oil (CPS).

2.2.1.2. Preparation of PAC containing microemulsions. Microemulsions containing 10–14 mg of PAC per gram of formulation with and without 14 mg of CsA were prepared by dissolving the appropriate amount of drug in methanol. Myvacet oil was subsequently added and methanol evaporated under vacuum. PAC was first dissolved in methanol to expedite the dissolution process and ensure that PAC was completely solubilized in the oil. The surfactant or surfactant mixture was added followed by water to make the microemulsion. Cremophor EL: Ethanol (1:1 w/w, CE), containing 6 mg/ml PAC was used as a reference in this project.

2.2.1.3. Physical characterization of PAC microemulsions.

2.2.1.3.1. Droplet size determination. The droplet size of the dispersed phase of the microemulsions was determined using photon correlation spectroscopy. Measurements were performed using a Zetasizer nano series instrument equipped with an avalanche photodiode (Malvern Instruments, Inc., Southborough, MA). The z-average droplet size for each microemulsion was determined at room temperature at a viscosity of 0.8872 cp and refractive index at 1.33. Microemulsions were diluted 1:60 with water before measurements.

2.2.1.3.2. Diffusion NMR. Self-diffusion coefficients (D) of specific molecules can be determined by NMR, typically by two types of pulse sequences, pulse-gradient spin-echo sequence (PGSE) [20] and pulse-gradient stimulated echo sequence (PGSTE) [21]. In liquid pharmaceutical preparations, D of a drug molecule is directly related to its mobility within its surrounding microenvironment. NMR spectroscopy was used to determine D of PAC and myvacet oil in the microemulsions. NMR measurements were performed at 25 °C on a Bruker Avance 400 NMR spectroscopy system equipped with a BBI 400 MHz S1 5-mm probe with Z-gradient (Bruker Biospin, Billerica, MA). Water in the microemulsions was replaced with deuterium oxide (D_2O) and approximately 0.75 ml of each microemulsion was transferred to a 0.5-mm NMR tube (Wilmad, Buena, NJ) for each experiment.

The peaks of PAC in the microemulsions were assigned, and unique proton signals from PAC were identified in the cyclic region around 7–8 ppm which did not overlap with any of the components in the microemulsion. The selected signals were then used to determine D utilizing a PGSTE sequence and the data processed using the Topspin 1.0 software. D for specific protons was calculated by a nonlinear fit of the intensity (I) decay at different gradient strengths according to the following equation:

$$I(\delta, \Delta, g) = I_0 e^{-D\delta^2\gamma^2 g^2(\Delta - \frac{\delta}{3})}, \quad (1)$$

where δ is the gradient pulse length, γ is the gyromagnetic ratio of the observed nucleus, g is the gradient strength and Δ is pulse interval. Initially the D of PAC dissolved in myvacet oil was measured. This value was compared to the D of pure myvacet oil. The D of PAC was subsequently determined in the various microemulsions containing 10% D_2O.

2.2.2. In situ assessment of PAC in the formulations: single pass perfusion studies

2.2.2.1. Experimental. The permeability of PAC with and without CsA in the microemulsions and the reference CE were compared from the gastrointestinal tract in male CD® IGS rats [226–250 gm, Charles River Laboratories, Wilmington, MA]. All animals used for this study were treated according to protocols evaluated and approved by the IACUC of the Stratton Veterans Administration Medical Center, Albany, NY. Rats were fasted for approximately 18 hr with free access to water to clear the gut prior to surgery. They were anaesthetized with ketamine:xylazine (7.25:1 v/v, Ben Venue Laboratories Inc., Bedford, OH; Phoenix Scientific, Inc., St. Joseph, MO) intraperitoneally half an hour before surgery and the abdominal wall opened. A heating pad and heating lamp were used to maintain the body temperature of the rat. The small intestine (approximately 13–15 cm in length) was isolated separately in situ and ligated at the beginning and end of the proper segment with a canula. Perfusion fluid (Hepes buffer) was passed slowly through the tract to clear the gut. The perfusion fluid consisting of the representative formulations [microemulsion or CE containing 0.16 mg/ml PAC with and without 0.23 mg/ml CsA spiked with 0.5μCi of ^{14}C PEG] was infused (0.2 ml/min) into the segments for thirty minutes to achieve steady state. ^{14}C PEG was used to account for water absorption that may occur during the experiment. Serial samples (0.5 ml) were taken at the end of the proper segments for up to 1.5 h after steady state. Samples were stored in a −80 °C freezer until assayed. PAC and ^{14}C PEG were analyzed by HPLC and liquid scintillation counting, respectively.

2.2.2.2. HPLC analysis of PAC in perfusate. PAC concentration in perfusate was determined using a high-performance liquid chromatography (HPLC) method developed in our laboratory [22]. 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 × 4.6 mm, 4 μm) column and a Synergi Hydro C18 guard column (4 × 3.0mm, 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.

Linearity was demonstrated over a concentration range of 0.45–9 μg/ml (R^2 = 0.9997) for PAC in methanol with a retention time of 10.8 min. 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 method was also reproducible with within-day and between-day variations of 5.75% and 5.97%, respectively.

2.2.2.3. Data analysis. The concentration of PAC in the perfusate leaving the intestinal segment corrected for water absorption was determined at each time point. The apparent in situ permeability coefficient, P_{eff} was calculated from the fraction of drug remaining in the intestinal segment using the following equation:

$$P_{eff} = -\frac{Q}{2\pi r l} \ln C_t/C_{initial} \times DPM_{initial}/DPM_t \quad (2)$$

where Q is the infusion rate (0.2 ml/min), $C_t/C_{initial} \times DPM_{initial}/DPM_t$ is the fraction of the permeant remaining in the intestinal lumen of length l (cm) and effective radius r (cm) at time t. The permeability coefficients for PAC from the microemulsions and CE through rat intestine were compared statistically using ANOVA and the Student–Newmann–Keuls test for individual differences (PRIMER of Biostatistics, ver. 6.03).

2.2.3. In vivo assessment of PAC in the formulations: pharmacokinetic study

2.2.3.1. Experimental. The in vivo preclinical pharmacokinetic parameters of PAC, elimination half-life ($t_{1/2}$), total clearance (CL), area-under-the-curve (AUC) and volume of distribution at steady-state (V_{ss}), were determined after a single oral dose of PAC in the microemulsions or CE in rats. All animals used for this study were treated according to protocols evaluated and approved by the IACUC of the Stratton Veterans Administration Medical Center, Albany, NY. Jugular-vein canulated male rats (CD-IGS, 264–395 g, Charles River Laboratories Wilmington, MA) were given a single dose of PAC (20 mg/kg) in either the microemulsions or CE. Five to seven rats in each formulation group were given approximately 500 μl of the formulation (weight normalized) diluted to a total volume of 1.5 ml with normal saline administered by oral gavage. Blood samples (0.5 ml) were taken from the jugular vein at 0, 10, 40, 90, 150, 240, 360, 450 and 480 min post-dosing. The blood samples were centrifuged and plasma collected and stored in a −80 °C freezer. Samples were assayed for PAC by tandem mass spectroscopy coupled to HPLC.

2.2.3.2. LC-MS/MS analysis of PAC in plasma.

2.2.3.2.1. Extraction procedure. PAC was extracted from the plasma samples on solid phase extraction cartridges [Strata, C-18E, 200 mg/3 ml, Phenomenx, Torrence, CA]. Briefly, each cartridge

was preconditioned with 2 × 1 ml of acetonitrile followed by 2 × 1 ml of water. Four hundred microliters of acetonitrile:water (30:70) and an internal standard, *N*-benzylbenzamide (N-BB, 1 ng/ml final concentration) were added to 200 μl of rat plasma. The sample was then applied and subsequently washed with 2 × 1 ml of methanol:water (50:50). The extract was collected with 2 × 1 ml of acetonitrile and evaporated under vacuum. The residue was reconstituted with methanol and assayed for PAC by LC–MS/MS.

2.2.3.2.2. LC–MS/MS. Concentrations of the standard solution of PAC and N-BB in methanol and plasma samples were determined using a modified published LC–MS/MS method [23]. A Shimadzu LC-20AB system (Shimadzu Scientific Instruments, Columbia, MD) equipped with a binary pump, a micro vacuum degasser, and an autoinjector was used. The chromatographic separation was performed by injecting an aliquot of the standard or sample (10 μl) onto a Thermo Hypersil reversed phase C_{18} column (100 × 2.1 mm, 4 μm, Keystone Scientific, Bellefonte). The mobile phase was a gradient mixture of methanol (solvent A) and 2 mM ammonium acetate buffer (solvent B) adjusted to a pH of 5.0 with acetic acid. The gradient program to separate the analyte was started at 60% by volume of solvent B, held for 10 min, at a flow rate of 0.2 ml/min. The gradient was increased to 85% solvent A in 9 min, held for 8 min, reversed back to 40% solvent B in 1 min, and then held for 7 min.

An Applied Biosystems (Concord, ON, Canada) API 2000 tandem mass spectrometer equipped with an electrospray source was used online with the HPLC system described above. Target analyte ions were monitored using the multiple reaction monitoring (MRM) mode with the parent ion ($[M+H]^+$) → product ion transitions as follows *m/z* 854.4 → 286.2 and 854.4 → 267.9. The pseudomolecular ion ($[M+H]^+$) was selected in the first MS quadrupole and the collision cell was adjusted to obtain the most abundant product ions, *m/z* 286.2 and 267.9 which were used for quantification and confirmation. The optimized MS/MS parameters used to analyze PAC were as follows: nitrogen curtain gas, 15 psi; ion spray voltage, 5500 V; declustering potential, 24 V; ion source temperature, 300 °C; dwell time, 300 ms; collision energy, 24.5 eV.

Linearity of the target analyte was investigated by constructing 6-point extracted calibration curves at concentrations of 0.05, 0.1, 0.5, 1, 3 and 5 μg/ml. The internal standard (N-BB) and an extracted calibration curve were used to correct for variability in the analytical extraction procedure and the effect of the ionization enhancement or suppression produced by the sample matrix. Calibration curves showed excellent linearity, with satisfactory coefficients of determination ($R^2 > 0.991$). The limit of detection (LOD) defined as the lowest concentration of the target compound to give a signal-to-noise ratio >3 determined by analysis of blanks was 0.05 μg/ml. 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 and found to be 68%. The precision and accuracy of the method was determined by spiking plasma with PAC and N-BB standards to produce PAC concentrations of 0.05, 0.5 and 5 μg/ml and 0.01 μg/ml N-BB after extraction procedures in triplicate. The method was precise and accurate in the sample analysis with coefficient of variations of less than 13%.

2.2.3.3. Data analysis. A plot of plasma PAC concentration versus time was constructed for each formulation and analyzed for model-dependent pharmacokinetic parameters (WinNonlin, version 5.2). The goodness of fit criteria used in selecting the most appropriate model included the weighted residual sum of squares (WRSS) and the Akaike information criterion (AIC). The suitability of the model to estimate the pharmacokinetic parameters was also determined by inspecting the graphs of predicted concentrations (weighted) versus observed concentrations as well as the correlation of variation for each parameter. The pharmacokinetic parameters – V_{ss}, CL, AUC and $t_{1/2}$ of PAC in the four formulations were derived and compared statistically by a one way ANOVA followed by the Student–Newmann–Keuls test for individual differences (PRIMER of Biostatistics, version 6.03).

3. Results and discussion

3.1. Formulation development

3.1.1. Component screening and phase diagrams

Phase diagrams were utilized in the development of microemulsions to determine their macroscopic phase behavior [24] and to compare the efficiency of different surfactants used in terms of water incorporation. The goal in the formulation of microemulsions is to have the lowest possible surfactant content with optimal solubilization of lipophilic components (in this case PAC). However, relatively high surfactant contents are often needed to form microemulsions. The microemulsion existence field for five surfactants or surfactant blends, lecithin: butanol, capmul, capmul: polysorbate 80, centromix CPS and capryol 90: polysorbate 80 when combined with myvacet oil is represented in Fig. 1. The shaded area represents the microemulsion region, and an undefined multi-phase region is indicated with no color. The largest area is seen with LBM (48.5%) followed by CPS (45.15%) with CM (20%) showing the least area (Table 1). Adding polysorbate 80 to capmul produced a more hydrophilic surfactant blend accounting for the increase in area from 20 (CM) to 27.6% (CPM). There appears to be a correlation between the HLB of the surfactant and the microemulsion region. Increasing the HLB values (i.e. making the surfactants more hydrophilic) correlated with an increase in the microemulsion area, the exception being LBM (Table 1). We can conclude that the lecithin:butanol surfactant mixture is most efficient at incorporating water compared to the other surfactants screened. Potentially, dilutability of this surfactant:oil mixture could prevent the precipitation of PAC. Microemulsions were developed for oral administration of PAC.

3.1.2. Microemulsion structure and droplet size determination

The structure of the microemulsions was assigned based on the HLB of the surfactants. Microemulsions containing surfactants with HLB values in the range of 3–6 were designated as water in oil (w/o), while those in the 8–18 range were oil in water (o/w) microemulsions. Hence, the LBM and CM systems are w/o and the CP-P80, CPM and CPS systems are o/w microemulsions. The particle size of the dispersed phase in the microemulsions ranged from 687 to 1010 nm for w/o microemulsions and from 272 to 363 nm for o/w microemulsions. In general the droplet sizes for the o/w microemulsions were smaller than that from w/o microemulsions (Table 1).

3.1.3. Diffusion NMR

D of a drug molecule is likely to vary in different media (myvacet oil, o/w and w/o microemulsions). The *D* of a compound is inversely related to its molecular size or the viscosity of the medium. This indicates the degree of molecular mobility, interactions, association, or aggregation. The *D* value of PAC dissolved in myvacet oil was determined as 5.81×10^{-11} m^2/s, which was close to the *D* of pure myvacet oil, (5.61×10^{-11} m^2/s). From these results it appears that PAC is located in the oil phase of the microemulsions rather than at the surfactant–oil interface. The *D* of PAC in CM, LBM and CPM containing 10% of D_2O was 2.24×10^{-11}, 1.97×10^{-11} and 0.51×10^{-11} m^2/s, respectively. The molecular mobility of the

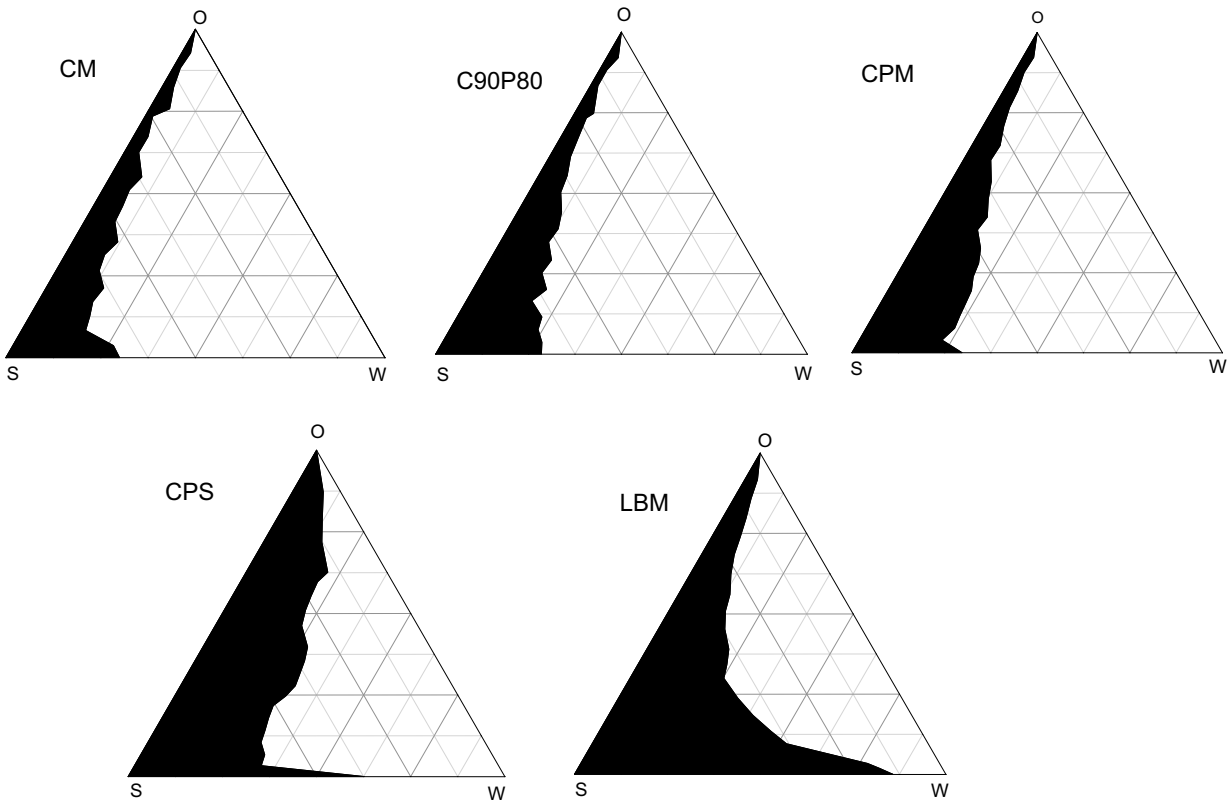

Fig. 1. Ternary and pseudoternary phase diagrams of five microemulsions. Surfactant or surfactant blends combined with oil are as follows – **CM**-capmul MCM: myvacet oil; **CP-P80** – capryol 90: polysorbate 80 (30:70): myvacet oil; **CPM** – capmul MCM: polysorbate 80 (30:70): myvacet oil; **CPS** – centromix CPS: butanol (67:33): myvacet oil; **LBM** – lecithin: butanol (67:33): myvacet oil.

Table 1
The hydrophile–lipophile balance (HLB) of surfactant or surfactant mixture, microemulsion region, structure, droplet size and permeability of PAC in five microemulsions compared to CE

Formulation	HLB	Microemulsion region (%)	Structure (based on HLB)	*z*-Average diameter[a] (nm)	Permeability[a] $\times 10^{-5}$ (cm/s)	
					PAC	PAC + CsA
CE	N/A	N/A	N/A	N/A	1.85 (1.03)	3.39 (1.18)[†]
CM	6	20	w/o	1010	19.7 (2.06)[*]	30.8 (5.76)[†]
LBM	5	48.5	w/o	687	5.71 (0.624)[*]	7.18 (1.08)[†]
CP-P80	8.7	23.9	o/w	325	4.47 (0.67)[*]	5.82 (1.36)[†]
CPM	8.7	27.6	o/w	272	4.23 (1.93)[*]	3 (1.12)
CPS	10.35	45.15	o/w	363	4.13 (1.55)[*]	6.18 (1.03)[†]

[a] Surfactants or surfactant mixtures were combined with myvacet oil at a 70:30 w/w ratio and diluted 1:60 with water.
[*] Significantly different from CE (PAC alone), $p < 0.05$.
[†] Significantly different from PAC alone, $p < 0.05$.

PAC was four times faster in CM and LBM than CPM suggesting that these systems are more likely to have an oil continuous phase and are w/o microemulsions. The molecular mobility of PAC as measured by *D* is affected by the nature of the surfactant and the structure of the microemulsion.

3.2. In situ assessment: single pass perfusion study

In situ single pass perfusion studies were performed with LBM, CM, CP-P80, CPM, and CPS containing PAC compared to CE, in the presence and absence of the pgp/CYP3A4 inhibitor CsA in male CD-IGS rats, demonstrating the effect of formulation on the permeability of PAC through rat small intestine (Fig. 2 and Table 1). PAC permeability was 3- and 11-fold higher from LBM and CM, respectively, than that from CE through the rat small intestine. However, PAC permeability from CPM, CP-P80, and CPS was twice that from CE. A significant increase in PAC permeability was observed in all formulations except CPM in the presence of CsA. The capmul: polysorbate 80 mixture appears to be less effective at increasing the permeability of PAC compared to capmul alone. A more substantial increase in permeability was seen with CM. This enhancement may be attributed to the pgp inhibitory effect of the surfactants, oil and/or the membrane perturbation effect of the surfactants. Both of these effects have been investigated in the literature showing the use of surfactants to increase the intestinal absorption of drugs [7–9,25].

There does not appear to be any correlation between the microemulsion area and permeability of PAC in the microemulsions (Table 1). However, PAC in CM a w/o microemulsion containing the relatively more lipophilic surfactant had the highest permeability (19.7×10^{-5} cm/s) followed by LBM a w/o microemulsion, although the permeability of PAC from this microemulsion is not significantly different from the other microemulsions tested ($4.13–4.47 \times 10^{-5}$ cm/s).

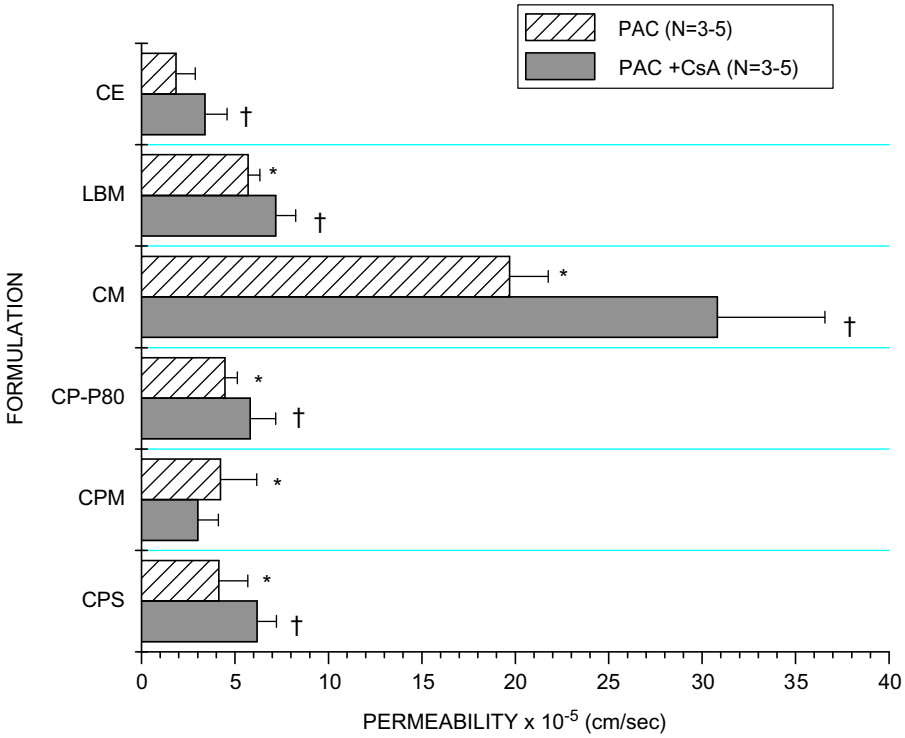

Fig. 2. Paclitaxel permeability from five microemulsions (LBM, CM, CP-P80, CPM and CPS) compared to CE with and without CsA. *significantly different form CE (PAC alone), $p < 0.05$ †significantly different from PAC alone, $p < 0.05$.

Several authors suggest that w/o microemulsions show no obvious correlation between droplet size and the bioavailability of a drug, rather the type of surfactant becomes a more important factor. The o/w microemulsions on the other hand are known to show a relationship between droplet size and bioavailability [25]. In this study, CM containing a lipophilic surfactant, with the largest droplet size exhibited the largest permeability. More such systems will need to be studied to make a conclusion as to the effect of droplet size on permeability.

3.3. In vivo assessment: pharmacokinetic study

The disposition of PAC in the microemulsions compared to CE was studied in male CD-IGS rats after a single oral dose (20 mg/kg). Model dependent parameters were determined for PAC in CM, LBM, CPM and CE and compared statistically. The pharmacokinetics of PAC in CM and LBM was determined because these two systems had the highest permeability values. CPM was included for the purposes of comparing o/w microemulsions to w/o microemulsions. PAC concentrations appeared to decline in a monoexponential manner for all the formulations (Fig. 3) after peak concentration was attained except CPM, which showed an apparent bioexponential decline. The concentration of PAC in CPM rose sharply after administration, followed by a rapid decline from 100 to 300 min. Thereafter, the concentration remained constant from 300 to 480 minutes. The time for maximum concentration of PAC to occur was similar for all three formulations except for LBM which was later.

A one-compartment model with equal first-order input and output, no lag time was used to obtain the pharmacokinetic parameters of PAC in the formulations. This model was selected based on acceptable goodness of fit criteria. There was a good correlation between the observed and predicted concentrations of PAC from the formulations, and the correlation of variation for each parameter was less than 30%.

The mean pharmacokinetic parameters of PAC from the formulations are summarized in Table 2. The area under the curve or the extent of absorption (AUC) of PAC in CM was significantly larger than that of PAC in the other formulations (Table 2). The clearance and volume of distribution normalized by bioavailability (F) of PAC in CM (5.82 L/min/kg) was three times less than that in CE (20.85 L/min/kg) and the other microemulsions, although not significantly different in terms of Vd. The half-life of PAC was comparable for all formulations. Data suggest that PAC in CM was most protected from being cleared and not widely distributed which resulted in a higher systemic exposure.

Table 3 summarizes the trend observed between D of PAC in the microemulsion to permeability across rat intestine and AUC. It appears that as the molecular mobility of PAC increases in the microemulsions, the permeability is increased as a result giving rise to an increase in the AUC in CM compared to CE, LBM and CPM.

4. Summary and conclusion

Microemulsions were developed for PAC which could incorporate significant amounts of water. The lecithin:1-butanol surfactant mixture was most efficient at incorporating water and capmul the least. Extensive water incorporation lends to dilutability, hence potentially preventing precipitation of PAC. The z-average droplet size of w/o microemulsions is smaller than that in o/w microemulsions. CM containing a lipophilic surfactant, with the largest droplet size exhibited the largest permeability than all the other microemulsions tested including CE. This large permeability of PAC from CM may be attributed to the pgp inhibitory effect of the surfactants, oil and/or the membrane perturbation effects of the surfactants. In the pharmacokinetic studies, the AUC of PAC in CM was significantly higher than that in LBM, CPM and CE. This higher systemic exposure of PAC in CM was as a result of a reduced clearance and limited extent of distribution. The enhanced permeability and AUC appears to be related to the

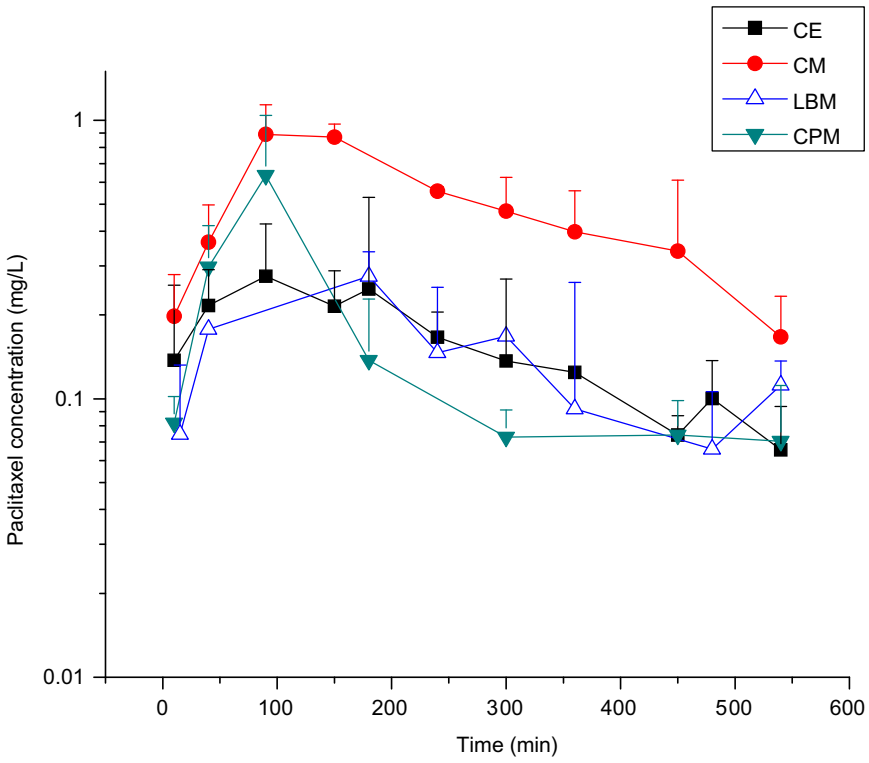

Fig. 3. Mean pharmacokinetic profiles of PAC from CM, LBM and CPM compared to CE after a single 20 mg/kg oral dose in rats. ($N = 5–7$).

Table 2
Comparison of the mean pharmacokinetic parameters of PAC from three microemulsions compared to CE after a single 20 mg/kg oral dose in rats ($N = 5–7$)

Formulation	AUC (mg/L min)	Half-life (min)	CL/F (L/min/kg)	V (L/kg)
CPM	104.37 (38.50)*	79.19 (37.4)	18.73 (8.64)*	2205.76 (1577.47)
LBM	123.11 (36.05)*	95.31 (19.89)	14.51 (5.62)*	1919.1 (438.73)
CM	292.87 (63.17)*	93.46 (15.27)	5.82 (1.5)	763.25 (110.37)
CE	100.92 (34.04)*	81.84 (17.76)	20.85 (8.7)*	2564.53 (1633.12)

* Significantly different from CM at $p < 0.05$.

Table 3
The relationship between diffusion coefficient (D) in the microemulsions to permeability and area-under-the-curve (AUC) of PAC

Formulation	D ($\times 10^{-11}$ m^2/s)	Permeability ($\times 10^{-5}$ cm/s)	AUC (mg/L min)
CPM	0.51	4.23 (1.93)*	104.37 (38.5)
LBM	1.97	5.71 (0.62)*,†	123.11 (36.05)†
CM	2.24	19.7 (2.07)*	292.87 (63.17)*
CE	ND	1.85 (1.03)	100.92 (34.04)

ND, not determined.
* Significantly different from CE at $p < 0.05$.
† Significantly different from CM at $p < 0.05$.

D of PAC, as increased molecular mobility of PAC corresponded to an increased permeability and AUC. Other microemulsions with various structures would have to be evaluated to determine conclusively the relationship between D, permeability and AUC of PAC.

Acknowledgments

This work was supported by the Blythe and Scholarship of Discovery grants, Albany College of Pharmacy, Albany, NY. This work was also supported in part by the Office of Research and Development, Stratton Veterans Administration Medical Center, Albany, NY. We are grateful to Dr. Alexander Shekhtman at SUNY, Albany, NY, for providing NMR instrument time and technical support.

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