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ORIGINAL ARTICLE

Physicochemical stability, pharmacokinetic, and biodistribution evaluation of paclitaxel solid dispersion prepared using supercritical antisolvent process

Srinivasan Shanmugam[1,*], Jae-Hyun Park[1,*], Sang-Cheol Chi[2], Chul Soon Yong[3], Han-Gon Choi[4], and Jong Soo Woo[1]

[1]Pharm. R&D Institute, Hanmi Pharm. Co., Ltd., Hwasung, Gyeonggi 445-913, Republic of Korea, [2]College of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi 440-746, Republic of Korea, [3]College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea, and [4]College of Pharmacy, Hanyang University,1271, Sa-3-Dong, Ansan 426-791, Republic of Korea

Abstract

Aim: To investigate the physicochemical stability, pharmacokinetics (PK), and biodistribution of paclitaxel (PTX) from paclitaxel solid dispersion (PSD) prepared by supercritical antisolvent (SAS) process.

Methods: Physicochemical stability was performed in accelerated (40°C 70 ± 5% RH) and stress (60°C) storage conditions for a period of 6 months and 4 weeks, respectively. PK and biodistribution studies were performed in rats following i.v. administration of PTX equivalent to 6 and 12 mg/kg formulations.

Results: Physical stability of PSD showed excellent stability with no recrystallization of the amorphous form. Chemical stability of PSD in terms of % PTX remaining was 98.2 ± 0.6% at 6 months and 97.9 ± 0.3% at 4 weeks of accelerated and stress conditions, respectively. The PK study showed a nonlinear increase in AUC with increasing dose, that is, 100% increase in dose (from 6 to 12 mg/kg) resulted in 405.90% increase in AUC. Unlike PK study, the organ distribution study of PTX from PSD showed linear relationship with dose escalation. The order of organ distribution of PTX from highest to lowest for both PSD and Taxol® was liver>kidney>lung>brain.

Conclusions: This study demonstrated excellent physicochemical stability with insight information on the PK and biodistribution of PTX from PSD prepared by SAS process.

Keywords: Paclitaxel solid dispersion, supercritical antisolvent process, accelerated/stress stability, pharmacokinetics, organ distribution

Introduction

Paclitaxel (PTX), a diterpenoid natural product, is one of the most effective antineoplastic agents that has been widely prescribed to treat a wide variety of tumors, including ovarian carcinoma, breast cancer, head and neck cancers, non-small lung cancer, prostatic cancer, and advanced forms of Kaposi's sarcoma.[1,2] One of the major limitations associated with this potent drug is its low aqueous solubility due to its extremely hydrophobic nature.[3,4] Therefore, various approaches to solubilize PTX were carried out for more than a decade and the most successful one was Taxol® (Bristol-Myers Squibb), the commercially available formulation for intravenous administration, which is 6 mg/mL of PTX in a 50:50% v/v mixture of Cremophor EL and dehydrated ethanol.[5–8]

Although Cremophor EL has been used to administer other drugs, such as cyclosporine[9] and teniposide,[10] the amount present in Taxol® to solubilize PTX is significantly

*Srinivasan Shanmugam and Jae-Hyun Park equally contributed to this work.
Address for Correspondence: Jong Soo Woo, Pharm. R&D Institute, Hanmi Pharm. Co. Ltd., Hwasung, Gyeonggi, 445–913, Korea. E-mail: jswoo@hanmi.co.kr; Han-Gon Choi, College of Pharmacy, Hanyang University,1271, Sa-3-Dong, Ansan 426-791, Republic of Korea. E-mail: hangon@hanyang.ac.kr

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higher.[11] While information on the blood concentration and pharmacokinetics (PK) of Cremophor EL in human is limited, LD_{50} of Cremophor EL in dogs following i.v. administration was reported to be 0.64 mL/kg.[12–14] Intravenous administration of a standard dose of Taxol® (315 mg) would administer about 26 mL of Cremophor EL into systemic circulation, which is considerably quite high to produce toxic side effects.[15–18] Consequently, the clinical application of Taxol® encountered many problems, including serious or even fatal hypersensitivity episodes due to histamine induction by Cremophor EL[19,20] and possible precipitation after dilution and leaching of the diethylhexylphtalate (DEHP) from polyvinylchloride (PVC) infusion sets, necessitating the use of plasticizer-free containers or bags and causing inconvenience to medical staff and pain to patients.[21,22]

Numerous alternative delivery systems have been proposed to overcome the aforementioned problems, such as mixed micellar solution,[23] liposomes,[24] cyclodextrin complexation,[25] poly(ε-caprolactone) microspheres,[26] and emulsion.[27] However, all these formulations have demonstrated problems of complicated preparative procedure and/or invariably low stability. Other methods such as encapsulation of PTX in water-insoluble biodegradable polymers were also unsuccessful.[28–30] Eventually, development of PTX formulation without Cremophor EL for intravenous use with improved safety, solubility, and stability is highly warranted.

In order to satisfy the increasing demands of the pharmaceutical industry, it is necessary to utilize a method that would be capable of improving the solubility as well as stability.[6–8] The most common and probably the most widely used method to stabilize a drug formulation is solid dispersion.[31–34] It was stated that stabilization of amorphous drug in solid dispersions was mainly the consequence of drug–polymer interactions, rather than antiplasticizing effect of the polymer dispersion.[35–39] Supercritical antisolvent (SAS) technique, analogous to spray drying, allows drug–polymer interaction in molecular level and aids in generation of small, even, and easily wettable particles that are difficult or even impossible to obtain by traditional techniques such as milling, crystallization, and spray drying.[40,41]

In our earlier works, we reported preparation and characterization of paclitaxel solid dispersion (PSD)[42] using SAS process and evaluated its *in vivo* toxicity in ICR mice.[43] We showed that PSD could be prepared with hydrophilic polymers and surfactant mixture by SAS process that produced nanosized particles with enhanced solubility of PTX in water (>20 mg/mL for PSD vs. 0.7 μg/mL for pure PTX). Besides, toxicity studies performed in ICR mice with PSD exhibited lower toxicity and higher safety profile compared with Taxol® in terms of LD_{50} (160 mg/kg PSD vs. 31.3 mg/kg Taxol®), nephrotoxicity (no significant change in creatinine clearance up to 50 mg/kg of PSD vs. death of all animals at 15 mg/kg dose of Taxol®), and hemolytic activity (10% with PSD vs. 40% with Taxol®).[43]

In drug-development process, physicochemical stability is a crucial factor that determines the success of any drug candidate. Therefore, in the present study, we prepared PSD using our previously reported SAS process[42] and evaluated its physicochemical stability in terms of recrystallization and % amount of active remaining as well as formation of related substances upon storage in accelerated condition (40°C 70 ± 5% RH) for 6 months and stress (60°C) condition for 4 weeks. This work was also aimed at investigation of plasma PK and biodistribution (organ distribution) of PTX in rats after intravenous administration of PSD and commercial formulation Taxol®.

Materials and methods

Materials

The following materials were purchased from various companies and then used as received. PTX (Natural pharmaceuticals, Inc., Beverly, MA, USA), hydroxypropyl β-cyclodextrin (HP-β-CD; ISPTechnologies, Wayne, NJ, USA), polyoxyl 40 hydrogenated castor oil (HCO-40; BASF Co., Ltd., Aktiengesellschaft, Ludwgshafen, Germany), polyvinylpyrrolidone C-30 (PVP C-30; ISP Technologies, Wayne, NJ, USA), dichloromethane (Daejung Chemicals & Metals Co., Ltd. Shiheung-Si, Gyeonggi-Do, Korea), carbon dioxide (high purity of 99.99%; Gyeonggi Gas Co. Ltd., Shiheung-Si, Gyeonggi-Do, Korea), acetonitrile (HPLC grade; Burdick & Jackson, Muskegon, MI, USA), and ethanol (HPLC grade; Burdick & Jackson). All other chemicals were of reagent grade and used without any further purification.

Preparation of solid dispersion

The SAS process for preparing PSD was performed by our previously reported method.[42] The SAS process parameters and equipment used for SAS process were described in detail in our earlier article.[42] In brief, CO_2 from the storage tank was delivered into top of the particle formation chamber using homemade plunger pump until equilibrium pressure (1200 psi) and temperature (40°C) achieved. Then, the drug solution (flow rate 0.3 mL/min), prepared by dissolving appropriate amounts of PTX, hydrophilic polymers HP-β-CD/PVP C-30, and surfactant HCO-40 in a mixture of dichloromethane and ethanol (3/2, v/v), and supercritical CO_2 (flow rate 10 mL/min) were co-injected through the two-flow spray nozzle in the particle formation chamber filled with supercritical CO_2. After the injection of drug solution, fresh CO_2 was introduced into the chamber to remove residual solvent. During the SAS process, the pressure of the chamber was controlled constantly using a back pressure regulator. The PSD formed on the walls and the bottom of the chamber was collected after reducing the chamber pressure to atmospheric pressure.[42]

Osmolarity analysis

OSMOMAT 030-D (Gonotec, Germany) instrument was used to measure the freezing point of samples in terms of milliosmolarity (mOsm). Two standard solutions, water for injection and sodium chloride, were used to calibrate

the equipment. In order to measure the osmolarity of PSD and Taxol®, each sample with PTX equivalent to 6 mg was diluted with 0.9% sodium chloride solution, and about 200 µL of this solution was used to measure the osmolarity. Each sample was tested in quintuple and the total osmolality of aqueous solutions was determined by comparative measurements of the freezing points of pure water to that of test solutions. Universally, water is considered to have a freezing point of 0°C and a solution with saline concentration of 1 Osmol/kg has a freezing point of –1.858°C.[44]

Stability analysis

In order to investigate the physical and chemical stability of the prepared PSD, all samples were kept in glass vials with rubber-stopper and stored at specified stability conditions. The 6-month accelerated stability study and 4-week stress stability study were performed at 40°C with 75 ± 5% relative humidity (RH) and 60°C, respectively, in stability chamber (FTL-600, Fine Scientific Instruments, Korea). The samples were analyzed at 0, 1, 2, 4, and 6 months for accelerated study and at 0, 1, 2, and 4 weeks for stress study. The amount of PTX was analyzed by a validated HPLC method reported in US Pharmacopeial monograph (USP28). All the analyses were tested in triplicate.

Pharmacokinetic and biodistribution evaluation

Animals

Healthy, male Sprague-Dawley rats (4–6 weeks old, about 250 g) were supplied by Samtacho (Kyeonggi, Korea) and quarantined for 1 week prior to use. Animals were maintained on sawdust bedding free of any known chemical contaminants in a 12-h photoperiod (light on at 08:00 and off at 20:00) in our animal facility at 23 ± 2°C and 50–80% RH (TECNIPLAST, Italy). The animals were provided with Purina Certified Rodent Chow No. 5002 meal (Ralston Purina, St. Louis, MO) and had free access to water. Animal care and procedures were in accordance with N.I.H. guidelines and were approved by our Institutional Animal Care and Use Committee (IACUC).

LC/MS analysis of PTX in plasma and organs

The analyses of PTX levels in all plasma and organ samples were measured by LC/MS. The Waters Alliance HT Chromatography System (Waters Corp., Milford, MA) equipped with system control was used. All of the mass spectrometry data were acquired and analyzed using MassLynx 3.5 (Micromass, Manchester, UK). A Waters XTerra® MS C_{18} column (150 × 4.6 mm, 3.5 µm particle size) was used with column temperature of 40°C and the injection volume was 10 µL. Acetonitrile/water/10 mM ammonium acetate (46/47/7, v/v/v) was used as mobile phase at a flow rate of 0.2 mL/min. The quantitative determination of PTX was performed with the Waters ZQ 4000 mass spectrometer. Data were acquired in the electrospray ionization (ESI) mode with positive ion detection under application of single ion recording (SIR). A cone voltage of 25 V and capillary voltage of 3.00 kV were used. The desolvation temperature was maintained at 150°C and nitrogen was used as both nebulizer gas and desolvation gas with flow rate of 50 and 250 L/h, respectively. PTX and internal standard econazole nitrate were detected at m/z values of 854.3 $(M+H)^+$ and 383.1 $(M+H)^+$, respectively, with dwell time of 0.5 sec. Linearity of PTX was investigated by constructing six-point extracted calibration curves at concentration range of 0.025 to 10 µg/mL. The internal standard used was econazole nitrate at a concentration of 0.2 µg/mL. Calibration curves showed excellent linearity with satisfactory coefficients of determination (R^2 > 0.9996). The method was precise and accurate with coefficient of variations of <11%.

Prior to extraction, frozen plasma samples were thawed in a water bath at ambient temperature and the organ samples were homogenized in a mixed solution of acetonitrile and water (50/50, v/v) by Ultra-Turrax homogenizer at 21,000 rpm for 15 min under ice bath. For analysis, 0.2 mL aliquot of either plasma or homogenate was spiked with 0.2 mL of internal standard solution (0.2 µg/mL in acetonitrile) and vortexed for 5 min. The solution was centrifuged for 3 min at 12,000 *g* and 0.2 mL of supernatant was mixed with 0.2 mL of 10 mM ammonium acetate (pH 3.5) and vortexed for 5 min. The solution was centrifuged for 3 min at 12,000 *g* and 10 µL of the supernatant was injected into LC/MS.

In vivo protocol

To evaluate the PK and biodistribution profile of prepared PSD, male Sprague-Dawley rats weighing 250 ± 20 g were randomly divided into four groups (n = 8). PTX equivalent to a dose of 6 and 12 mg/kg of Taxol® and PSD were diluted in 0.9% saline solution and intravenously administered to rats through the tail vein. Five rats in each group were used for PK study while the remaining animals were used for biodistribution study. The femoral vein was cannulated with 23-gauge polyethylene cannula under anesthesia with diethyl ether. About 0.5 mL of blood samples were collected into heparinized tube at 0, 0.016, 0.083, 0.25, 0.50, 1, 2, 3, 5, 7, and 24 h after dosing. The collected blood samples were centrifuged at 10,000 *g* for 10 min and the plasma was stored at –20°C until analysis. For biodistribution study, the designated rats were killed at 1 h after i.v. administration. Organ samples, such as brain, lung, kidney, and liver, were collected immediately by dissection and frozen at –20°C until analysis.

Pharmacokinetic data analysis

The plasma concentration of PTX vs. time profile was analyzed by a two-compartmental method using WinNonlin program for windows (Pharsight, Cary, NC). The relative bioavailability (BA) of PSD to reference injection (Taxol®) was calculated using the following equation:

$$\text{Relative BA}\,(\%) = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{reference}}} \times \frac{\text{Dose}_{\text{reference}}}{\text{Dose}_{\text{test}}}$$

where AUC is the area under plasma drug concentration curve from time 0 to the last sampling time. The PK

parameters were analyzed for statistical significance by unpaired Student's *t*-test with significance level of $P < 0.05$.

Results and discussion

In this study, PTX was precipitated from a mixture of dichloromethane and ethanol (3/2, v/v) using our previously reported SAS process.[42] Formulation composition of PSD prepared was shown in Table 1. A thorough characterization of PSD was described in our earlier report.[42] Mean particle size of prepared PSD was 0.37 µm with homogeneous distribution of particles. Precipitation time of PTX formulation upon dilution is the crucial factor to assess the stability of formulation intended for prolonged infusion time. It was reported that Taxol® upon dilution in dextrose saline forms a hazy dispersion as PTX is not actually in solution and precipitation of particles with clinically unacceptable sizes eventually occurs necessitating the use of in-line filters with infusion sets when it is infused for >24 h.[45] In case of PSD, a clear solution with PTX concentration of up to 10 mg/mL was achieved with reasonable stability in terms of precipitation time of >70 h (Table 2).

Wherever possible, parenteral products intended for intravenous infusion should be iso-osmotic; typically, osmolarities between 280 and 290 mOsm/L are targeted during formulation to prevent any side effects produced by hyper- or hypo-osmotic pressure. The osmolalities of PSD and Taxol® measured at concentrations of 300 and 1000 µg/mL were 308 and 365 mOsm/kg for PSD, and 748 mOsm/kg and not detectable for Taxol®, respectively.

Table 1. Composition of paclitaxel solid dispersion (PSD) produced by supercritical antisolvent (SAS) process.

Ingredient	Amount (mg)	Use
Paclitaxel	5	Active ingredient
HP-β-CD	100	Hydrophilic polymer
PVP C-30	165	Hydrophilic polymer
HCO-40	250	Surfactant
α-Tocopherol	3	Antioxidant
Total	523	

Table 2. Physical properties of paclitaxel solid dispersion (PSD) produced by supercritical antisolvent (SAS) process.

Properties	PSD	Taxol®
Bulk density	0.32	NA
Solubility[a]	>20 mg/mL	6 mg/mL
Precipitation time[b]	>70 h	<27 h[d]
Turbidity[c]	Clear	Slightly hazy[d]
pH (0.6 mg/mL in water)*	4.4 ± 0.1	5.8 ± 0.1
Mean particle size	0.37 ± 0.13 µm	NT

NA, Not applicable; NT, not tested.
*USP specification of pH for intravenous paclitaxel injection is between 3.0 and 7.0.
[a]Solubility of pure PTX powder was 0.7 µg/mL.
[b]Precipitation time was the time taken for PTX to precipitate after formulation equivalent to 1000 µg/mL PTX was mixed in water.
[c]Turbidity measured visually upon dilution in dextrose saline.
[d]The precipitation time and turbidity of Taxol® as stated in Taxol® Injection Patient Information, 2003.

Taxol® showed high values of osmolarity even at 300 µg/mL of PTX, which is the concentration of conventional clinical administration protocol.[1–3] Thus, Taxol® could increase the osmotic pressure of the fluid/blood and eventually produce side effects at high concentration with prolonged infusion. However, in case of PSD, osmolality of 0.9% sodium chloride solution containing PTX concentration of 300 and 1000 µg/mL showed 308 and 365 mOsmol/kg, respectively. These results indicated that osmolality of PSD in 0.9% sodium chloride solution was not greatly affected by PTX concentration and so, PSD can be administered even in higher concentration range without any side effects associated with osmotic pressure changes.

Physical stability of PSD

Generation of amorphous forms of a drug by solid dispersion techniques has been a subject of intensive research for decades since by this way a substantial enhancement of drug solubility as well as increased stability during storage can be achieved.[46] However, it has been well known that transforming the physical state of drug, that is, from crystalline to amorphous or partially amorphous state, as well as solubilization and supersaturation by the carrier leads to a high-energy and high-disorder state, resulting in enhanced kinetic solubility.[47,48] For this reason, solid dispersions have a natural tendency to revert back to their stable crystalline form during prolonged storage period leading to change in stability and solubility of the solid dispersions.[28] Therefore, the physical stability of PSD was investigated using DSC to see the recrystallization pattern of PTX. DSC thermograms of PSD obtained during 6-month accelerated (40°C, 75% RH) and 4-week stress (60°C) conditions were shown in Figure 1. The thermogram of pure PTX powder exhibited an endothermic peak at about 216.6°C with an enthalpy of 12.979 J/g (data not shown), corresponding to its melting point.[42] On the other hand, PSD prepared by SAS process showed no endothermic peak due to conversion of crystalline PTX into amorphous form and/or formation of inclusion complex with polymers during SAS process.[49] Regarding the physical stability, there was no endothermic peak of PTX until 6 months in accelerated and 4 weeks in stress conditions. Studies have shown that exposure of amorphous powders to high temperatures and humidity can cause crystallization of the amorphous drug.[50] However, this remarkably good physical stability of PSD during long storage period even at high temperature and humidity indicated that hydrophilic polymers utilized in the preparation of PSD (HP-β-CD and PVP C-30) were appropriate to stabilize the amorphous state of drug due to inhibition of drug recrystallization.[51–53]

Chemical stability of PSD

To evaluate the chemical stability of PTX, stability studies were performed at accelerated (40°C and 75 ± 5% RH) and stress (60°C) conditions for a period of 6 months and 4 weeks, respectively. The % PTX remaining, % related substances, and pH of PSD and Taxol® during 6-month

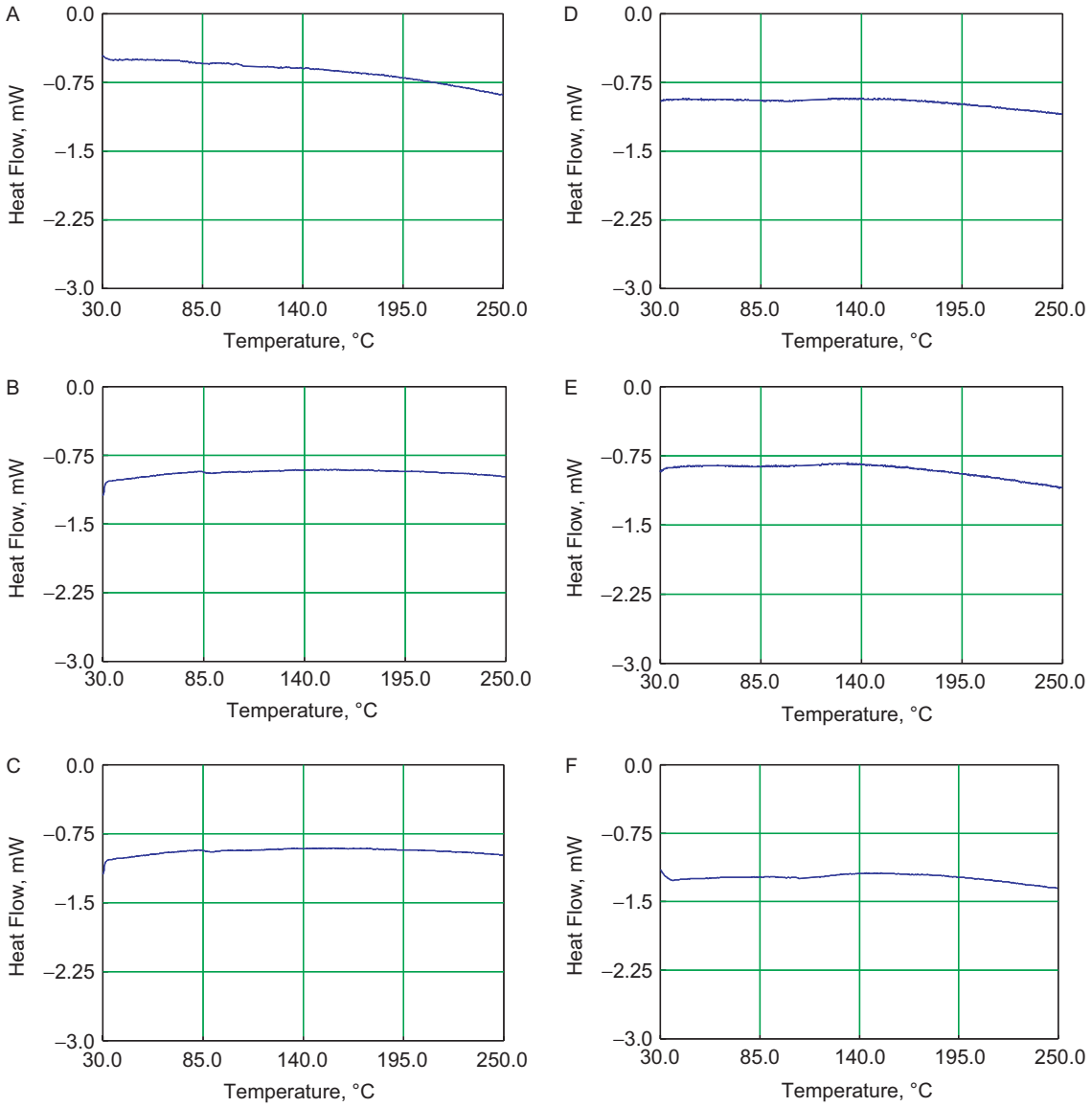

Figure 1. DSC thermograms of paclitaxel solid dispersion (PSD) according to storage periods; accelerated condition (40°C/70 ± 5% RH): (A) 1 month, (B) 2 months, and (C) 6 months; stress condition (60°C): (D) 1 week, (E) 2 weeks, and (F) 4 weeks.

accelerated study were presented in Table 3. The chemical stability profile of PSD was not significantly different from reference product Taxol® and no significant degradation of the active from the initial value was seen. The % of PTX remaining after 6 months of storage was 98.2 ± 0.6% and 104.8 ± 0.6% for PSD and Taxol®, respectively. This was a reduction of 0.91% and 0.85% of initial values of PSD and Taxol®, respectively. The pH measured after dissolving appropriate amount of formulations into water showed that the pH was consistent throughout the study period with a value of 4.3 ± 0.1 and 5.8 ± 0.1 for PSD and Taxol®, respectively.

In terms of related substances, the amount of baccatin and ethyl ester side chain was appeared to be the major by-products with a value of 0.032% and 0.054% for PSD, and 0.038% and 0.021% for Taxol® after 6 months of storage, respectively. Formation of by-products constitutes the most serious problem of any active drug since it proves that drug is not stable during storage and thus its effectiveness would be reducing dramatically if by-products are inactive.[54,55] In this study, although the contents of baccatin and ethyl ester side chain appeared to increase a little with time, their values were very low and quite below the specification level of ≤0.8 and ≤0.4 for baccatin and ethyl ester side chain, respectively.[56] These results indicated possible interaction of PTX reactive group with polymers that might reduce or hinder the movements of PTX reactive groups leading to its stabilization.

It was well-recognized that residual water associated with drugs in the solid state can have significant effects on a variety of physicochemical properties such as chemical degradation and solubility.[57,58] However, these are not the only factors affecting drug stability. For example, it was mentioned that different used carriers with high glass transition temperature and similar water absorbance have different effects on drug stabilization.[31–34]

Table 3. Six-month accelerated stability study of paclitaxel solid dispersion (PSD) and Taxol® performed at 40°C and 75 ± 5% RH storage condition.

Samples	Items tested	Limits (%)	Period (months) 0	1	2	4	6
Paclitaxel solid dispersion (PSD)	Content of paclitaxel	90.0–110.0	99.1 ± 0.8	98.7 ± 0.5	98.6 ± 0.6	NT	98.2 ± 0.6
	pH (0.6 mg/mL in water)	3.0–7.0	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	NT	4.3 ± 0.1
	Related compounds						
	Baccatin III	≤0.8	0.002 ± 0.001	0.002 ± 0.001	ND	NT	0.032 ± 0.001
	Ethyl ester side chain	≤0.4	0.003 ± 0.001	0.003 ± 0.001	0.060 ± 0.006	NT	0.054 ± 0.004
	10-Deacetylpaclitaxel	≤0.8	0.042 ± 0.013	0.042 ± 0.008	0.070 ± 0.004	NT	0.031 ± 0.002
	10-Deacetyl-7-epipaclitaxel	≤0.5	0.005 ± 0.001	0.005 ± 0.001	ND	NT	ND
	7-Epipaclitaxel	≤0.6	ND	ND	ND	NT	ND
Taxol® (Bristol-Myers Squibb)	Content of paclitaxel	90.0–110.0	105.7 ± 0.3	106.6 ± 0.1	—	105.0 ± 0.8	104.8 ± 0.6
	pH	3.0–7.0	5.9 ± 0.2	5.9 ± 0.1	5.9 ± 0.2	5.8 ± 0.2	5.8 ± 0.3
	Related compounds						
	Baccatin III	≤0.8	ND	0.016 ± 0.005	0.019 ± 0.003	0.040 ± 0.004	0.038 ± 0.001
	Ethyl ester side chain	≤0.4	ND	ND	0.011 ± 0.001	0.017 ± 0.002	0.021 ± 0.005
	10-Deacetylpaclitaxel	≤0.8	ND	ND	0.025 ± 0.007	0.004 ± 0.001	0.038 ± 0.003
	10-Deacetyl-7-epipaclitaxel	≤0.5	ND	ND	ND	ND	ND
	7-Epipaclitaxel	≤0.6	0.030 ± 0.007	0.045 ± 0.005	0.049 ± 0.003	0.060 ± 0.008	0.055 ± 0.007

All data are expressed as mean value ($n = 3$).
ND, Not detectable; NT, not tested.

For example, drug dispersions in hydroxypropyl methyl cellulose (HPMC) are more susceptible to physical instability under accelerated stress conditions of the solid dispersion although this effect is much more reduced in solid dispersions where polymeric carriers such as Plasdone and polyvinyl pyrrolidone (PVP) are present.[59] The results of the stress stability study performed for PSD for a period of 4 weeks at 60°C were shown in Table 4. There was no significant increase in drug loss (remaining amount 97.9% after 6 months) or pH change or related substances (baccatin and ethyl ester side chain) even at high temperature of as high as 60°C for 4 weeks. This was further proof that PSD produced by SAS process was quite stable due to stabilization of PTX by the polymers, and led to the prediction that the formulation would also be stable at room temperatures with no change in physicochemical parameters for even longer periods of storage.[60]

Pharmacokinetic study

To assess the PK behavior of PTX in Taxol® and PSD prepared using SAS method, the plasma profile of PTX in Sprague-Dawley rats was obtained after intravenous administration of each formulation. The mean plasma concentration–time profile of PTX after intravenous administration of PTX equivalent to 6 and 12 mg/kg of PSD and 6 mg/kg of Taxol® injection was shown in Figure 2. PK profile of PTX at higher dose of 12 mg/kg was also investigated to see the effect of formulations on systemic and organ exposure in rats. PK investigation of PTX equivalent to 12 mg/kg of Taxol® was discontinued due to sudden death of animals or severe side effects following intravenous administration. However, PSD was well-tolerated in both doses tested and the PK parameters of PTX obtained by fitting the data to a two-compartmental model were shown in Table 5. The concentration profile followed biphasic pattern in plasma with initial rapid distribution phase ($t_{1/2\alpha} < 0.5$ h) followed by slow elimination phase for both Taxol® and PSD.[61] There were no significant differences noted with PK parameters among Taxol® and PSD formulations at a dose of 6 mg/kg. The PK profile of PSD at PTX equivalent to 12 mg/kg dose showed a disproportionate increase in AUC of PTX. PSD at a dose equivalent to 6 mg/kg of PTX resulted in mean AUC of 9.57 ± 2.10 μg/mL/h, whereas dose equivalent to 12 mg/kg of PTX resulted in AUC of 38.85 ± 0.64 μg/mL/h (Table 5). This finding is in accordance with other clinical studies that revealed a nonlinear disposition of PTX with other formulations suggesting chances of greater-than-expected increase in systemic exposure for a given increase in dose of PTX.[62–65] Thus, a 100% increase in dose (from 6 to 12 mg/kg) resulted in 405.90% increase in AUC.

Absence of PK profile of Taxol® at a dose of PTX equivalent to 12 mg/kg precluded the assessment of BA of PSD, but the relative BA of PSD to that of Taxol® at a dose of PTX equivalent to 6 mg/kg was 64.36%. Although BA of PSD at this dose was less than that of Taxol®, it could be possible that the BA of PSD be similar or even higher than Taxol® at 12 mg/kg due to the demonstrated nonlinear PK of PSD. The clearance of PTX at 6 mg/kg dose was similar for both formulations, but there was a significant decrease in clearance of PTX at 12 mg/kg of PSD compared with 6 mg/kg ($P < 0.05$). The clearance of PTX decreased from 658.59 ± 144.27 mL/h/kg at a dose of 12 mg/kg to 154.52 ± 2.53 mL/h/kg at 6 mg/kg dose of PTX. Thus, both reduction in the clearance and an over proportional increase in C_{max} and AUC of PTX with increasing dosages indicated that both drug elimination and distribution were affected.[67] This characteristics of

Table 4. Four-week stress stability study of paclitaxel solid dispersion (PSD) performed at 60°C storage condition.

Items tested	Limits (%)	Period (weeks) 0	1	2	4
Content of paclitaxel	90.0–110.0	99.1 ± 0.8	99.0 ± 0.3	99.4 ± 0.3	97.9 ± 0.3
pH (0.6 mg/mL in water)	3.0–7.0	4.4 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	4.4 ± 0.3
Related compounds					
Baccatin III	≤0.8	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.003 ± 0.001
Ethyl ester side chain	≤0.4	0.003 ± 0.001	0.008 ± 0.003	0.008 ± 0.002	0.014 ± 0.006
10-Deacetylpaclitaxel	≤0.8	0.042 ± 0.012	0.074 ± 0.009	0.141 ± 0.057	0.125 ± 0.042
10-Deacetyl-7-epipaclitaxel	≤0.5	0.005 ± 0.002	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.003
7-Epipaclitaxel	≤0.6	ND	ND	0.003 ± 0.001	0.003 ± 0.001

All data are expressed as mean value ($n = 3$).
ND, Not detectable.

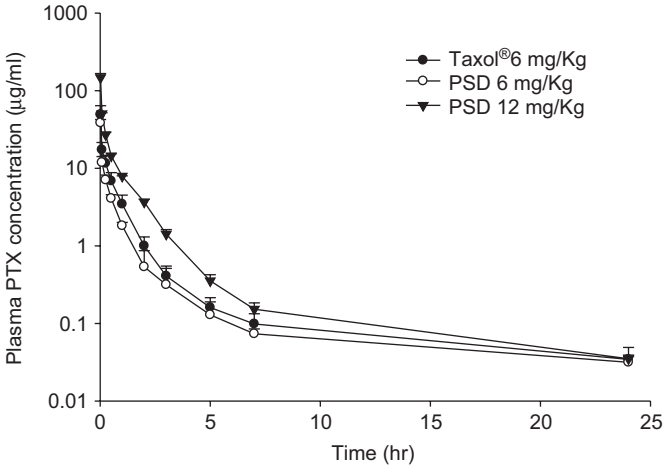

Figure 2. Mean plasma concentration–time profile of paclitaxel in rat plasma after i.v. administration of 6 mg/kg dose of Taxol® and 6 and 12 mg/kg dose of paclitaxel solid dispersion (PSD) in male SD rats. Mean ± SE ($n = 5$).

PTX may be due to saturable processes in distribution and elimination/metabolism.[66–69] The PK of PTX is mainly dependent on extrarenal mechanisms that include both metabolism and biliary elimination.[69] At higher doses of PTX, saturation of metabolism occurs, which leads to decreased clearance leading to nonlinear PK profile (high C_{max} and AUC).[68,69]

Biodistribution study

An attempt was made to investigate the organ distribution behavior of PTX after intravenous administration of PSD (PTX equivalent to 6 and 12 mg/kg) to rats along with Taxol® (PTX equivalent to 6 mg/kg) as control. After intravenous administration, PTX was found to be widely distributed into most organs and the distribution pattern of PTX into different organs is shown in Figure 3. Higher concentrations of PTX were found in all organs tested (liver, lung, and kidney) except brain, where the concentration of PTX was negligible. Earlier investigations of drug penetration into brain tumors indicate that both physiological variables (i.e. blood flow, membrane integrity, and hypoxia) and drug-specific parameters (i.e. molecular weight and lipophilicity) are important determinants.[70] Besides, PTX appears to be a substrate of the multidrug resistance protein P-gp, and it is likely that this transporter contributes to its limited access to the brain. P-gp is expressed in high levels in cultured brain capillary endothelial cells and in intact brain capillaries.[70,71] It is localized at the luminal surface of the endothelium, and therefore is in the correct location to restrict permeation of a variety of drugs into the CNS including PTX.[69–71] Eventually, the above-mentioned parameters might have contributed to the poor penetration of PTX into brain.

PTX concentrations measured in liver, lung, and kidney after 6 mg/kg of PTX dose were 8.19 ± 1.02, 3.81 ± 0.15, and 5.47 ± 0.25 µg/g for PSD, and 7.63 ± 0.69, 3.40 ± 0.53, and 4.71 ± 0.39 µg/g for Taxol®, respectively. The order of organ distribution of PTX from highest to lowest for both PSD and Taxol® was liver>kidney>lung>brain. Although these results suggested relatively higher organ distribution of PTX from PSD compared with Taxol®, there was no statistical significance between them. Concentration of PTX in plasma was 2.01 ± 0.44 and 1.23 ± 0.03 µg/mL for PTX equivalent to 6 mg/kg of Taxol® and PSD, respectively. Figure 3 (secondary *y*-axis) shows plasma distribution pattern of PTX from PSD and Taxol® formulations. Plasma concentration of PTX equivalent to 12 mg/kg of PSD was 5.08 ± 0.59 µg/mL. To investigate the possibility of PSD injection at high concentration, we performed organ distribution study with PTX equivalent to 12 mg/kg of PSD. The organ distribution of PTX measured in liver, lung, and kidney for PTX equivalent to 12 mg/kg

Table 5. Pharmacokinetic parameters of paclitaxel obtained after i.v. administration of 6 mg/kg dose of Taxol® and 6 and 12 mg/kg dose of paclitaxel solid dispersion (PSD) in male SD rats.

		Taxol®	PSD	
Parameters	Units	6 mg/kg	6 mg/kg	12 mg/kg
$AUC_{0\text{-}24\,h}$	μg/mL/h	14.87 ± 5.59	9.57 ± 2.10	38.85 ± 0.64
MRT	h	3.48 ± 0.89	3.98 ± 0.37	3.26 ± 0.23
$t_{1/2\alpha}$	h	0.31 ± 0.02	0.24 ± 0.01	0.24 ± 0.05
$t_{1/2\beta}$	h	7.92 ± 0.61	8.02 ± 0.81	5.26 ± 1.07
K_{10}	mL^{-1}	1.62 ± 0.16	1.96 ± 0.07	1.79 ± 0.17
K_{12}	mL^{-1}	0.59 ± 0.24	0.85 ± 0.04	1.14 ± 0.36
K_{21}	mL^{-1}	0.12 ± 0.03	0.12 ± 01	0.24 ± 0.07
Cl	mL/h/kg	469.99 ± 176.80	658.59 ± 144.27	154.52 ± 2.53*

All values are expressed as mean ± S.E. ($n = 5$).
*Significance in comparison with PTX equivalent to 6 mg/kg of PSD ($P < 0.05$).

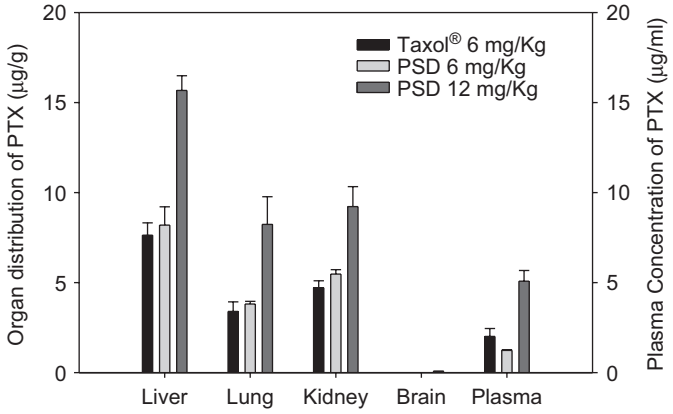

Figure 3. Mean paclitaxel concentration in organs (biodistribution) at 1 h following i.v. administration of 6 mg/kg dose of Taxol® and 6 and 12 mg/kg dose of paclitaxel solid dispersion (PSD). Mean ± SE ($n = 3$).

of PSD was 15.67 ± 0.82, 8.23 ± 1.54, and 9.22 ± 1.11 μg/g, respectively. Unlike the nonlinear increase of PTX in plasma with increasing dose, PTX levels in organs were linear. These findings were consistent with earlier report that upon PTX dose escalation, an overproportional or nonlinear increase of PTX concentration in plasma and dose proportional or linear increase of PTX concentration in organs were observed.[62]

Conclusion

PSD was prepared by SAS process with a mean particle size of <0.37 μm. Physicochemical stability of PSD was excellent with no recrystallization of amorphous drug and insignificant amount of related substance formation during the tested period. PK study performed in rats showed fatal side effects with 12 mg/kg Taxol®, whereas all doses of PSD were well-tolerated and illustrated a nonlinear increase of AUC in plasma with increasing dose. The organ distribution study revealed excellent distribution of PTX into all organs except brain and showed linear relationship with dose escalation. Conclusively, through this study, we prepared PSD by SAS process (a Cremophor-free formulation that showed enhanced solubility and safety in earlier study) and demonstrated excellent physicochemical stability with insight information on the PK and biodistribution of PTX in rats. Besides, our works suggested that efficacy of PTX may be increased by administrating escalated doses of PTX using PSD prepared by SAS process due to its higher solubility, stability, and safety profile. However, further studies are warranted to investigate the PK profile and organ accumulation profile of PTX at elevated doses of PSD to interpret any possible toxicity related to high exposure of PTX.

Declaration of interest

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