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Enhanced oral bioavailability of paclitaxel by D-α-tocopheryl polyethylene glycol 400 succinate in mice

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ABSTRACT

Paclitaxel is widely used to treat several types of solid tumors. The commercially available paclitaxel formulation contains Cremophor/ethanol as solubilizers. This study evaluated the effects of D-α-tocopheryl polyethylene glycol 400 succinate (TPGS 400) on the oral absorption of paclitaxel in mice. Mice were given an intravenous (18 mg/kg) or oral (100 mg/kg) dose of paclitaxel solubilized in Cremophor/ethanol or in TPGS 400/ethanol formulations. Paclitaxel plasma concentrations and pharmacokinetic parameters were determined. The maximal plasma concentrations of paclitaxel after an oral dose were 1.77 ± 0.17 and 3.39 ± 0.49 μg/ml for Cremophor/ethanol and TPGS 400/ethanol formulations, respectively, with a similar time at 40–47 min to reach the maximal plasma concentrations. The oral bioavailability of paclitaxel in TPGS 400/ethanol (7.8%) was 3-fold higher than that in Cremophor/ethanol (2.5%). On the other hand, the plasma pharmacokinetic profiles of intravenous paclitaxel demonstrated a superimposition for the two formulations. Furthermore, TPGS 400 concentration-dependently increased the intracellular retention of Rhodamine 123 in Caco-2 cells and enhanced paclitaxel permeability in monolayer Caco-2 cultures. TPGS 400 at concentrations up to 1 mM did not inhibit testosterone 6β-hydroxylase, a cytochrome P450 isozyme 3A in liver microsomes metabolizing paclitaxel. Our results indicated that TPGS 400 enhances the oral bioavailability of paclitaxel in mice and the enhancement may result from an increase in intestinal absorption of paclitaxel.

1. Introduction

Paclitaxel is one of the most important anticancer drugs developed in the last two decades, with significant activity against ovarian, head and neck, bladder, breast, and lung cancers (Rowinsky et al., 1993). Paclitaxel is administered to patients via i.v. infusion. Since paclitaxel is practically water insoluble at a very low aqueous solubility of less than 0.77 μM (Wenk et al., 1996), it is solubilized in a mixture of Cremophor EL (polyoxyethylated castor oil) and ethanol (1:1, w/w), which comprises a commercially available formulation for Taxol® (Adams et al., 1993; Singla et al., 2002). However, the use of Cremophor EL is associated with hypersensitivity reactions (Weiss et al., 1990) and caused leakage of plasticizers from polyvinyl chloride (PVC) infusion bags and polyethylene tubing (Goldspiel, 1995). Efforts searching for an optimal paclitaxel formulation with greater antitumor activity and reduced adverse effects have been significantly active. Formulation for oral administration of paclitaxel would offer advantages over the i.v. infusion, such as better patient compliance and void of adverse effects caused by Cremophor EL (Theis et al., 1995). However, several reports indicated that low oral absorption of paclitaxel is mainly due to its low solubility as well as the efflux pump function for which the drug molecules are pumped out by the multidrug efflux transporter P-glycoprotein (P-gp) in the intestinal lumen (Bardelmeijer et al., 2000, 2004; Sparreboom et al., 1997). These findings have recruited investigational efforts toward the development of an oral formulation without Cremophor EL for paclitaxel (Choi and Jo, 2004), synthesis of oral absorbable paclitaxel analogs (Lavelle, 2002), and oral dosing regimens with the addition of a P-gp inhibitor (van Asperen et al., 1997).

P-gp is primarily expressed in the luminal surface of epithelial cells from several tissues such as small intestines, liver, kidneys, and the endothelial cells comprising the blood–brain barriers (Thiebaut et al., 1987). In the small intestines, P-gp is co-localized with cytochrome P-450 at the apical membrane of the cells (Johnson et al., 2002). A protective function of P-gp against exogenous compounds in the blood–brain barrier, and a lower body clearance

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and reduced fecal excretion of vinblastine and paclitaxel were reported (van Asperen et al., 1996). Wacher et al. showed that P-gp may limit oral drug bioavailability in the gut by controlling drug transport from the intestinal lumen and by affecting access to cytochrome P-450 isozyme 3A (CYP3A) (Wacher et al., 1998). P-gp inhibitors KR30031, MS-029, SDZ PSC 833, and GF120918 enhanced the oral absorption of paclitaxel in animals (Bardelmeijer et al., 2004; Kimura et al., 2002; Woo et al., 2003; Varma and Panchagnula, 2005). It has also been shown that the absorption of orally administered paclitaxel was increased by a co-administration of cyclosporine A by competing both P-gp and CYP3A4 in patients (Meerum Terwogt et al., 1999). These observations suggest an important role of P-gp in the absorption and disposition of xenobiotics, including drug molecules, in animals and humans.

D-α-Tocopheryl polyethylene glycol 400 succinate (TPGS 400) comprised of a hydrophilic portion and a lipophilic portion is a water-miscible form of vitamin E derivative prepared by the esterification of the acid group of D-α-tocopheryl acid succinate with polyethylene glycol (PEG) 400. TPGS 400 is a low molecular weight liquid analog of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS 1000), a United States Pharmacopeia-National Formulary standardized pharmaceutical excipient for formulation as an emulsifier or a solubilizer. It was reported that TPGS 1000 interacted with P-gp in the intestines and thus increased the oral absorption of cyclosporine A (Dintaman and Silverman, 1999). With a similar function to that of Cremophor EL and TPGS 1000, TPGS 400 is a new liquid solubilizer under investigation. In the present study, we examined the effects of TPGS 400 on the permeability of a P-gp substrate paclitaxel through the monolayer Caco-2 cells and the oral pharmacokinetics of paclitaxel in mice.

2. Materials and methods

2.1. Materials

Caco-2 cells were purchased from The Bioresources Collection and Research Center of Food Industry Research and Development Institute (Hsinchu, Taiwan). Culture media and supplements were from Invitrogen Taiwan, Ltd. (Taipei, Taiwan). TPGS 400 and TPGS 1000 were gifts from Eastman Chemical Co. (Kingsport, TN, USA). 4-Hydroxybenzoic acid *N*-hydroxyl ester for paclitaxel analysis was purchased from TCI (Tokyo, Japan). Acetonitrile and methanol of HPLC grades were purchased from J.T. Baker (Philipsburg, NJ, USA) and Echo Chemical Co. (Brookfield, CT, USA). Mouse and human liver microsomes, and azamulin were purchased from BD Gentest (Woburn, MA, USA). Ethanol was purchased from Merck (Germany). Paclitaxel, baccatin III, Cremophor EL, testosterone, propanolol, and NADPH were purchased from Sigma (St. Louis, MO, USA). 6β-Hydroxy testosterone was purchased from Ultrafine Chemicals (Manchester, UK). All other chemicals and reagents were of analytical grade and were obtained commercially.

2.2. Measurement of Rhodamine 123 efflux by Caco-2 cells

Caco-2 cells were seeded and cultured at a density of 1×10^4 cells/well in 96-well black plates with clear bottom (Cat#3603, 4.71 cm^2/well) purchased from Costar (Corning, NY, USA) with DMEM for 21–24 days until the cell population reached 90% confluence. Cells were exposed to 13 μM Rhodamine 123 from Sigma (St. Louis, MO, USA) in the presence of TPGS 400 at a concentration range of 0.00001–0.1% for 2, 4, and 6 h, respectively, followed by rinses of the cells with phosphate buffered saline (PBS) four times. To ensure solubilization of the TPGS 400, 1% TPGS 400 in ethanol was prepared freshly for each experiment and serially diluted further in the culture media to the indicated concentrations. The fluorescence intensity was measured using a FluoroCount™ Plate Reader from Packard (Ramsey, Minnesota, USA) with the wavelengths of excitation at 485 nm and emission at 530 nm. This assay is based on the reduction of fluorescence in viable Caco-2 cells. The data presented are the mean ± S.D. of at least three independent experiments, each performed in triplicates.

2.3. Visualization of Rhodamine 123 retention in Caco-2 cells

Caco-2 cells were cultured on the same type of 96-well microplates used for the Rhodamine 123 uptake study described above. After tight junctions were formed among the monolayer cells, the cells were exposed to 13 μM Rhodamine 123 in the presence of TPGS 400 at a concentration range of 0.00001–0.1% for 2, 4 or 6 h followed by rinses with PBS four times. Fluorescent (Ex: 488 nm; Em: 530 nm) and bright-field images were taken using an inverted microscope DMIL system purchased from Leica (Wetzlar, Germany).

2.4. Permeability assay in Caco-2 cultures

The permeability of paclitaxel was evaluated using the previously reported methods with modifications (Dintaman and Silverman, 1999). Caco-2 cells were cultured in the growth medium: DMEM medium containing 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 40 μg/ml gentamicin, and 1× MEM vitamin solution. The Caco-2 cells were harvested and seeded on the upper chamber (apical side) of 6-well plate of Transwell™ from Corning (Corning, NY, USA) at a density of 3×10^5 per well. The growth medium was exchanged with fresh medium every other day. The transmonolayer electrical resistance caused by the confluent Caco-2 cells of each well was measured by a Millicell-ERS voltohmmeter from Millipore (Billerica, MA, USA). The Caco-2 permeability assays were performed with a Millicell-ERS reading >2300 Ω cm^2. The upper chamber (apical side) was filled with 1 ml of the growth medium containing 20 μM of paclitaxel in the absence or presence of various concentrations of TPGS 400. The lower chamber (basal side) was filled with 2 ml of the growth medium followed by incubation at 37 °C with 5% CO_2/95% air. Growth media of 200 μl each in the lower chamber were then collected at 1, 2, 3, and 4 h after the paclitaxel application, and stored at −80 °C until LC/MS analysis.

2.5. Animals and paclitaxel treatments

Male B ALB/c mice of 6–8 weeks old were purchased from The National Laboratory Animal Center (Taipei, Taiwan). The use and care of the animals were approved by the Institutional Animal Care and Use Committee of The National Health Research Institutes. Mice of 23 ± 3 g body weight were given with paclitaxel by either oral gavage at 100 mg/kg or intravenous bolus injection at 18 mg/kg from tail vein in two different vehicle formulations: Cremophor EL/ethanol (1:1, v/v) and TPGS 400/ethanol (1:1, v/v). After the administration of paclitaxel, blood samples were collected via cardiac puncture at 10 min, 0.5, 1, 2, 4, 8, 16 and 24 h from those dosed orally (n = 6 for each time point), and 2, 5, 15 min, 1, 4, 8, and 24 h from those intravenously administered (n = 3 for each time point) in potassium EDTA-containing centrifuge tubes. Plasma samples were obtained after centrifugation (1000 × g for 15 min) and stored at −20 °C until analysis.

2.6. Chemical analysis of paclitaxel in plasma and in culture medium

The analytical conditions utilized were adopted from the previously described with minor modifications for samples from plasma (Lee et al., 1999) and from the culture medium (Dintaman and Silverman, 1999) Briefly, 150 μl of plasma sample was mixed with 150 μl of internal standard solution (10 μg/ml of 4-hydroxybenzoic acid *N*-hydroxyl ester in acetonitrile), vortexed for 30 s, and centrifuged at 15,000 × *g* for 20 min. The supernatant of 150 μl was transferred into HPLC injection vials. Fifty microliter was injected onto a C_{18} guard column (13 mm, particle size 5 μm, Upchurch Scientific, USA) connected with a Zorbax 300SB-C_{18} Column (2.1 mm × 150 mm, particle size 5 μm, Agilent Technologies, USA) using an Agilent 1100 LC/DAD system. The detection wavelength was set at 230 nm and the limit of detection was 5 ng/ml. Data were acquired and processed with HP Chemstation chromatography manager software from Hewlett-Packard (Santa Clara, CA, USA). The isocratic mobile phase was composed of acetonitrile with 0.1% phosphoric acid:deionized water (55:45, v/v) and delivered at a flow rate of 1.3 ml/min. The retention times for paclitaxel and the internal standard were 3.4 and 4.9 min, respectively.

The collected growth medium of 50 μl each was spiked with an equal volume of baccatin III as internal standard dissolved in acetonitrile and the mixture was then centrifuged at 20,000 × *g* for 20 min. The supernatant of 50 μl was analyzed by LC/MS (Agilent 1100 LC/MSD series) with a Zorbax Eclipse XDB-C_{18} column. The mobile phase consisted of a gradient mixture of acetonitrile and 10 mM ammonium acetate in 0.1% formic acid. The flow rate was 0.5 ml/min. The retention times were 3.8 and 4.1 min, and monitored *m*/*z* were 587 and 854 for baccatin III and paclitaxel, respectively.

2.7. Pharmacokinetic analysis

Plasma concentration–time data from the two dosing regimens, 100 mg/kg oral gavage and 18 mg/kg intravenous bolus, were analyzed by both non-compartmental analysis to obtain pharmacokinetic parameters, $AUC_{(0-\infty)}$: area under concentration–time curve from 0 to infinity; Cmax: peak concentration; Tmax: time to reach peak concentration; CL: total body clearance; $T_{1/2}$: terminal half-life; were obtained using WinNonLin (Pharsight Corp., Mountain View, CA, USA). The absolute bioavailability (*F*%) of paclitaxel after the oral administration compared to the intravenous bolus administration was calculated as $(AUC_{oral}/AUC_{i.v.}) \times (Dose_{i.v.}/Dose_{oral}) \times 100$. Analysis of variance (ANOVA) was used to determine a significant difference between experimental groups.

2.8. Measurement of cytochrome P450 isozyme 3A (CYP3A) activity

Similar to those previously reported (Yao et al., 2006), experimental procedures were adopted with modifications for the present study. The CYP3 A-catalyzed oxidation was allowed to carry out for 10 min in a volume of 0.2 ml reaction mixture containing 1 mM NADPH, 3 mM $MgCl_2$, and 79 mM potassium phosphate buffered at pH 7.4, in the presence of a probe substrate for CYP3A, testosterone at 60 μM and a testing sample, TPGS 400, TPGS 1000, or Cremophor EL in the human or mouse liver microsomal homogenate of 0.2 mg protein/ml at 37 °C with constant shaking in a 96-well plate on a temperature-controlled heating block. After adding 1 μl of the testing sample solution to the reaction mixture without NADPH, the plate was pre-incubated at 37 °C for 5 min followed by initiation of the reaction by an addition of 20 μl of 10 mM NADPH in phosphate buffer. Incubation with ethanol alone was carried out as negative control. The final concentration of vehicle ethanol used to dissolve testosterone and reference inhibitor azamulin in the incubation mixture was 1% or less. All CYP3A-catalyzed enzymatic reactions were conducted in duplicates. Azamulin was preincubated with liver microsomes and NADPH for 10 min prior to adding the probe substrate to initiate the reaction for its mechanistic basis (Stresser et al., 2004). At the end of the incubation, 80 μl of ice-cold acetonitrile containing internal standard (propanolol) was added into each incubation well to terminate the reaction. The reaction mixtures were then vortexed and centrifuged at 20,800 × *g* for 20 min at room temperature. Supernatants were collected for further detection of the reaction product 6β-hydroxy testosterone using a LC/MS system consisted of Agilent 1100 Series LC System and Series mass spectrometer (Palo Alto, CA, USA). Zorbax Eclipse XDB-C8 (5 μm, 150 × 3.0 mm i.d., Agilent) was used and the column temperature was set at 25 °C. Mobile phase consisted of solvent A (10 mM ammonium acetate containing 0.1% formic acid) and solvent B (acetonitrile) in a gradient system: 30% B to 90% B (0–2 min), 90% B to 98% B (2–6 min), 98% B to 30% B (6–8 min), and 30% B (8–10 min). The flow rate was 0.5 ml/min. The retention times for 6β-hydroxy testosterone and propanolol were 4.4 and 3.4 min, respectively. Data acquisition was via selected ion monitoring (SIM) mode. The relative testosterone 6β-hydroxylase activity was calculated by dividing the peak area ratio for 6β-hydroxy testosterone from reaction wells containing the testing agent with that from wells containing vehicle ethanol alone. The concentrations (IC_{50}) inhibiting 50% testosterone 6β-hydroxylase activity were estimated using SigmaPlot® from SPSS Inc. (Chicago, IL, USA).

Table 1
Concentration-dependent increases of Rhodamine 123 retention in Caco-2 cells by TPGS 400[a]

TPGS 400 (%)	Time (h)		
Vehicle control	2	4	6
0	7.7 ± 1.8[b]	8.8 ± 0.2	8.4 ± 1.1
0.00001	8.0 ± 2.1	9.0 ± 1.9	8.40 ± 0.1
0.0001	9.0 ± 1.1	9.8 ± 1.8	8.6 ± 1.3
0.001	9.4 ± 1.1	10.3 ± 1.7	9.2 ± 0.4
0.01	10.4 ± 2.5	12.7 ± 1.6*	10.1 ± 0.1
0.1	11.5 ± 2.3	15.6 ± 4.5	13.9 ± 5.2

* $p < 0.05$ vs. vehicle control without TPGS 400.
[a] Caco-2 cells were treated with TPGS 400 of various concentrations in the presence of 13 μM Rhodamine 123.
[b] Values expressed in mean ± S.D. are folds of increase in fluorescence intensity compared to that at time zero.

3. Results

3.1. Inhibition of the efflux of Rhodamine 123 in Caco-2 monolayer cells by TPGS 400

Rhodamine 123, a P-gp substrate, has been used to probe the active transport activity of the membrane-bound P-gp (Lee et al., 1994). Fig. 1 shows that the TPGS 400 inhibited Rhodamine 123 efflux from Caco-2 cells and increased its retention inside the Caco-2 cells as visualized by fluorescence microscopy. The amount of Rhodamine 123 trapped inside the Caco-2 cells increased with the increment of TPGS 400 concentration. TPGS 400 treatments for 4 h appeared to keep more Rhodamine 123 inside the Caco-2 cells. The retention of Rhodamine 123 is time-dependent and concentration dependent by TPGS 400 treatments. The fluorescent intensity of Rhodamine 123 inside the Caco-2 cells treated with TPGS 400 of various concentrations were quantified and summarized in Table 1.

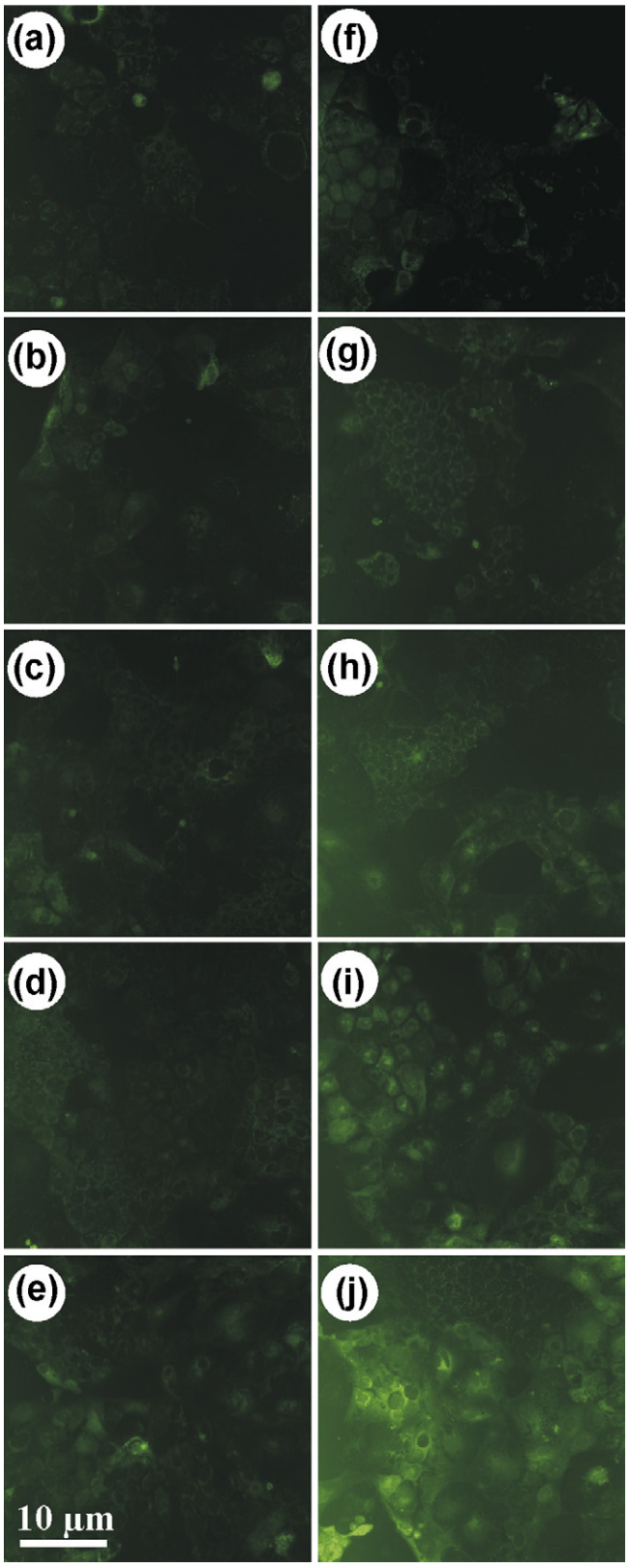

Fig. 1. Concentration- and exposure time-dependent retention of Rhodamine 123 by TPGS 400 treatments in Caco-2 cells. Caco-2 cells were grown on Transwell™ plates and Rhodamine 123 of 13 μM was applied to the apical side in the absence or presence of TPGS 400 of various concentrations for 2 and 4 h. The images were taken after a 2-h treatment with Rhodamine 123 alone (a), and TPGS 400 at 0.00001% (b), 0.0001% (c), 0.001% (d), and 0.1% (e), respectively; and 4-h treatment with Rhodamine 123 alone (f), and TPGS 400 at 0.00001% (g), 0.0001% (h), 0.001% (i), and 0.1% (j), respectively.

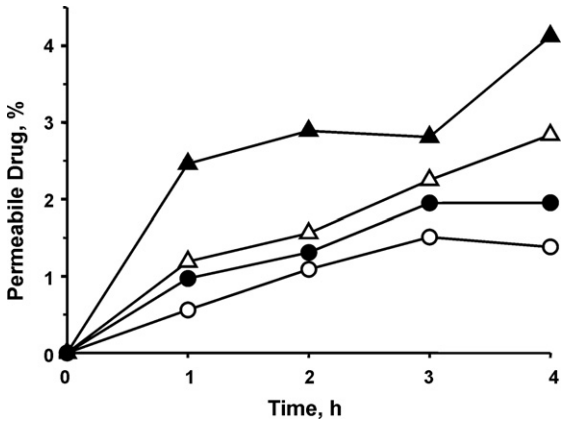

Fig. 2. Enhanced permeability of paclitaxel through Caco-2 monolayer cultures by TPGS 400. The percentage of paclitaxel (20 μM) permeable through the Caco-2 cell monolayer was measured in the absence or presence of TPGS 400 of various concentrations at paclitaxel alone (○); paclitaxel + 0.0001% TPGS 400 (●); paclitaxel + 0.001% TPGS 400 (△); paclitaxel + 0.01% TPGS 400 (▲); mean ± S.D., $n = 3$ per time point.

A trend of time- and concentration-dependency was observed in both qualitative and quantitative assays. Our results showed that the inhibition of P-gp activity by TPGS 400 indicated a blockade in the permeability from the basal side to apical side. The results further suggested that TPGS 400 interferes P-gp mediated transmembrane transport.

3.2. Effects of TPGS 400 on paclitaxel permeability in Caco-2 monolayer cultures

The percentage of paclitaxel permeable through the monolayer Caco-2 cells is increased with the increase of TPGS 400 concentrations as shown in Fig. 2. The penetration of paclitaxel through the Caco-2 monolayer from the upper chamber (apical side) to the lower chamber (basal side) was facilitated by TPGS 400 in a concentration-dependent manner. For example, the average percentage of the paclitaxel molecules penetrated after 4 h in incubation in the presence of TPGS 400 at 0%, 0.0001%, 0.001% and 0.01% were 1.33, 1.95, 2.84 and 4.13%, respectively. The results indicated that TPGS 400 affects the trans-monolayer transport of paclitaxel in Caco-2 culture system.

3.3. Pharmacokinetics of orally administered paclitaxel formulations

Plasma concentration profiles of paclitaxel after a single oral administration of paclitaxel at 100 mg/kg formulated in Cremophor EL/ethanol or in TPGS 400/ethanol were shown in Fig. 3. The plasma concentrations of paclitaxel in TPGS 400/ethanol were significantly ($p < 0.05$) higher than those in Cremophor EL/ethanol at any given measured time point except the first sampling time points at 10-min. The pharmacokinetic parameters of paclitaxel formulated in the two formulation vehicles: Cremophor EL/ethanol and TPGS 400/ethanol as shown in Table 2. The AUC of paclitaxel in TPGS 400/ethanol was 3.1-fold higher than that in Cremophor EL/ethanol. Accordingly, the total body clearance of paclitaxel in Cremophor EL/ethanol was 3.3-fold higher than that of TPGS 400/ethanol. The peak plasma concentration (Cmax) of paclitaxel in TPGS 400/ethanol and in Cremophor EL/ethanol was 3.39 ± 0.50 and 1.79 ± 0.17 μg/ml, respectively, with a similar Tmax, time to reach Cmax. The Cmax of paclitaxel in TPGS 400/ethanol was significantly higher than that in Cremophor EL/ethanol and a significant longer half-life for the TPGS 400/ethanol group was also observed

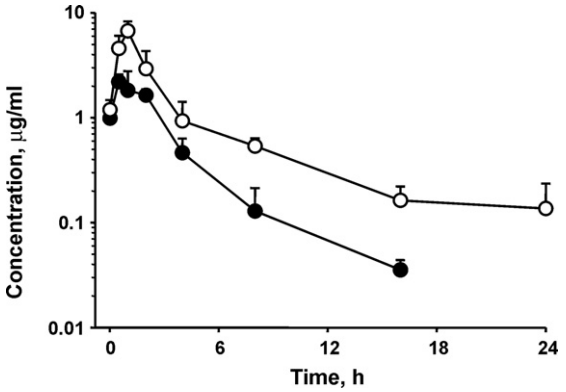

Fig. 3. Pharmacokinetics of paclitaxel after an oral administration to mice. Mice were given a dose of 100 mg/kg paclitaxel in the ethanol formulation with Cremophor EL (●) or TPGS 400 (○), mean ± S.D., $n = 6$ per time point.

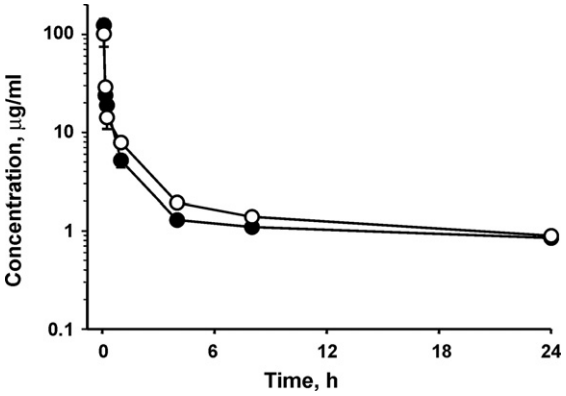

Fig. 4. Pharmacokinetics of paclitaxel after an intravenous administration to mice. Mice were given an intravenous dose of 18 mg/kg paclitaxel in the ethanol formulation with Cremophor EL (●) or TPGS 400 (○), mean ± S.D., $n = 3$ per time point. A superimposition between the two plasma concentration–time curves was observed.

($p < 0.05$). The calculated absolute bioavailability of paclitaxel delivered in TPGS 400/ethanol and Cremophor EL/ethanol formulations were 7.8% and 2.5%, respectively.

3.4. Pharmacokinetics of intravenously administered paclitaxel formulations

The plasma concentration profiles of paclitaxel after a single intravenous bolus administration of paclitaxel at 18 mg/kg formulated in Cremophor EL/ethanol or in TPGS 400/ethanol were shown in Fig. 4. The plasma concentrations of paclitaxel of the TPGS 400/ethanol formulation were not significantly different from that of the Cremophor EL/ethanol formulation ($p = 0.32$). The pharmacokinetic parameters of paclitaxel formulated in the two vehicles: Cremophor EL/ethanol and TPGS 400/ethanol were shown in Table 2. The AUCs of paclitaxel for the two TPGS 400/ethanol and Cremophor EL/ethanol groups were 3572 ± 199 and 3539 ± 103 min μg/ml, respectively, and were not different from each other ($p = 0.45$). The two plasma concentration cures of obtained from the two vehicles were super-imposable to each other.

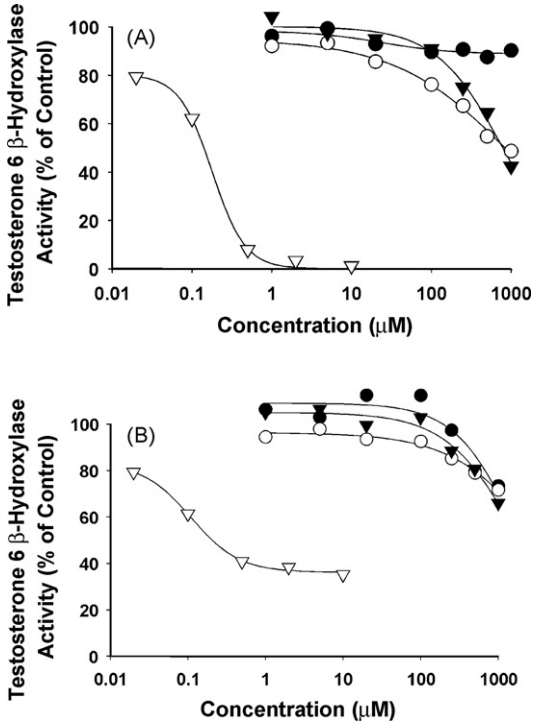

Fig. 5. Effects of TPGS 1000, TPGS 400 and Cremophor EL on the activities of testosterone 6β-hydroxylase in (A) human and (B) mouse liver microsomes. Microsomes were incubated with a range of concentrations of TPGS 400 (●), TPGS 1000 (○), Cremophor EL (▼), and azamulin (▽). Enzymatic activities of the corresponding incubation concentrations were expressed with means in μM from two separate experiments in duplicates.

3.5. Effects of TPGS analogs and Cremophor EL on CYP3A activity

Inhibitions of testosterone 6β-hydroxylase activity in mouse and human liver microsomes by TPGS 400, TPGS 1000 and Cremophor EL were observed in relatively high concentrations as shown in Fig. 5. The results indicated that the three testing

Table 2
Pharmacokinetic parameters of formulated paclitaxel after an intravenous or oral dose in mice

Pharmacokinetic parameters	Oral administration (100 mg/kg)		Intravenous administration (18 mg/kg)	
	Cremophor EL	TPGS 400	Cremophor EL	TPGS 400
$AUC_{(0-\infty)}$ (min μg/ml)	499 ± 42	1556 ± 188*	3539 ± 103	3572 ± 199
Tmax (min)	40 ± 9	47 ± 18	–	–
Cmax (μg/ml)	1.79 ± 0.17	3.39 ± 0.50*	–	–
CL (min/(ml kg))	0.20 ± 0.02	0.06 ± 0.01*	5.4 ± 0.2	5.3 ± 0.3
$T_{1/2}$ (min)	163 ± 10	283 ± 29*	1150 ± 679	866 ± 294
F (%)	2.5	7.8	–	–

Mice were orally gavaged with a dose of 100 mg/kg paclitaxel in the ethanol formulation containing either Cremophor EL or TPGS 400 (mean ± S.D., $n = 6$ per time point for each formulation group). Another group of mice were intravenously administered with a dose of 18 mg/kg paclitaxel in the ethanol formulation containing either Cremophor EL or TPGS 400 (mean ± S.D., $n = 3$ per time point for each formulation group). $AUC_{(0-\infty)}$, area under concentration–time curve from 0 to infinity; Cmax, peak concentration; Tmax, time to reach peak concentration; CL, total body clearance; $T_{1/2}$, terminal half-life; F, absolute oral bioavailability.

* $p < 0.05$, significant difference between two formulations.

Table 3
Effects of TPGS 400, TPGS 1000 and Cremophor EL on testosterone 6β-hydroxylase in mouse and human liver microsomes

	Human	Mouse
TPGS 400	>1000	>1000
TPGS 1000	823[a]	>1000
Cremophor EL	788	>1000
Azamulin	0.14	0.45

[a] IC_{50} values in μM were expressed as the average of two separate experiments with duplicates.

agents have little or no effect (IC_{50} > 1000 μM) on the activity of CYP3A-catalyzed testosterone 6β-hydroxylation in mouse liver microsomes as shown in Table 3. Similarly, TPGS 400, TPGS 1000, and Cremophor EL are also weak inhibitors with an IC_{50} of >1000, 823, and 788 μM, respectively, for the CYP3A4-catalyzed testosterone 6β-hydroxylation in human liver microsomes. The IC_{50}s of these agents were much higher than that of the reference inhibitor azamulin with an IC_{50} of 0.45 and 0.14 μM in mouse and human liver microsomes, respectively.

4. Discussion

Caco-2 monolayer cell culture system has been widely used as an in vitro model for evaluation of drug absorption across the intestinal tract (Nerurkar et al., 1997). We have demonstrated that TPGS 400 interferes with P-gp activity as visualized by the retention of Rhodamine 123 in Caco-2 cells. Our data showed that TPGS 400 enhanced the paclitaxel permeability in a concentration-dependent manner in Caco-2 monolayer cultures. The increased paclitaxel permeability might be resulted from altering the solubility of paclitaxel. However, the TPGS 400 concentrations used in the present in vitro studies were below its critical micelle concentration (0.28%), suggesting that TPGS 400 at these low concentrations shall not show a significant effect on the solubility of paclitaxel based on the classical micelle solubilization theory (Huibers et al., 1997) and, therefore, indicating that the TPGS 400-enhanced permeability of paclitaxel may be due to an inhibition of P-gp-mediated drug efflux in the intestinal epithelial cell monolayer. Moreover, the plasma AUC and bioavailability of orally administered paclitaxel formulated in ethanol-TPGS 400 were increased by approximately 3-fold compared to those of orally administered paclitaxel formulated in ethanol-Cremophor EL, a non-ionic surfactant used for Taxol® formulation. The use of TPGS 400 at high concentrations in the oral dosing formulation may then provide dual advantages by increasing drug solubility, which leads to a better delivery of hydrophobic drugs and renders drug molecules accessible to the intestinal tract, and by inhibiting the P-gp efflux system by which a decrease in drug efflux and a higher absorption through the intestinal mucosa may be achieved. The enhancement of paclitaxel oral bioavailability may, in part, be due to the increase of solubility of paclitaxel. However, the solubility of paclitaxel in TPGS 400 was not different from that in Cremophor EL (our unpublished data) suggesting that the increase of solubility of paclitaxel using TPGS 400 as a solubilizing vehicle does not play an important role on the increase of oral bioavailability of paclitaxel. Therefore, TPGS 400 may act via inhibiting the drug efflux pump, P-gp, to increase the absorption and oral bioavailability of paclitaxel. Taken together the in vitro and in vivo studies, our results suggest that the increases of the plasma levels of paclitaxel after oral administration is mainly due to a blockade in the P-gp efflux pump in the intestinal tract. Whether chemical stability of pacliatxel in the intestinal lumen also contributes to this differential pharmacokinetics is to be further investigated.

On the other hand, the present pharmacokinetic study of intravenously administered paclitaxel showed a superimposition of the two plasma concentration–time profiles and no difference in the plasma AUC between the two formulated paclitaxel. These results indicated that paclitaxel formulated in either Cremophor EL/ethanol or TPGS 400/ethanol share similar pharmacokinetic characteristics in the tissue disposition or elimination mechanisms between the two formulations administered systemically via the intravenous route. This observation further supports that the increase of paclitaxel oral bioavailability is mainly due to the P-gp inhibition taking place in the intestinal tract, i.e., due to an increase in absorption, not a decrease in the elimination process. The present study showed that TPGS 400 interferes with the P-gp activity in the intestines and, therefore, is able to significantly increase the oral bioavailability of paclitaxel in mice.

TPGS 400 and TPGS 1000 both consist of a hydrophilic polar head group and a lipophilic alkyl tail group and thus show an amphiphilic property. They are structurally different in the length of the PEG moiety. TPGS 400 is smaller averaged molecular weight (927 Da vs. 1513 Da), higher critical micelle concentration (0.28%, w/w vs. 0.02%, w/w), and lower hydrophilic–lipophilic balance value (8.3 vs. 13.2) as compared to TPGS 1000 (Wu and Hopkins, 1999). Previous reports showed that co-administered TPGS 1000 improved the oral bioavailability of vitamin E and cyclosporine A in humans and animals (Sokol et al., 1987, 1991; Boudreaux et al., 1993; Pan et al., 1996; Fischer et al., 2002) and a 1.5-fold increase in the plasma AUC of cyclosporine A in humans was observed (Chang et al., 1996). These enhancements of drug bioavailability may be due to enhanced permeability, inhibition of P-gp, or reduced intestinal metabolism (Dintaman and Silverman, 1999). The inhibitory activity of TPGS 1000 showed specificity on P-gp, not the other multi-drug resistant proteins, such as MRP2 (Bogman et al., 2003), and its underlying mechanism for P-gp inhibition is via an inhibition on the P-gp-associated ATPase (Collnot et al., 2007). Chang et al. suggested that TPGS 1000 may interact with P-gp in the intestine to increase cyclosporine A absorption (Chang et al., 1996). TPGS 1000 has been suggested as an emulsifier and a matrix material in the formulation of paclitaxel nanoparticles (Mu and Feng, 2002; Zhang and Feng, 2006). Nevertheless, a P-gp-independent mechanism for internalization of Rhodamine 123-labelled poly(ethylene glycol) 2000-phosphatidyl ethanolamine conjugates/TPGS 1000 micelles by Caco-2 cells was reported (Dabholkar et al., 2006), indicating that the P-gp inhibitory activity of free TPGS 1000 may not be obvious in conjugated TPGS 1000. It is not known whether the biological activity of TPGS 400 is also affected by conjugation process. The present study introduced TPGS 400 as a co-solvent for paclitaxel formulation and demonstrated its ability in increasing oral bioavailability of paclitaxel.

For a given medicine, oral formulations generally offer more advantages over the injectables for a better patient compliance, less frequency of clinic visits, ease of chronic therapeutic regimens, and lower cost. Paclitaxel is a potent anticancer drug. However, it is poorly oral absorbable in animals and in humans (Malingre et al., 2001) and is routinely administered into patients by the intravenous route. The oral absorption of paclitaxel was inhibited by the P-gp efflux pump in the intestine mucosa (Sparreboom et al., 1997) and paclitaxel was metabolized by CYP3A in both the liver and epithelial cells of small intestine (Choi et al., 2004). Enhancement of oral bioavailability of paclitaxel may be achieved via an increase in intestinal epithelial permeability, P-gp inhibition, or reduced intestinal metabolism (Sonnichsen et al., 1995). The weak inhibition activity of TPGS 400, TPGS 1000 and Cremophor EL on CYP3A-catalyzed testosterone 6β-hydroxylation in both mouse and human liver microsomes indicated a low possibility of interference on the

paclitaxel metabolism in liver and intestine by these solubilizers. It is, therefore, suggested that the increased oral bioavailability of paclitaxel is not caused by inhibitions of both intestinal and hepatic metabolism mediated by CYP3 A. Attempts have been made to increase the oral bioavailability of paclitaxel, including reformulated paclitaxel, synthetic derivatives or pro-drugs of paclitaxel, and co-administration with a P-gp inhibitor (Bardelmeijer et al., 2004; Kimura et al., 2002; Woo et al., 2003; Varma and Panchagnula, 2005). In agreement with a previous report on TPGS 1000, we found that TPGS 400 increases oral absorption of paclitaxel in mice. Both TPGS 400 and TPGS 1000 may, therefore, be used to enhance the oral bioavailability of drugs in patients. Furthermore, TPGS 400 did not show clinical signs of toxicity nor increase tumor incidences at high daily oral dose levels of greater than 1000 mg/kg for 104 weeks in toxicological studies in mice and rats (EFSA, 2007). PEG 400 is used as a pharmaceutical excipient in a broader spectrum than PEG 1000. Whether TPGS 400 is better than or comparable to TPGS 1000 in general as a formulation excipient remains to be further investigated.

We here reported that TPGS 400 enhances the oral bioavailability of paclitaxel in mice, in part via interfering the activity of intestinal P-gp, increasing the drug permeability, and thus enhancing absorption in the intestinal tract. The enhancement of oral absorption of paclitaxel formulated using TPGS 400 warrants further development of oral formulations for paclitaxel and its Taxane analogs in clinical uses. Whether TPGS 400 can be used as a formulation excipient for the other poorly orally absorbable drugs remains to be evaluated in the future.

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