Enhanced Oral Absorption of Paclitaxel in a Novel Self-Microemulsifying Drug Delivery System with or without Concomitant Use of P-Glycoprotein Inhibitors

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Received April 10, 2003; accepted October 17, 2003

Purpose. The objective of this study was to evaluate the pharmacokinetics of paclitaxel in a novel self-microemulsifying drug delivery system (SMEDDS) for improved oral administration with or without P-glycoprotein (P-gp) inhibitors.

Methods. Paclitaxel SMEDDS formulation was optimized, in terms of droplet size and lack of drug precipitation following aqueous dilution, using a ternary phase diagram. Physicochemical properties of paclitaxel SMEDDS and its resulting microemulsions were evaluated. The plasma concentrations of paclitaxel were determined using a HPLC method following paclitaxel microemulsion administrations at various doses in rats.

Results. Following 1:10 aqueous dilution of optimal paclitaxel SMEDDS, the droplet size of resulting microemulsions was 2.0 ± 0.4 nm, and the zeta potential was −45.5 ± 0.5 mV. Compared to Taxol, the oral bioavailability of paclitaxel SMEDDS increased by 28.6% to 52.7% at various doses. There was a significant improvement in area under the curve (AUC) and time above therapeutic level (0.1 μM) of paclitaxel SMEDDS as compared to those of Taxol following coadministration of both formulations with 40 mg cyclosporin A (CsA)/kg. The oral absorption of paclitaxel SMEDDS slightly enhanced following coadministration of tacrolimus and etoposide, but plasma drug concentrations did not reach the therapeutic level. The nonlinear pharmacokinetic trend was not modified after paclitaxel was formulated in SMEDDS.

Conclusions. The results indicate that SMEDDS is a promising novel formulation to enhance the oral bioavailability of paclitaxel, especially when coadministered with a suitable P-gp inhibitor, such as CsA.

KEY WORDS: microemulsion; paclitaxel; P-glycoprotein inhibitors; pharmacokinetics; self-microemulsifying drug delivery system (SMEDDS).

INTRODUCTION

Paclitaxel, which disrupts tubulin dynamics, has a significant clinical activity against a broad range of tumor types including breast, lung, head and neck, bladder, and platinum-refractory ovarian carcinoma (1). Paclitaxel has a low therapeutic index and is practically insoluble in water (2). Its plasma concentration above a threshold value of 0.1 μM (equivalent to 85.3 ng/ml) was proven to be pharmacologically active (3). The commercially available product, Taxol, is currently formulated for systemic administration in a mixture of ethanol and polyoxyethylated castor oil (Cremophor EL); with the latter apparently primarily responsible for drug-related hypersensitivity reactions rather than the drug itself (4). Moreover, Cremophor EL contributes to the nonlinear pharmacokinetic behavior of paclitaxel (5).

An attractive approach to overcome the hypersensitivity reactions resulting from systemic administration of Cremophor EL might be the design of oral formulations of paclitaxel (1,6,7), which would offer additional advantages over intravenous dosing, including elimination of the need for frequent visits to the outpatient clinic and easier chronic administration. However, preclinical studies have suggested that paclitaxel was not significantly absorbed after oral administration, and the bioavailability in humans was less than 6% (7,8). Many reasons have been proposed to account for the poor oral bioavailability of paclitaxel. The most likely explanations are its affinity to the membrane-bound drug efflux pump P-glycoprotein (P-gp) and metabolism by cytochrome P450 3A4 (CYP3A4) (9–12), poor water solubility, and hydrophobicity (2).

Numerous studies clearly showed that in both animals and patients, the oral bioavailability of paclitaxel was greatly improved when the drug was administered with P-gp inhibitors, such as cyclosporin A (CsA) or its analogs (7,9,10) or KR30031, a verapamil analog (12). CsA, which inhibits the functions of both P-gp and CYP3A4, has been shown to improve paclitaxel oral bioavailability *in vivo* through enhancing oral absorption and decreasing elimination (13). Oral bioavailability of paclitaxel in mice increased from 9.3% up to 67% with coadministration of CsA (13) and increased 10-fold with SDZ PSC 833, a nonimmunosuppressive CsA analog and P-gp inhibitor (9). Phase II studies in cancer patients of weekly oral paclitaxel in two doses of 90 mg/m^2 on the same day, with 10 mg/kg of CsA given orally 30 min before, showed that oral paclitaxel was safe and active (14,15). Moreover, systemic paclitaxel exposure (i.e., C_{max}, AUC) did not increase as the absolute oral dose of paclitaxel was increased from 180 to 540 mg, suggesting that oral absorption of paclitaxel was a saturable process (10). A similar saturation was noted following coadministration of paclitaxel with P-gp inhibitors (12,15).

Self-emulsifying drug delivery systems (SEDDS) have been developed for enhancing the oral absorption of lipophilic drugs (16,17). SEDDS, which are isotropic mixtures of oils and surfactants, can disperse in the gastrointestinal (GI) lumen to form microemulsions (transparent dispersed systems with oil droplet size of less than 30 nm) or fine opaque emulsions (either submicrometer emulsions with oil droplet size of 50–200 nm or coarse emulsions with oil droplet size larger than 500 nm) upon dilution with water or GI fluids (16). The SEDDS of halofantrine base yielded a 6- to 8-fold improvement in absolute oral bioavailability relative to the solid halofantrine hydrochloride tablet formulation (18). Furthermore, the commercial success of the self-microemulsifying drug delivery system (SMEDDS) (SEDDS that upon

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0724-8741/04/0200-0261/0 © 2004 Plenum Publishing Corporation

aqueous dilution form only microemulsion) formulation Neoral (cyclosporin A) as well as the recent commercialization of novel self-emulsifying formulations, such as Norvir (ritonavir) and Fortovase (saquinavir) have raised the interest in such promising emulsion-based delivery systems to improve the oral bioavailability of lipophilic drugs (19).

Recently, some excipients, such as D-α-tocopheryl polyethylene glycol succinate 1000 (TPGS) and Cremophor, which may be part of SEDDS, can inhibit both presystemic drug metabolism and intestinal efflux mediated by P-gp resulting in an increased oral absorption of cytotoxic drugs (20,21). Deoxycholic acid sodium salt (DOC-Na) can increase membrane fluidity (22) and inhibit P-gp located in the intestine (23). Thus, it is worthy to investigate the synergic potential of SMEDDS composed of these excipients for oral paclitaxel absorption and activity prolongation. The objective of the current study was therefore to evaluate in rats the pharmacokinetics of paclitaxel SMEDDS alone and in combination with P-gp inhibitors to assess the intrinsic effect of the dosage form on the improvement of paclitaxel oral absorption.

MATERIALS AND METHODS

Materials

Paclitaxel was purchased from Farmachem (USP; Lugano, Switzerland). Vitamin E and deoxycholic acid sodium salt (DOC-Na) were purchased from Sigma (St. Louis, MO, USA). D-α-tocopheryl polyethylene glycol succinate 1000 (TPGS) was a gift from Eastman Chemical (Kingsport, TN, USA). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil) and EL were obtained from BASF (Ludwigshafen, Germany). All excipients and solvents were either pharmacopoeial or HPLC grade.

Taxol [each milliliter contains 6.0 mg paclitaxel, 527 mg of purified Cremophor EL, and 49.7% (v/v) dehydrated alcohol] and etoposide [Vepasid; each milliliter contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg Tween 80, 650 mg polyethylene glycol 300, and 30.5% (v/v) alcohol] were purchased from Bristol-Myers-Squibb Company (Princeton, NJ, USA). CsA (Neoral; 100 mg gelatin capsule includes 11.9% v/v dehydrated alcohol and polyoxyl 40 hydrogenated castor oil) was bought from Novartis Corporation (Basel, Switzerland). Tacrolimus (Prograf; each milliliter contains 5 mg tacrolimus, 200 mg polyoxyl 60 hydrogenated castor oil, and 80.0% (v/v) dehydrated alcohol) was purchased from Fujisawa Healthcare, Inc. (Dublin, Ireland).

Methods

Preparation of Paclitaxel SEDDS

A ternary phase diagram was constructed in order to determine the optimum SMEDDS. The blank formulations consisted of 10% w/w DOC-Na, 20% w/w propylene glycol, and varying amounts of vitamin E, TPGS, and Cremophor RH 40. TPGS and Cremophor, which are both semisolid at room temperature, were melted on a 40°C water bath. Vitamin E, DOC-Na, and propylene glycol were then added and mixed until a homogeneous oil phase was obtained. A predetermined quantity of paclitaxel (0.5–2.5% w/w) was dissolved in 40-fold or more absolute ethanol and added to the blank formulation. After completely mixing the drug into the oil formulation, ethanol was evaporated under vacuum (Labconco Corporation, Kansas City, MO, USA) at room temperature. Paclitaxel emulsions were formed following 1:10 dilution of SEDDS with distilled water.

Characterization of Paclitaxel Emulsions

Droplet Size. Emulsions were formed following 1:10 dilution of paclitaxel SEDDS with distilled water, saline, and glucose solutions. The droplet size of the emulsions was determined by the photon correlation spectroscopy (PCS) method using a Coulter Model N4SD type particle sizer (Coulter, Hialeah, FL, USA). The refractive index of each medium was used for measuring the droplet size of resultant emulsions.

Zeta Potential. The zeta potential of the emulsions was measured utilizing a Malvern Zetasizer 3000 (Malvern Instruments, Malvern, UK).

Paclitaxel Precipitation. The possible drug precipitation was followed up following aqueous dilution using an Olympus 201 optical imaging light microscope equipped with a Sony DXC-390P video camera (Tokyo, Japan).

Animal Study

Experiments were performed on male Sprague-Dawley (S.D.) rats weighing 200–250 g which fasted overnight for 12–14 h with free access to water. The experimental procedure was approved by the Committee on Use and Care of Animals at the Hebrew University of Jerusalem. Following 1:10 dilution of optimal SMEDDS containing 0.5, 1.25, or 2.5% w/w paclitaxel with saline (intravenous) or water (oral), paclitaxel microemulsions were intravenously (39 rats/dose) and orally (24 rats/dose) administered at 2.0, 5.0, or 10.0 mg paclitaxel/kg doses. CsA, tacrolimus, and etoposide scaled from usual human doses were orally administered 30 min prior to oral administration of paclitaxel. Blood samples were collected into heparinized tubes at 0.5, 1, 2, 4, 6, 8, 12, and 24 h time points following oral dosing. Additional samples were collected at 1, 5, and 15 min, 1.5 h, and 3.0 h post intravenous dosing. At each time point, three animals were sacrificed. All blood samples were immediately placed on ice upon collection and centrifuged at 4000 rpm for 15 min to obtain the plasma. Aliquots were stored at –20°C until analysis.

Analysis of Paclitaxel

Prior to extraction, 0.05–2.0 ml of rat plasma, which was diluted to a total of 2.0 ml with double-distilled water for intravenous administration or with plasma for oral administration, was mixed with 50 μl docetaxel internal standard (0.3 μg) in methanol. Extraction of paclitaxel was accomplished by adding 4.0 ml of *tert*-butyl methyl ether and vortex-mixing the sample for 1.0 min. The mixture was then centrifuged for 10 min at 4000 rpm, after which 3.0 ml of the organic layer was transferred to a clean tube and evaporated to dryness under vacuum (Labconco Corporation) at 20°C. Approximately 200 μl mobile phase was used to reconstitute the residue, and 80 μl aliquot was injected into the high performance liquid chromatograph (HPLC) equipped with a Hypersil BDS C_{18} (5 μm, 250 × 4.6 mm) analytical column and a Betasil C_{18} guard column. The detection wavelength of paclitaxel was 227 nm.

The mobile phase was acetonitrile–water (48:52) and was pumped at a flow rate of 1.5 ml/min. The analysis was carried out at room temperature (24). The retention time of paclitaxel and docetaxel was 12.4 and 11.0 min, respectively. The detection limit of paclitaxel was 10 ng/ml, and the range of linear response was 25–800 ng/ml ($r^2 > 0.9992$). At the concentrations of 25, 200, and 800 ng/ml, the observed recovery of paclitaxel was 96.8–101.6%, and the intra-day and inter-day assay variations were less than 6%.

Pharmacokinetic Data Analysis

Plasma paclitaxel concentrations obtained from rats at each time point were determined to provide mean concentration and standard deviation (SD). Plasma pharmacokinetic parameters were obtained from the pooled concentration–time data of each experiment with statistical moment algorithm using the WinNonlin (version 1.1; SCI software, Statistical Consulting Inc., Apex, NC, USA). The area under the curve ($AUC_{0\text{-}24}$) and area under the first moment curve ($AUMC_{0\text{-}24}$) from 0 to 24 h were calculated using the linear trapezoidal method. The $AUC_{0\text{-}\infty}$ was calculated by dividing the concentration of 24-h point (C_{24}) by the elimination rate constant (k) as follows:

$$AUC_{0-\infty} = AUC_{0-24} + C_{24}/k$$

The area under the first moment curve (AUMC) was calculated as follows:

$$AUMC_{0-\infty} = AUMC_{0-24} + (T_{24} \cdot C_{24})/k + C_{24}/k$$

The absolute bioavailability in 24 h ($F_{a,\ 0\text{-}24}$) and at infinity ($F_{a,\ 0\text{-}\infty}$) at the same dose was calculated as:

$$F_{a,0-24} = \frac{(AUC_{0-24})_{oral,SMEDDS}}{(AUC_{0-24})_{i.v,SMEDDS}} \times 100$$

$$F_{a,0-\infty} = \frac{(AUC_{0-\infty})_{oral,SMEDDS}}{(AUC_{0-\infty})_{i.v,SMEDDS}} \times 100$$

The relative bioavailability in 24 h ($F_{r,\ 0\text{-}24}$) and at infinity ($F_{r,\ 0\text{-}\infty}$) at the same dose was calculated as:

$$F_{r,0-24} = \frac{(AUC_{0-24})_{oral,SMEDDS}}{(AUC_{0-24})_{oral,Taxol}} \times 100$$

$$F_{r,0-\infty} = \frac{(AUC_{0-\infty})_{oral,SMEDDS}}{(AUC_{0-\infty})_{oral,Taxol}} \times 100$$

The mean residence time in 24 h ($MRT_{0\text{-}24}$) and at infinity ($MRT_{0\text{-}\infty}$) was determined as follows:

$$MRT_{0-24} = AUMC_{0-24}/AUC_{0-24}$$

$$MRT_{0-\infty} = AUMC_{0-\infty}/AUC_{0-\infty}$$

RESULTS

SEDDS Preparation

The ternary diagram depicted in Fig. 1 shows the different types of emulsions obtained following 1:10 aqueous dilution of SEDDS which contained constant concentrations of 1.25% paclitaxel, 10% DOC-Na, and 20% propylene glycol. The formulations located in area B formed microemulsions and/or micellar solutions, but paclitaxel precipitation appeared within 6 h. The formulations located at area C formed submicrometer emulsions or coarse emulsions with droplet size larger than 100 nm, and no paclitaxel precipitation was noted within 6 h. The formulations located in area A formed microemulsions that remained physically stable for at least 6 h with no paclitaxel precipitation. The SEDDS formulation with a combination of vitamin E (28.5% w/w), TPGS (43.0% w/w), and Cremophor RH 40 (28.5% w/w) located at the center of area A was chosen as the optimal SMEDDS formulation.

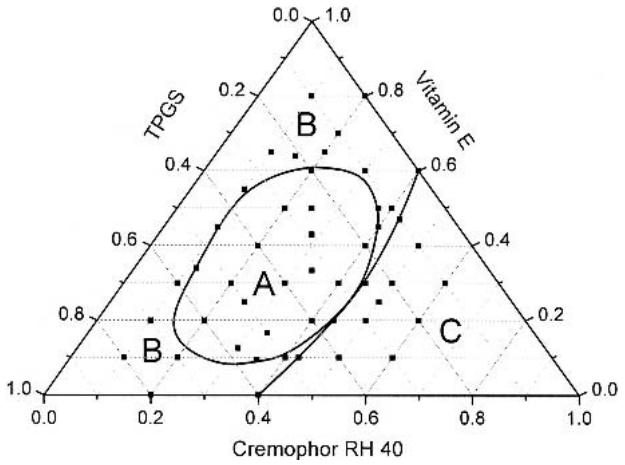

Fig. 1. The ternary diagram of SMEDDS containing 1.25% paclitaxel, 10% DOC-Na, and 20% propylene glycol following 1:10 dilution with distilled water. (A) Microemulsions stable for at least 6 hours with no paclitaxel precipitation. (B) Microemulsions and/or micellar solutions with paclitaxel precipitation within 6 hours. (C) Emulsions or opaque dispersions with droplet size larger than 100 nm whereas no paclitaxel precipitation noted within 6 hours.

Characterization of Paclitaxel Microemulsions

Following 1:10 aqueous dilution of the optimal SMEDDS formulation, the droplet size of the microemulsions was 2.0 ± 0.4 nm for SDP weight results. The resulting microemulsions were negatively charged, and the zeta potential value was −45.5 ± 0.5 mV. The pH values of the microemulsions were around 7.5.

Stability Study

Following 1:10 aqueous dilution, the paclitaxel microemulsions remained physically stable for at least 6 h with no paclitaxel precipitation. Similar results were observed following SMEDDS dilution with simulated gastric and intestinal fluids (USP XXII). The physical stability of paclitaxel in microemulsions decreased with the increase of paclitaxel concentration in the optimal SMEDDS formulations. No precipitate from microemulsions was noted following aqueous dilution of 0.5% w/w paclitaxel SMEDDS for over 2 months.

Pharmacokinetics of Paclitaxel SMEDDS

Paclitaxel plasma concentration data after intravenous administration were analyzed by the noncompartmental analysis. Figures 2A and 2B show the drug logarithmic concentration–time profiles after intravenous administration of Taxol and paclitaxel SMEDDS at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The pharmacokinetic parameters

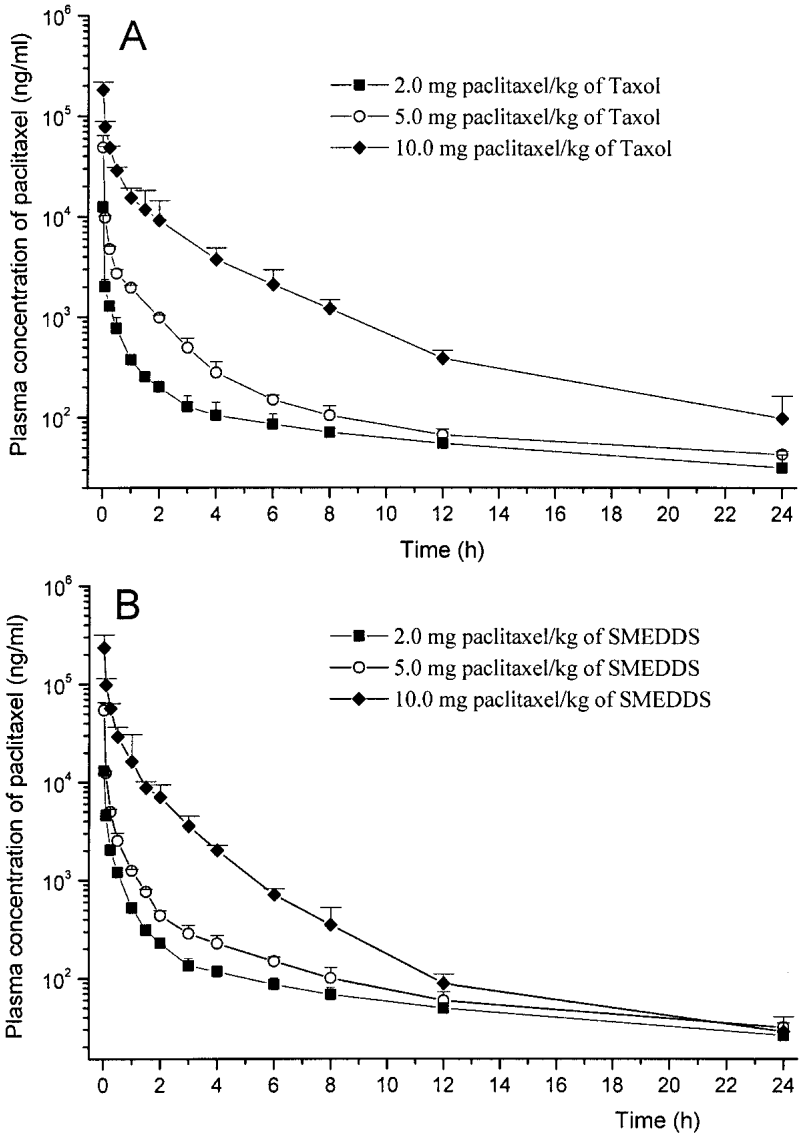

Fig. 2. The drug logarithmic concentration–time profiles after intravenous administration of 2.0, 5.0, and 10.0 mg/kg doses of (A) Taxol and (B) paclitaxel SMEDDS. Data are expressed as mean ± SD (n = 3).

Table I. Pharmacokinetic Parameters of Paclitaxel After Intravenous Administration

	2.0 mg paclitaxel/kg		5.0 mg paclitaxel/kg		10.0 mg paclitaxel/kg	
Parameters	Taxol (0.6% w/v)	SMEDDS (0.5% w/w)	Taxol (0.6% w/v)	SMEDDS (1.25% w/w)	Taxol (0.6% w/v)	SMEDDS (2.5% w/w)
T_{max} (h)	0.0	0.0	0.0	0.0	0.0	0.0
C_{max} (ng/ml)	19742	169670	73125	78531	225610	235210
$t_{1/2\ \beta}$ (h)	14.31	12.28	15.24	11.53	5.74	6.90
AUC_{0-24} (ng · h/ml)	3242.2	3929.9	10584	9605.1	83705	72563
$AUC_{0-\infty}$ (ng · h/ml)	3894	4392	11526	10130	84518	72846
Cl (ml/h · kg)	513.6	455.4	433.8	493.6	118.3	137.3
$AUMC_{0-24}$ (ng · h^2/ml)	14474	13484	24364	19481	183690	87083
$AUMC_{0-\infty}$ (ng · h^2/ml)	43590	32762	67652	40807	209940	96718
MRT_{0-24} (h)	4.5	3.4	2.3	2.0	2.2	1.2
$MRT_{0-\infty}$ (h)	11.2	7.5	5.9	4.0	2.5	1.3
V_{ss} (ml/kg)	5748.2	3396.6	2546.4	1988.3	293.9	182.3

of paclitaxel calculated using a statistical moment method are outlined in Table I.

The clearance (Cl) of paclitaxel in Taxol was 513.6, 433.8, and 118.3 ml/h·kg at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The clearance of paclitaxel in SMEDDS was 455.4, 493.6, and 137.3 ml/h·kg at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The $AUC_{0-\infty}$ of paclitaxel in Taxol was 3894.4 ng·h/ml at the dose of 2.0 mg paclitaxel/kg and increased to 11526 ng·h/ml at the dose of 5.0 mg paclitaxel/kg and 84518 ng·h/ml at the dose of 10.0 mg paclitaxel/kg. The $AUC_{0-\infty}$ of paclitaxel in SMEDDS was 4392.1 ng·h/ml at the dose of 2.0 mg paclitaxel/kg and escalated to 10,130 ng·h/ml and 72,846 ng·h/ml at doses of 5.0 mg paclitaxel/kg and 10.0 mg paclitaxel/kg, respectively. The maximum concentration (C_{max}) and AUC_{0-24} of paclitaxel increased disproportionately with higher doses, and the clearance of paclitaxel decreased with the increase in dose, indicating the nonlinear or saturable pharmacokinetic behavior of the drug in Taxol and SMEDDS. The MRT and steady-state volume of distribution (V_{ss}) decreased with the increase in doses, and the MRT and V_{ss} of paclitaxel SMEDDS were lower compared to those of Taxol.

Paclitaxel plasma concentration–time profiles following oral administration of Taxol and paclitaxel SMEDDS are plotted in Fig. 3. Table II shows the pharmacokinetic parameters calculated using the noncompartmental analysis. The values of C_{max} for all doses of paclitaxel SMEDDS were between 48 and 54 ng/ml, which are higher than the corresponding C_{max} (42–45 ng/ml) of Taxol. The time to maximum concentration (T_{max}) was 6.0 h for paclitaxel SMEDDS; and it was 1 h for Taxol. Compared with Taxol, the $F_{r,\,0\text{-}24}$ values of paclitaxel SMEDDS were 134.0, 132.8, and 120.2% and $F_{r,\,0\text{-}\infty}$ were 128.6, 144.1, and 138.4% at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The $F_{a,\,0\text{-}24}$ values of paclitaxel SMEDDS were 20.5, 9.3, and 1.2% and $F_{a,\,0\text{-}\infty}$ were 35.7, 20.7, and 2.9% at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. For the same dose (10.0 mg paclitaxel/kg) but different concentrations (0.5 and 2.5% w/w) of paclitaxel in SMEDDS,

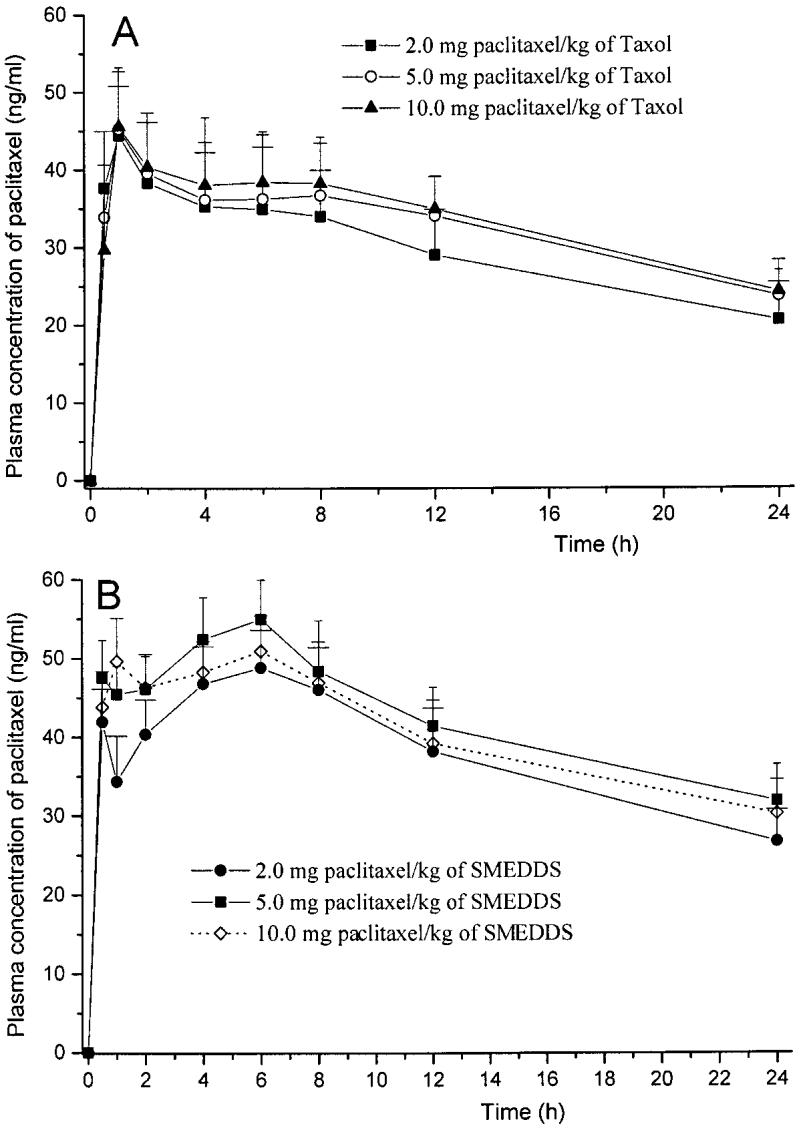

Fig. 3. The plasma paclitaxel concentration–time profiles after oral administration of 2.0, 5.0, and 10.0 mg/kg doses of (A) Taxol and (B) paclitaxel SMEDDS. Data are expressed as mean ± SD (n = 3).

Table II. Pharmacokinetic Parameters of Paclitaxel SMEDDS and Taxol after Oral Administration

Parameters	2.0 mg paclitaxel/kg		5.0 mg paclitaxel/kg		10.0 mg paclitaxel/kg		
	Taxol (0.6% w/v)	SMEDDS (0.5% w/w)	Taxol (0.6% w/v)	SMEDDS (1.25% w/w)	Taxol (0.6% w/v)	SMEDDS (2.5% w/w)	SMEDDS (0.5% w/w)
T_{max} (h)	1.0	6.0	1.0	6.0	1.0	6.0	6.0
C_{max} (ng/ml)	44 ± 6	48 ± 5	45 ± 7	54 ± 5	45 ± 8	50 ± 5	51 ± 8
AUC_{0-24} (ng · h/ml)	601.5	806.0	674.0	895.0	697.0	837.5	970.5
$AUC_{0-\infty}$ (ng · h/ml)	1220.1	1569.5	1453.6	2094.4	1506.6	2084.8	2300.7
$AUMC_{0-24}$ (ng · h^2/ml)	7308.5	9577.5	8296.5	10804	8613.0	10228	10958
$AUMC_{0-\infty}$ (ng · h^2/ml)	41291	50318	53432	85995	55352	92024	97230
MRT_{0-24} (h)	12.2	11.9	12.3	12.1	12.4	12.2	11.3
$MRT_{0-\infty}$ (h)	33.8	32.1	36.8	41.1	36.7	44.1	42.3
$F_{r,\ 0-24}$ (%)	100	134.0	100	132.8	100	120.2	139.2
$F_{r,\ 0-\infty}$ (%)	100	128.6	100	144.1	100	138.4	152.7
$F_{a,\ 0-24}$ (%)		20.5		9.3		1.2	1.3
$F_{a,\ 0-\infty}$ (%)		35.7		20.7		2.9	3.2

the AUC of 0.5% w/w paclitaxel SMEDDS was slightly higher than that of 2.5% w/w paclitaxel SMEDDS.

Figure 4 shows the paclitaxel plasma concentration–time profiles following oral administration of Taxol and paclitaxel SMEDDS with CsA at 40 mg/kg dose. Table III shows the pharmacokinetic parameters calculated using the noncompartmental analysis. The $F_{r,0-24}$ values of paclitaxel SMEDDS were 188.8, 252.0, and 250.5% and the $F_{r,0-\infty}$ were 144.8, 176.7, and 179.2% at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The C_{max} of paclitaxel escalated to 164.0, 225.0, and 239.0 ng/ml and T_{max} was 1.0, 4.0, and 4.0 h at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The C_{max} values of paclitaxel SMEDDS coadministered with CsA, which were much higher than the therapeutic level of paclitaxel (0.1 μM), increased by 3.4, 4.2, and 4.8-fold compared to those of paclitaxel SMEDDS alone at 2.0, 5.0, and 10.0 mg paclitaxel/kg doses, respectively. The $T_{>0.1}$ (time above the threshold of 0.1 μM) lasted about 4.5, 8.0, and 8.1 h and $AUC_{>0.1}$ (AUC above the threshold of 0.1 μM) were 185.5, 590.5, and 730.5 ng·h/ml at 2.0, 5.0, and 10.0 mg paclitaxel/kg, respectively (Table III and Fig. 4). The $T_{>0.1}$ and $AUC_{>0.1}$ of Taxol were 4.6 h and 220.5 ng·h/ml at the dose of 5.0 mg paclitaxel/kg. Following coadministration with 40 mg CsA/kg, the values of AUC_{0-24}, C_{max}, $T_{>0.1}$ and $AUC_{>0.1}$ of paclitaxel in SMEDDS at 5.0 mg/kg dose increased by 49.8, 28.6, 74.0, and 168% compared to the corresponding values of Taxol. The values of MRT_{0-24} and $MRT_{0-\infty}$ decreased significantly following paclitaxel SMEDDS coadministration with CsA compared to the values of paclitaxel SMEDDS or Taxol administered alone at various doses.

When the paclitaxel SMEDDS was coadministered with 20 mg etoposide/kg or with 8.0 mg tacrolimus/kg, paclitaxel plasma levels slightly improved between 0.5 and 4 h compared to that of paclitaxel in SMEDDS alone (Fig. 5). After coadministration with etoposide, the values of $F_{r,0-24}$ and $F_{r,0-\infty}$ of paclitaxel were 139.2 and 131.6%, respectively, close to the values obtained with tacrolimus. The C_{max} of paclitaxel was 84.1 ng/ml, which is near the therapeutic threshold. The C_{max} of paclitaxel increased slightly after coadministration with tacrolimus but did not reach the therapeutic level, and

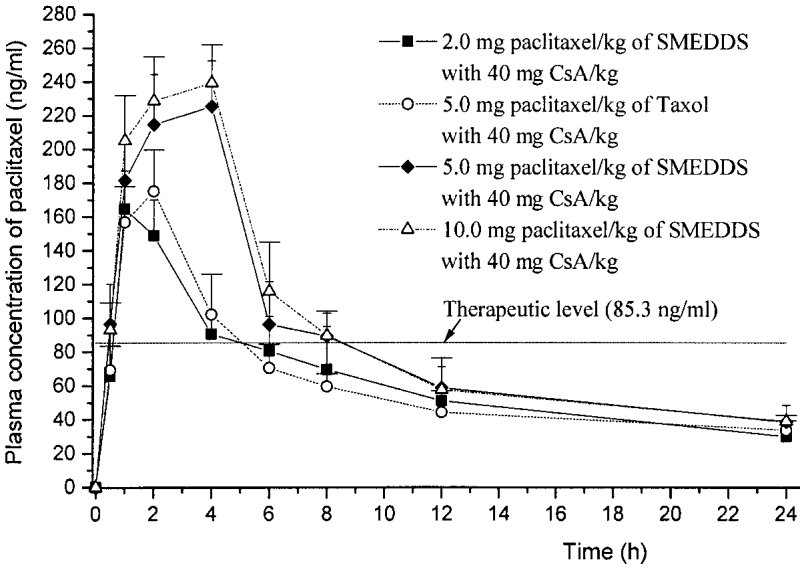

Fig. 4. The plasma paclitaxel concentration–time profiles after oral administration of various doses of different paclitaxel formulations with 40.0 mg CsA/kg. Data are expressed as mean ± SD (n = 3).

Table III. Pharmacokinetic Parameters of Paclitaxel after Oral Administration with P-Glycoprotein Inhibitors

	2.0 mg/kg			5.0 mg/kg			10.0 mg/kg
Parameters	SMEDDS (0.5% w/w) CsA (40.0 mg/kg)	SMEDDS (0.5% w/w) Etoposide (20.0 mg/kg)	SMEDDS (0.5% w/w) Tacrolimus (8.0 mg/kg)	Taxol (0.6% w/v) CsA (40.0 mg/kg)	SMEDDS (1.25% w/w) CsA (40.0 mg/kg	SMEDDS (1.25% w/w) Tacrolimus (8.0 mg/kg)	SMEDDS (2.5% w/w) CsA (40.0 mg/kg)
T_{max} (h)	1.0	1.0	1.0	2.0	4.0	1.0	4.0
C_{max} (ng/ml)	164 ± 23	84 ± 10.2	58 ± 9	175 ± 25	225 ± 27	67 ± 12	239 ± 24
AUC_{0-24} (ng · h/ml)	1135.5	837.5	838.0	1134.0	1698.5	921.0	1746.0
$AUC_{0-\infty}$ (ng · h/ml)	1767.0	1605.6	1675.9	2203.6	2568.2	2119.7	2699.5
$AUMC_{0-24}$ (ng · h^2/ml)	12817	10124	10003	12512	16739	11250	17125
$AUMC_{0-\infty}$ (ng · h^2/ml)	41264	50409	56114	72853	57517	84918	63318
MRT_{0-24} (h)	11.3	12.1	11.9	11.0	9.9	12.2	9.8
$MRT_{0-\infty}$ (h)	23.4	31.4	33.5	33.1	22.4	40.1	23.5
$F_{r,\ 0-24}$ (%)	188.8	139.2	139.3	168.2	252.0	136.6	250.5
$F_{r,\ 0-\infty}$ (%)	144.8	131.6	137.4	151.6	176.7	145.8	179.2
*$T_{>0.1}$ (h)	4.5			4.6	8.0		8.1
†$AUC_{>0.1}$ (ng · h/ml)	185.5			220.5	590.5		730.5

CsA, cyclosporin A.
* $T_{>0.1}$: Time above the threshold of 0.1 μM.
† $AUC_{>0.1}$: AUC above the threshold of 0.1 μM.

the values of $F_{r,0-24}$ and $F_{r,0-\infty}$ increased only by 36.6 to 45.8% at 2.0 and 5.0 mg paclitaxel/kg doses, respectively (Table III).

The pharmacokinetics of paclitaxel followed a nonlinear trend after oral and intravenous administration, and this trend was preserved when the drug was orally coadministered at increasing doses with P-gp inhibitors, such as CsA and tacrolimus (Fig. 6).

DISCUSSION

SMEDDS Preparation

Paclitaxel has a poor solubility in water, and its solubility in lipophilic solvents, such as soybean oil, is also quite low and precludes the use of simple oil-in-water emulsion formulations (6). SEDDS could significantly improve the oral bioavailability of poorly absorbed or lipophilic drugs (16). Some of the SEDDS formulations are more sensitive to composition changes caused by drug addition (16). In all cases, preformulation solubility and phase diagram studies are required in order to design an optimal self-emulsifying drug vehicle. In the current study, based on the preliminary experiments, vitamin E used in the paclitaxel SMEDDS formed the oil phase in the microemulsions resulting from the dilution of SMEDDS in aqueous phase. It was already reported that vitamin E could be considered a good solvent for paclitaxel (25). Cremophor has widely been used as a vehicle for the solubilization of paclitaxel and other hydrophobic drugs (4). TPGS might also improve paclitaxel solubility in SMEDDS, and the self-emulsifying ability of the formulations (25).

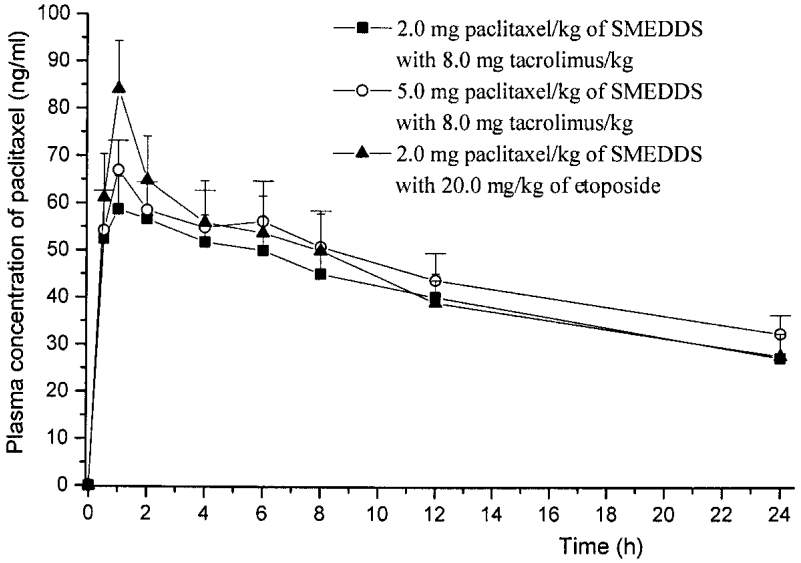

Fig. 5. The plasma paclitaxel concentration–time profiles after oral administration of 2.0 and 5.0 mg paclitaxel/kg SMEDDS with 8.0 mg tacrolimus/kg and 2.0 mg paclitaxel/kg SMEDDS with 20.0 mg etoposide/kg. Data are expressed as mean ± SD (n = 3).

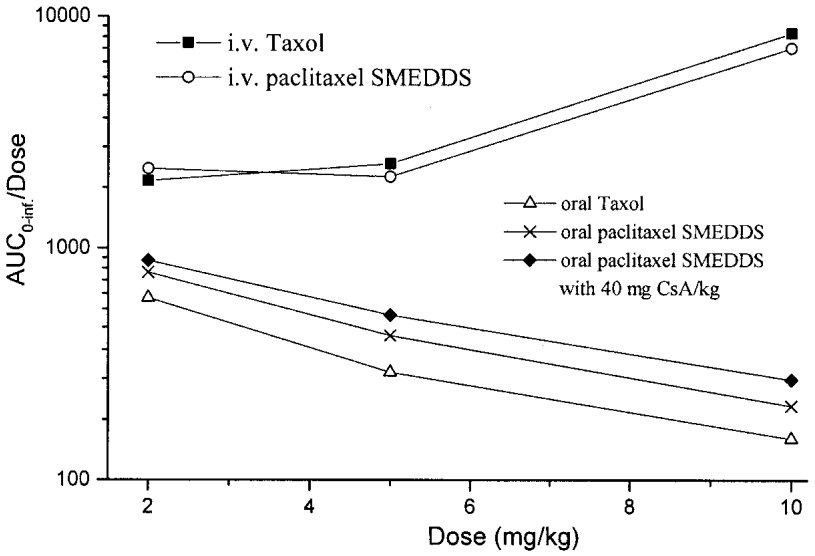

Fig. 6. Relationship between dose-adjusted $AUC_{0-\infty}$ and administered doses for intravenous and oral administrations of paclitaxel SMEDDS and Taxol with and without CsA.

DOC–Na, a natural water-soluble surfactant, was chosen in the formulations to improve solubility and oral absorption of paclitaxel. In addition, DOC–Na can form micelles, greatly increase the solubility of polycyclic compounds (26), and improve the solubilization capacity of oils and lipophilic drugs in the formation of o/w microemulsions (27). Furthermore, TPGS, Cremophor, and DOC–Na might moderately inhibit the P-gp efflux system, leading to improvement of paclitaxel oral absorption (20,21,23).

Pharmacokinetics of Paclitaxel SMEDDS

Following aqueous dilution, SMEDDS formed o/w microemulsions whereas Taxol formed mostly micelles. Cremophor EL could affect the blood distribution of paclitaxel as a result of entrapment of paclitaxel in micelles and protein binding alteration (28). When the dose of Taxol increased from 2.0 to 10.0 mg paclitaxel/kg, the dose of injected Cremophor EL increased 5-fold. However, the dose of the excipients in paclitaxel SMEDDS remained constant when the drug dose increased. The low amount of Cremophor RH 40 in the SMEDDS formulation might be beneficial to the oral absorption of paclitaxel (29).

There are two peaks in the concentration–time profiles after oral administration of paclitaxel SMEDDS (Fig. 3B). The first one between 0.5 and 2 h might be due to the absorption of paclitaxel in the stomach and in the duodenum where the P-gp activity is less pronounced (11). The second peak between 4 and 8 h might be the result of the delayed absorption of paclitaxel in the jejunum and ileum through lymphatic transport, which is known to be mediated by o/w microemulsions especially if they contain vitamin E, TPGS, and DOC-Na (Table II) (17,18,30,31). Bile salts can decrease duodenal and jejunal brush-border membrane vesicle integrity, increase membrane fluidity and passive proton permeability (22), which might increase the absorption of paclitaxel in the gut. These deductions can be supported by reported evidences showing a synergistic antitumor effect of mitomycin C and bile salts against L1210 cells owing to a probable increase in membrane fluidity by bile salts that resulted in an enhanced uptake of mitomycin C by the cells (32). Other possible reasons for the enhanced uptake of paclitaxel from the GI tract might be the solubilization of the drug in the SMEDDS (20,21,26,27) and protection of the drug from chemical as well as enzymatic degradation in the oil droplets.

For the same dose (10.0 mg paclitaxel/kg) but different concentrations (0.5 and 2.5% w/w) of paclitaxel in the SMEDDS, the AUC of paclitaxel SMEDDS with 0.5% w/w paclitaxel was slightly higher than that of SMEDDS with 2.5% w/w paclitaxel. It seems that the increase in excipient concentrations had a moderate effect on improving the absorption of paclitaxel from SMEDDS, which might be due to some inhibition of P-gp efflux pump by TPGS and Cremophor (20,21). However, the possibility that the drug at 2.5% w/w concentration might have precipitated at the gut wall and thus resulted in a decrease in drug oral absorption cannot be excluded.

For a single oral dose, the C_{max} of paclitaxel did not reach the therapeutic level when the drug was administered alone in SMEDDS at different doses despite the presence of DOC-Na, TPGS, and Cremophor in the formulation. The low plasma concentration and poor oral bioavailability of paclitaxel were due not only to the overexpression of P-gp by the intestinal cells, but also to the significant first-pass extraction by cytochrome P450-dependent process (11). Although, it has been reported that multiple dose regimens of moderate doses of the drug would result in higher AUC values and better systemic exposure with a paclitaxel level above 0.1 μM (7,33). It can be deduced from the overall data presented that SMEDDS alone cannot overcome the efflux effect of the P-gp in the case of paclitaxel. Thus, when different doses of paclitaxel SMEDDS were coadministered with 40 mg CsA/kg, there was a substantial increase in the C_{max} and AUC values compared to those obtained with paclitaxel SMEDDS alone (Table III).

The actual results conform with the data reported by various authors and particularly by van Asperen et al. (13) who have administered i.v. and orally both paclitaxel and CsA in mice at doses similar to doses used in the current study.

They clearly showed that the effect of cyclosporine on the increased systemic exposure of orally administered paclitaxel was the result of both a significantly decreased clearance and an increased uptake which enhanced the oral bioavailability of paclitaxel. The C_{max} values were well above the therapeutic level. Even more striking was the fact that at 5 mg paclitaxel/kg dose, the drug was absorbed much more when formulated in SMEDDS than in Taxol following coadministration of both formulations with 40 mg CsA /kg (Fig. 4). There was a significant improvement in the relative bioavailability, $AUC_{>0.1}$ (by 168%), and $T_{>0.1}$ (by 74%) of the drug in SMEDDS as compared to those of Taxol (Table III). SMEDDS might have a delayed positive effect on the P-gp inhibitory effect of CsA either through increasing its oral absorption or enhancing the interaction of CsA with cytochrome P 450 at the level of the mature villus tip enterocytes of the small intestine (11,12), leading to further improvement in paclitaxel oral bioavailability. The lower exposure of paclitaxel in Taxol as compared to SMEDDS coadministered with CsA might be attributed to a reduced absorption of CsA in the presence of large concentrations of Cremophor micelles and/or to a possible precipitation of paclitaxel resulting from the dilution of Taxol in the GI tract (34). The significantly high C_{max} values clearly indicated that the therapeutic level could easily be achieved by a single-dose coadministration of paclitaxel SMEDDS with CsA.

The antineoplastic agent etoposide was reported as a substrate for the P–gp efflux pump (35); thus, the use of this drug could increase the bioavailability of paclitaxel. Because etoposide is also an anticancer drug, coadministration of paclitaxel with etoposide may be beneficial to patients because not only oral absorption of paclitaxel may be improved by etoposide, but also the two anticancer drugs might have synergic effects. Coadministration of paclitaxel SMEDDS with tacrolimus (8 mg/kg), another P-gp inhibitor, and CYP3A4 substrate (11) did not seem to improve greatly the oral bioavailability of the drug (Fig. 5). It only slightly increased the oral absorption of paclitaxel SMEDDS as compared to SMEDDS alone and did not enhance the oral absorption of paclitaxel to a therapeutic level.

The improvement in paclitaxel oral absorption when the drug was coadministered with CsA far more exceeded the effect achieved when the drug was coadministered with tacrolimus and etoposide tested P-gp inhibitors. It is noteworthy to note that not all the well-known P-gp inhibitors do enhance the oral absorption of paclitaxel. For the time being, only CsA and analogs and, most recently, a verapamil analog were shown to enhance markedly the oral bioavailability of paclitaxel (9,10,12,13). In addition to a multidrug efflux pump, phase I metabolism by intestinal cytochrome P450s is now becoming recognized as a significant factor in oral drug bioavailability (12). The results indicated that for an improved paclitaxel oral bioavailability, an efficient P-gp/CYP3A4 comodulation was necessary. Among all the modulators tested, CsA exhibited this comodulation most efficiently. Furthermore, SMEDDS can strengthen this comodulation by diminishing the CYP3A4 metabolism and/or the counter transport of absorbed drug back into the intestinal lumen. It can be deduced from the data depicted in Fig. 6, where the AUC/dose values increase with the i.v. administration and decrease with the oral administration either in the absence or presence of CsA, that apparently the P-gp and first-pass extraction in the liver reach saturation whereas the P-gp and first-pass extraction in the gut do not reach a saturation process at the doses of drug administered. This is also confirmed by other studies performed both in animal and humans where a marked increase in the oral administered dose did not increase the oral absorption of paclitaxel (10,12,15). The poor oral bioavailability of paclitaxel is due to P-gp and first-pass extraction in the gut where no complete saturation is reached. Thus, the oral bioavailability should decrease with the increase of doses of paclitaxel due to progressive saturation of P-gp. This hypothesis is partly supported by the differential oral absorption improvement of paclitaxel when coadministered with the various P-gp inhibitors that elicit different comodulation effects on P-gp and CYP3A4. Furthermore, with regard to the SMEDDS paclitaxel formulations, as the excipients known to moderately inhibit P-gp in SMEDDS proportionally increased with the increased doses, the relative bioavailability significantly increased particularly at the dose of 10 mg paclitaxel/kg as shown in Tables II and III. In addition, other factors such as possible precipitation of paclitaxel in gut at the dose of 10.0 mg paclitaxel/kg as previously stated cannot be excluded and would account for some bioavailability decrease.

CONCLUSIONS

A novel SMEDDS has been developed for the oral delivery of paclitaxel. The formulation contained vitamin E as an oil phase, DOC-Na, TPGS, and Cremophor RH 40 as surfactants to increase the solubility of paclitaxel. The surfactants might moderately inhibit the P-gp efflux system, leading to a slight improvement of paclitaxel oral absorption. The low amount of Cremophor and lymphatic transport of paclitaxel microemulsions in the gut might also be beneficial to the oral absorption of paclitaxel in SMEDDS, but the coadministration of CsA was needed to attain the required therapeutic paclitaxel levels in rats. Following coadministration with CsA, paclitaxel SMEDDS showed a higher bioavailability and much longer time above the therapeutic level than Taxol did. It appears that SMEDDS may be a promising delivery system for the efficient oral administration and enhancement of oral absorption of paclitaxel, especially when incorporated with an effective P-gp inhibitor and CYP3A4, such as CsA.

ACKNOWLEDGMENTS

Dr. Shicheng Yang was a recipient of the Valazzi-Pikowsky Fellowship Fund at the Hebrew University of Jerusalem. Dr. R. Neslihan Gursoy was financially supported through a postdoctoral fellowship sponsored by the Israeli Council for Higher Education.

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