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Research Paper

Preparation and evaluation of Cremophor-free paclitaxel solid dispersion by a supercritical antisolvent process

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Abstract

Objectives To avoid the major adverse effects induced by Cremophor EL formulated in the commercial paclitaxel products of Taxol.

Methods An injectable paclitaxel solid dispersion free of Cremophor was prepared by a supercritical antisolvent process and then was fully characterized and investigated with regard to its short-term and long-term stability. Pharmacokinetics in rats was also evaluated compared with the commercial product.

Key findings The solid dispersion system at a 1/20/40 weight ratio of paclitaxel/HP-β-CD/HCO-40 had a paclitaxel solubility of about 10 mg/ml, an almost 10 000-fold increase over its aqueous solubility. This system was physically stable for at least six months or four weeks in accelerated conditions (40 ± 2°C; RH: 75 ± 5%) and stress conditions (60°C), respectively. The precipitation time of paclitaxel solid dispersion in 0.9% sodium chloride injection at a concentration of 1000 μg/ml was above 70 h at room temperature. Intravenous administration of paclitaxel solid dispersion at a dose of 6 mg/kg revealed no significant differences when compared with the commercial product. However, our results obtained at a dose of 12 mg/kg showed a striking non-linear increase in the plasma Cmax and AUCall with increased dose. In addition, the concentrations of paclitaxel in various organs in the solid dispersion group were found to be higher than those of Taxol at 6 mg/kg, and the paclitaxel levels in these organs increased proportionately with increasing dose.

Conclusions Nano-scale paclitaxel solid dispersion without Cremophor EL provided advantageous results over Taxol with respect to the physicochemical properties, safety, clinic convenience and pharmacokinetic behaviour in rats.

Keywords Cremophor EL; paclitaxel; solid dispersion; supercritical fluid

Introduction

Paclitaxel, a white or whitish powder extracted from the bark of the Pacific yew tree *Taxus brevifolia*, has been one of the most effective drugs against ovarian cancer, breast cancer, head and neck cancer, non-small lung cancer and prostatic cancer in the past 10–20 years.[1,2] However, paclitaxel is an extremely hydrophobic drug that has a low aqueous solubility of 0.7 μg/ml.[3] Therefore, in most cases intravenous infusion is preferred. Generally paclitaxel injection (Taxol) is the main method of dosage for clinical application. Paclitaxel needs to be solubilized in certain co-solvents, such as 50% Cremophor EL and 50% ethanol, to form injectable solutions. Taxol must be diluted to a low concentration before use, hence an infusion time of 3–24 h is recommended. Patients are generally required to remain in hospital overnight because of the long infusion time, resulting in low patient compliance.

Although Cremophor EL was widely used in the development of oral dosage forms, such as a self-microemulsifying drug delivery system, for drugs with poor aqueous solubility,[4] the incorporation of Cremophor EL into injectable pharmaceutical formulation was limited. In the case of palitaxel, Cremophor EL causes hypersensitivity reactions and leaches diethylhexylphthalate from polyvinylchloride infusion sets, necessitating the use of plasticizer-free containers or bags and causing inconvenience to medical staff and pain to patients.[5] Therefore, numerous alternative delivery systems have been proposed to overcome these problems, such as mixed-micellar solutions,[6] liposomes,[7] cyclodextrin,[8]

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poly(ε-caprolactone) microspheres[9] and paclitaxel-containing emulsion.[10] However, there are problems related to the complicated preparative procedure and low stability of these formulations. Another method involves encapsulating paclitaxel in water-insoluble biodegradable polymers, such as poly lactic acid, poly(lactic-co-glycolic acid)[11,12] and solid lipid nanoparticles,[13] and these particles and solid lipid nanoparticles also allow sustained release of paclitaxel for chemotherapy. Thus, if we could develop a paclitaxel solid dispersion powder (without Cremophor EL) for injection that could be easily solubilized in injectable water, this would lead to improvements in safety and stability.

Supercritical fluid processes (SCF) have emerged as attractive methods in pharmaceutical development, for processes including polymer synthesis, drug delivery, powder production – especially for proteins and ceramics – powder coating, dyeing, impregnation and lithography. There are many excellent reviews outlining these processes in detail.[14] Since the provision of fine powders with a small size below 1 μm and a narrow particle size distribution is essential for parenteral drug delivery. In this study, a supercritical anti-solvent (SAS) method was employed to prepare paclitaxel solid dispersion. This method is analogous to spray drying in that a feed is continuously sprayed into supercritical carbon dioxide, but allows the generation of small, even, easily wettable particles that are difficult to obtain by traditional techniques such as milling, crystallization and spray drying.[15]

This study endeavoured to solve the problem of the poor solubility of paclitaxel in water and develop a pharmaceutically acceptable and better-tolerated injectable solid dispersion without Cremophor EL, which would have the potential to shorten the infusion time and reduce toxicity. The formulation was screened and optimized by solubility and stability study. The paclitaxel-loaded solid dispersion was prepared and its physicochemical properties were investigated by particle size distribution analysis, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). Short-term and long-term stability, pharmacokinetics and organ distribution studies of the solid dispersion in rats were also conducted and compared with the commercial product Taxol.

Materials and Methods

Materials

The following materials were purchased from various companies and then used as received. Taxol (Samyang Genex, Taejon, USA), paclitaxel ($C_{47}H_{51}NO_{14}$; Natural Pharmaceuticals, Inc., Westport, USA), saline (0.9% NaCl injectable solution; Choongwae Co, Seoul, Korea) hydroxypropyl-β-cyclodextrin (HP-β-CD; ISP, Tokyo, Japan), hydroxypropylmethyl cellulose (HPMC 2910, Shin-Etsu Chemical Co., Ltd, Tokyo, Japan), polyoxyl 40 hydrogenated caster oil (HCO-40; BASF Co., Ltd, Ludwigshafen, Germany), polyoxyethylene 40 stearate (Myrj 52S; Uniqema, Barcelona, Spain), polyvinylpyrrolidone C-30 (PVP C-30, ISP, Tokyo, Japan), polyvinylpyrrolidone (Kollidone 12PF K-12; BASF Co., Ltd, Vidalia, USA), hydroxypropylcellulose (HPC-L 11; Shinetsu Chemical Co., Ltd, Tokyo, Japan), polysorbates (Tween 20 and 80; Uniqema, Barcelona, Spain), polyoxyethylene 35 lauryl ether (Brij 35; Junsei Chemical Co. Ltd, Tokyo, Japan), lecithin (Degussa, Courbevoie, France), macrogoglycerides (Labrasol; Gattefosse, Lyon, France), dimethyl sulfoxide (Daejung Co., Daegu, Korea), dichloromethane (Daejung Co., Daegu, Korea), carbon dioxide (99.99% purity; Gyeonggi Gas Co., Ltd, Suwon, Korea), acetonitrile (HPLC grade, Burdick & Jackson, Muskegon, USA) and ethanol (HPLC grade, Burdick & Jackson, Muskegon, USA). All other chemicals were reagent grade.

Animals

Male Sprague-Dawley rats, 250 ± 20 g, were purchased from Charles River Company Korea (Orient, Seoul, South Korea). The healthy male SD rats were randomly divided into three groups ($n = 8$ for each group). All rats were fasted for 10–12 h before the experiments but allowed free access to water at a temperature of 20–23°C and a relative humidity of 50 ± 5%. Formulations of paclitaxel solid dispersion and Taxol (control) were diluted in 0.9% saline solution and administered to rats through the tail vein at the required dose. All animal care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology.[16] Furthermore, the protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Yeungnam University.

Preparation of solid dispersion

The supercritical fluid process for preparing the paclitaxel solid dispersion was performed as follows. First, dichloromethane and ethanol were mixed by agitating, and then, paclitaxel and polymer were added to prepare a solution while agitating. Before the solvent mixture was injected into the reactor using a small liquid pump, about 2 ml of a blank solvent, dichloromethane and ethanol 3 : 2 (v/v), was injected through a nozzle into the reactor to prevent the nozzle from clogging.

The supercritical fluid process used in this study was as follows. The precipitation chamber was first charged with supercritical carbon dioxide. After the predetermined operating conditions with the temperature of 40°C and pressure of 8.3 MPa were reached, a steady flow of CO_2 (10 ml/min) was established by adjusting the metering valve and the metering pump. The polymer solution (3%, w/v) was then injected at a flow rate of 0.3 ml/min into the high-pressure chamber through the capillary nozzle. As the injected mixture was sprayed via a capillary nozzle inside the reactor, a highly uniform solid dispersion was formed under the supercritical state.

Solubility and precipitation time of solid dispersion in water

To measure the solubility of the paclitaxel/polymer (PT-P) solid dispersions, paclitaxel/polymer/surfactant (PT-P-S) solid dispersions and paclitaxel solid dispersion (optimized final formulation) prepared by the supercritical fluid process, a saturated solubility test was performed. In brief, excess amounts of solid dispersion samples (equivalent to 3–20 mg of paclitaxel) and 1 ml of distilled water were placed in an Eppendorf tube. They were vigorously mixed for 30 min at 25 ± /0.5°C using a vortex mixer (Glas-Col multi-pulse

vortex; motor 30, pulser 30) and shaken at 25°C for 24 h. After reaching equilibrium, samples were centrifuged at 12 500*g* for 10 min. The supernatant was filtered through a 0.45-μm membrane filter, and the filtered solutions were diluted as required with ethanol.

The HPLC analysis was carried out with a Hitachi L-7100 pump, Hitachi UV detector L-7400, Rheodyne 7725 injector and Inertsil ODS-2 column (5 μm, 4.6 × 150 mm; GL Science, Tokyo, Japan), and the absorbance was measured at UV 228 nm. A 50% acetonitrile aqueous solution was employed as the mobile phase, and its flow rate and injection volume were 1 ml/min and 20 μl, respectively. The calibration curve showed that the absorbance was linear ($r^2 \geq 0.999$) to the paclitaxel concentration within the range of 0.5–20 μg/ml. The average intraday and inter-day variations (% RSDs and % deviations) of the assay for non-biological samples were less than 7.5%.

For precipitation studies, an amount of solid dispersion equivalent to 2.0 mg of paclitaxel and 2 ml of 0.9% sodium chloride injection was placed in an Eppendorf tube and vortexed for 10 min at 25 ± 0.5°C. This concentration was higher than the normal clinical range used, to enable a good test of the system. The onset of precipitation was viewed with the help of an Olympus BX51/BX52 optical microscope, through which particle formation was observed. Following these initial observations, the mixtures were placed on a test tube rotator and observed at various time intervals, with the samples being mixed by shaking for 3 s before each test.

Characterization of solid dispersion

The shape and surface morphology of the paclitaxel solid dispersion were investigated using a scanning electron microscope (Model XL30ESEMFEG; FEI Co., Hillsboro, USA). The paclitaxel solid dispersion for injection was placed in a sample dish and fixed using a carbon adhesive. The sample dish was placed on a stage of an ion-coating device and subjected to platinum coating for 2–3 min. Then the paclitaxel solid dispersion was examined by SEM under 20 kV.

The mean particle size (PS) and particle size distribution (PSD) of each sample was determined by laser light diffraction using a laser particle size analyser (Model SALD-2001; Shimadzu Co., Kyoto, Japan). The samples were dispersed in water and sonicated for 2 min to ensure a homogenous dispersion. The obtained dispersion was examined to determine the volume, mean diameter and size distribution.

The thermochemical property of the paclitaxel solid dispersion was characterized using DSC thermogram analysis (Rheometric Scientific, model: DLOS). After weighing about 3 mg of a sample placed in an aluminium pan with a lid using a microbalance (Sartorius), the aluminium pan was sealed with a cap to be used as a test sample. An empty aluminium pan with its cap was used as a control. The samples were purged with pure dry nitrogen at a flow rate of 70 ml/min. The temperature ramp speed was set at 10°C/min and the heat flow was recorded from 30 to 250°C. The temperature scale was calibrated with high purity standards. DSC analysis of pure paclitaxel had been carried out previously to identify the melting point peak. Then, the prepared particles were analysed to observe the specific heat change of the peak.

The physical states of the pure paclitaxel physical mixture and paclitaxel solid dispersion were evaluated with X-ray powder diffraction. Diffraction patterns were obtained using a BRUKER D8 FOCUS High Resolution Powder Diffractometer (BRUKER AXS, Karlsruhe, Germany) equipped with a scintillation counter detector and a divergent beam. This beam employed a Cu-Kal radiation source with a wavelength of $\lambda = 1.5418$ *Å* containing 2-mm slits over the range of 5–40°2-theta. X-ray diffraction data were collected at room temperature and scanned with a step size of 5°2-theta and a dwell time of 12 min at each step. The 20 values and the intensities of the peaks for pure ingredients and the solid dispersion system were compared. The generator was set at 40 kV and 40 mA.

Fourier transform IR spectra were obtained on a Bruker TENSOR 27 Optics system (Bruker Optics, Ettlingen, Germany). The scanning range was 600–4000 cm^{-1} and the instrument resolution was 1 cm^{-1}. The spectra of paclitaxel raw material, HP-β-CD, their physical mixtures and the paclitaxel solid dispersion were recorded. Samples were prepared in KBr disks prepared with a hydrostatic press at a force of 2.8 kN/cm for 3 min (Riken Seiki, Ojiya, Japan).

Osmolarity test

To measure the osmolarity of the paclitaxel solid dispersion and Taxol, an amount equivalent to 6 mg of paclitaxel per sample was diluted with 0.9% sodium chloride. About 200 μl of this solution was taken using a micropipette and the freezing point of samples was measured by OSMOMAT 030-D (Gonotec, Berlin, Germany). Each sample was tested in quintuple and the mean was calculated. The total osmolarity of aqueous solutions was determined by comparative measurements of the freezing points of pure water and of solutions. Whereas water has a freezing point of 0°C, a solution with a saline concentration of 1 Osmol/kg has a freezing point of −1.858°C.

Stability test

To evaluate the stability of the paclitaxel solid dispersion, the amount remaining, its degradation compounds and DSC were examined at intervals during storage. Taxol injection was used as the reference. For the accelerated test, Taxol and the paclitaxel solid dispersion powder were placed in a glass vial with a rubber-stopper and stored at 40°C, 75% relative humidity (RH) in humidified chambers, in a life tester (FTL-600, Fine Scientific Instruments, Seoul, South Korea). Samples were removed at 0, 1, 2, 4 and 6 months of storage. For the paclitaxel solid dispersion alone, a stress test was also conducted at 60°C in a humidified chamber and samples were removed at 0, 1, 2, and 4 weeks of storage. The amount of paclitaxel remaining was analysed by an HPLC method validated in the US Pharmacopeia monograph. The samples were tested in triplicate.

Intravenous administration and detection of paclitaxel *in vivo*

To evaluate the pharmacokinetic characteristics of the prepared paclitaxel solid dispersion, the experimental design was as follows: Group I rats received Taxol intravenously at a dose of 6 mg/kg paclitaxel. Group II and Group III received the paclitaxel solid dispersion intravenously at a dose of 6 mg/kg

and 12 mg/kg (as paclitaxel), respectively. Five rats per group were used for the pharmacokinetic study; the others were used for the organ distribution study. The femoral vein was cannulated with a 23-gauge polyethylene cannula under anaesthesia with diethyl ether. About 0.5 ml of blood was collected into a heparinized tube at time intervals (1, 5, 15, 30 and 60 min and 2, 3, 5, 7 and 24 h) after dosing. Then, 0.5 ml of blood from untreated rats was replaced. The collected blood samples were centrifuged at 10 000*g* for 10 min and the plasma was stored at −20°C until analysis. For the organ distribution study, rats were killed 1 h after intravenous bolus injection. Organ samples such as the brain, lung, kidney and liver were collected immediately by dissection and frozen at −20°C and stored until analysis.

Two hundred microlitres of internal standard (econazole nitrate 0.2 μg/ml in acetonitrile) was added to 200 μl of plasma, and vortex-mixed for 5 min, followed by centrifugation (3 min at 12 000*g*). The supernatant (200 μl) was added to 200 μl of 10 mM NH_4OAc (pH 3.5) and vortexed for 5 min, followed by centrifugation (3 min at 12 000*g*). A 10-μl volume of each sample was injected into the LC/MS system.

Separation was performed on a Waters Alliance HT Chromatography System (Waters Corp., Milford, MA, USA), and a Waters XTerra MS C_{18} column (150 × 4.6 mm, 3.5 μm particle size) was used. The column temperature was held at 40°C. Acetonitrile–water–10 mM NH_4OAc (46 : 47 : 7, v/v) was used as the mobile phase at a flow rate of 0.2 ml/min.

The quantitative determination of paclitaxel was performed with the Waters ZQ 4000 mass spectrometer. Data were acquired in the electrospray ionization (ESI) mode with positive ion detection and 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 the nebulizer gas and desolvation gas with a flow rate of 50 and 250 l/h, respectively. Paclitaxel and econazole nitrate were detected at m/z values of 854.3 $[M + H]^+$ and 383.1 $[M + H]^+$, respectively, with a dwell time of 0.5 s. Validation study showed that the standard curve is linear over the concentration range of 0.025–10 μg/ml for biological sample. The method had high extraction recovery (≥90%) and accuracy (≥90%), with an intraday and interday precision of ≤15%.

Pharmacokinetic data analysis

The area under the drug concentration–time curve from zero to infinity (AUC_{all}), the elimination constant (Kel) and half-life ($t^1/_2$) were calculated using a non-compartmental anaylsis (WinNonlin; professional edition, version 2.1; Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration of drug (Cmax) and the time taken to reach the maximum plasma concentration (Tmax) were obtained directly from the plasma data.

Statistical analysis

Non-parametric Kruskal–Wallis one-way analysis of variance was performed to evaluate any possible difference among groups. Values were reported as mean ± SD and differences were considered statistically significant at $P \leq 0.05$.

Results and Discussion

To develop a solid dispersion system of the poorly water-soluble paclitaxel using the supercritical fluid process, various hydrophilic polymers, such as HPMC2910, HP-β-CD, HPC-L, polyvinylpyrrolidone C-30 and polyvinylpyrrolidone K-12, were treated with paclitaxel. In the case of paclitaxel/polymer complex formulations, the solubility of paclitaxel increased to 8.2–22.4 μg/ml, which was much higher than that of the paclitaxel raw material (Table 1). HPMC2910 and HP-β-CD produced more soluble paclitaxel than the other tested polymers. HPMC was excluded from the polymer candidates given its potential for nephrotoxicity.[17,18] When the ratio of HP-β-CD as a hydrophilic polymer was altered incrementally, the solubility of paclitaxel changed correspondingly, the highest solubility being observed when the ratio of paclitaxel to HP-β-CD was 1/20 by weight (Figure 1).Unfortunately, the stability of the paclitaxel solid dispersion treated by the SAS process with hydrophilic polymers was not significantly improved in view of the occurrence of drug precipitation within 0.5 h in physiological saline, a potential hazard for clinical use.

To increase the solubility of paclitaxel and stabilize the solution for injection, the effect of surfactant addition was investigated. Several surfactants were thus added to paclitaxel/polymer solid dispersions and the change in the solubility of paclitaxel was evaluated.

Table 1 showed that paclitaxel solubility was increased by the use of surfactants. Myrj 52 and Tween 80 were good surfactants for the preparation of solid dispersions. However, the physical stability of the paclitaxel solid dispersion in a 0.9% saline solution prepared by these two surfactants was not good as suggested by relatively shorter precipitation time.

Table 1 Solubility and precipitation time of paclitaxel/polymer and paclitaxel/polymer/surfactant solid dispersion systems at 25°C

Systems	Solubility[c] in water (μg/ml)	Precipitation time[d] (h)
Paclitaxel/polymer systems[a]		
Without polymer	0.6 ± 0.6	<0.5
HP-β-CD	22.4 ± 3.1	<0.5
HPMC2910	21.1 ± 2.7	<0.5
HPC-L	13.4 ± 1.4	<0.5
PVP K-12	11.7 ± 1.3	<0.5
PVP C-30	8.2 ± 0.9	<0.5
Paclitaxel/polymer/surfactant systems[b]		
Without surfactant	190 ± 52	<0.5
Tween 80	15 360 ± 3 236	10
Tween 20	7 080 ± 320	10
Myrj 52	11 440 ± 1 860	48
Brij 35	3 400 ± 420	16
HCO-40	13 800 ± 2 150	70
Lecithin	230 ± 51	–[e]
Labrasol	350 ± 72	–[e]

[a]The ratio of paclitaxel to hydrophilic polymers was set at 1 : 3. [b]The ratio of paclitaxel/HP-β-CD/surfactant was fixed at 1 : 20 : 40. [c]Solubility is expressed as the solubility in water. Data are expressed as mean ± SD ($n = 3$). [d]Precipitation test was carried out with a paclitaxel concentration of 1000 μg/ml in saline solution. Data are expressed as mean value ($n = 3$). [e]Not tested

Moreover, liquid surfactants were not considered to be suitable for the preparation of the solid dispersion powder since they led to difficulties with drying. Thus HCO-40 was selected for paclitaxel solid dispersion, due to the solubility of the dispersion and its physical stability after dissolving in physiological saline.

The precipitation time of paclitaxel at a concentration of 1000 μg/ml in the absence of Cremophor EL was increased from 8 to 74 h as the ratio of paclitaxel to HCO-40 decreased from 1 : 20 to 1 : 55. At a ratio below 1 : 55, micellar systems of formulations were stable but had a high viscosity. Thus, the optimum final formulation of nano-scale paclitaxel solid dispersion for injection contained paclitaxel 5 mg, HP-β-CD 100 mg and HCO-40 200 mg. The solubility and precipitation time of the optimized final paclitaxel formulation were about >10 mg/ml and >70 h, respectively. Thus, it was concluded that the reconstituted paclitaxel solid dispersion could be diluted to a higher concentration than the normal clinical range, which made it possible to shorten the infusion time. Moreover, the diluted paclitaxel could be used within at least 24 h at clinical setting.

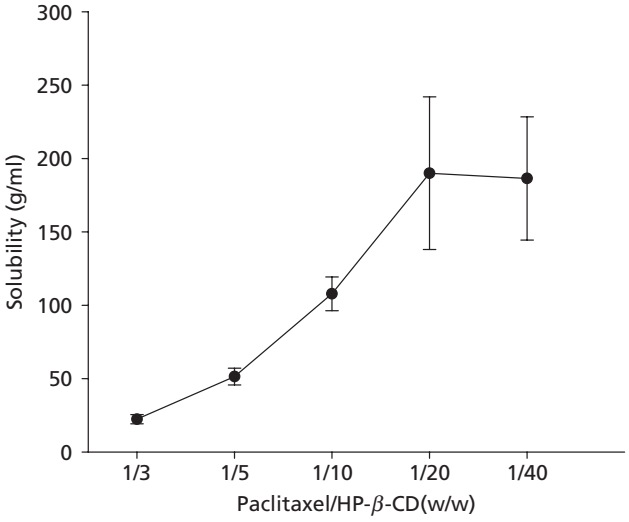
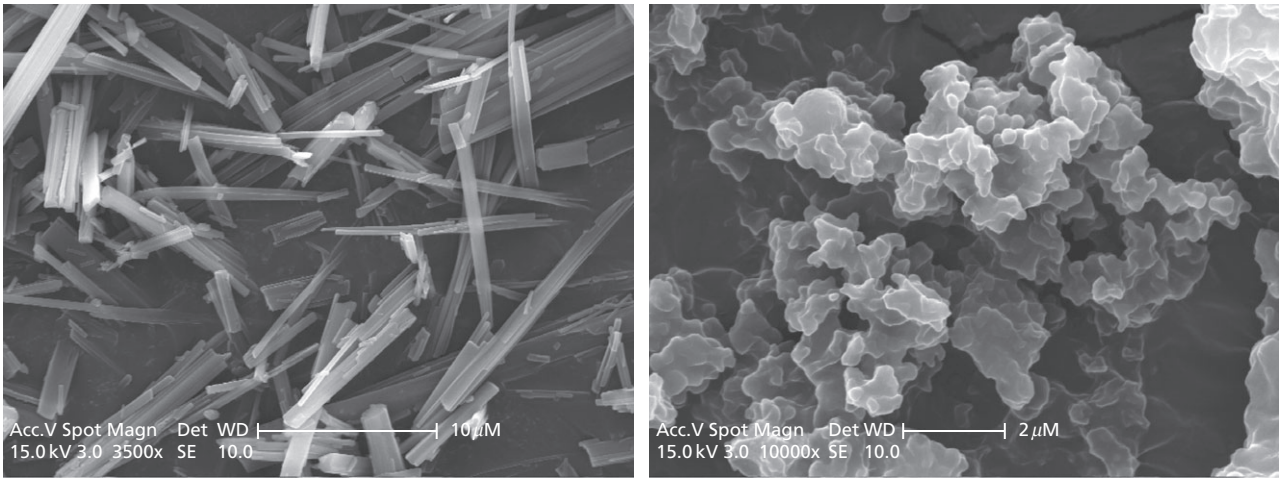


Figure 1 Effect of paclitaxel/HP-β-CD ratios on the solubility profiles of paclitaxel/polymer solid dispersion at 25°C. Data are expressed as mean ± SD ($n = 3$).

The crystalline particles of the paclitaxel solid dispersion powder in the optimized formulation were examined. Figure 2 shows SEM micrographs of paclitaxel pure material and paclitaxel solid dispersion powder. Paclitaxel raw material particles were long needle-shaped crystals with a wide particle size distribution (Figure 2a), whereas particles of SCF-treated paclitaxel were seen as pieces of spherical particles but appeared in the form of irregular particles (Figure 2b). Particles of paclitaxel solid dispersions formulated using the SAS process had a mean diameter of 370 ± 78 nm, which was well within the recommended limits for products intended for intravenous administration. The reduced particle size, increased surface area and the close contact between the hydrophilic polymers and paclitaxel solid dispersion might be responsible for the enhanced drug solubility in the solid dispersion.

DSC thermogram analysis provided qualitative and quantitative information on the physical status of the drug in the solid dispersion. The thermogram of pure paclitaxel exhibited an endothermic peak at about 216.6°C with an enthalpy of 12.979 J/g, corresponding to its melting point. In the case of paclitaxel solid dispersion particles prepared by the SAS process, the endothermic peak was absent (data not shown). These results are consistent with those for other SAS-processed particles.[19] This means that the physical state of paclitaxel changed from crystalline to amorphous or formed an inclusion complex during the SAS process. It is known that transforming the physical state of the drug to the amorphous or partially amorphous state leads to a high-energy state and high disorder, resulting in enhanced solubility. As a result, it was expected that solid dispersion particles would also have enhanced solubility.

FT-IR spectra are mainly used to determine whether there is any interaction between the drug and the polymeric carrier



(a) (b)

Figure 2 SEM micrographs. (a) paclitaxel raw material (X 3500); (b) solid dispersion (X 10 000).

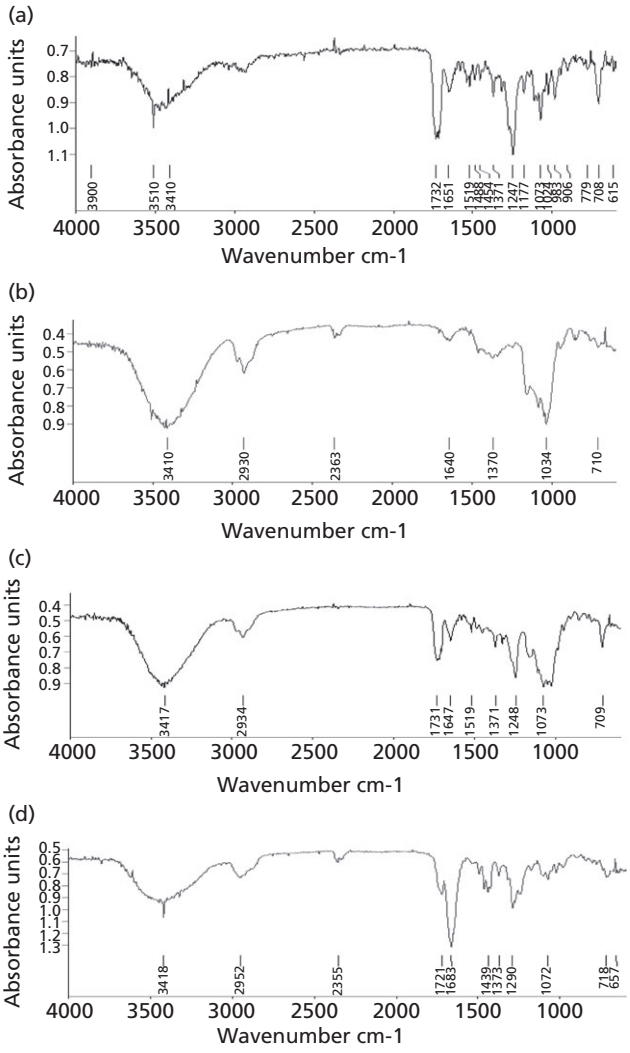


Figure 3 The FT-IR spectra. (a) paclitaxel raw material; (b) HP-β-CD as the hydrophilic polymer; (c) physical mixture of paclitaxel/HP-β-CD (1 : 3); (d) solid dispersion of paclitaxel/HP-β-CD.

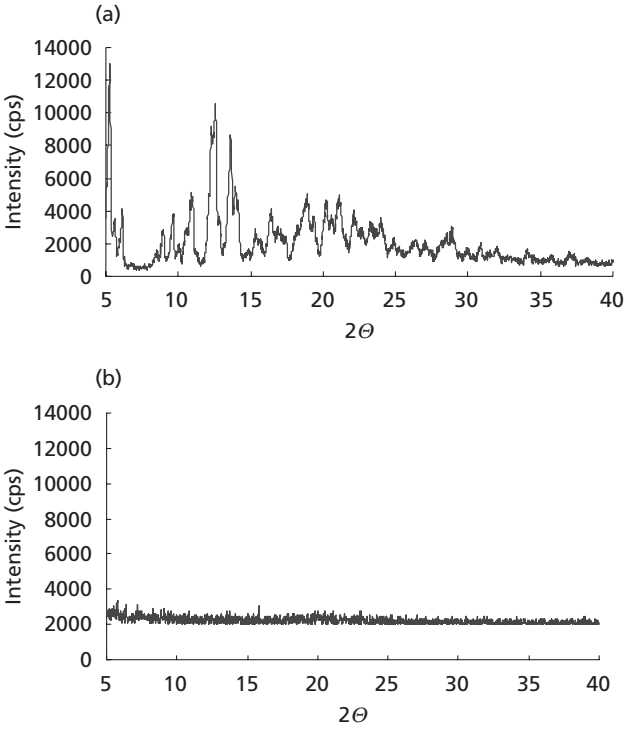


Figure 4 X-ray diffraction patterns. (a) paclitaxel raw material (X 3500); (b) solid dispersion.

used in the solid state.[20] As shown, the spectrum of paclitaxel (Figure 3a) exhibited the characteristic broad band of the OH group at 3510 cm^{-1}. The carbonyl stretching mode appeared at 1732 and 1651 cm^{-1}. Other characteristic bands were found at 1519 cm^{-1} (stretching vibration of C=C in the aromatic ring), 1247, 1177 cm^{-1} (C-O-C stretch) and 708 cm^{-1} (benzene ring with tetra-substitutions).

The spectrum of pure HP-β-CD (Figure 3b) illustrates the vibration of free OH between 3100 and 3700 cm^{-1} and that of the bound OH between 2800 and 3100 cm^{-1}, a shorter band between 1550 and 1700 cm^{-1}, and a large band that displayed distinct peaks in the region between 900 and 1200 cm^{-1}.[21] Broad bands of cyclodextrins overlap the main characteristic peaks of paclitaxel, and the paclitaxel peaks at 1732, 1651, 1371, 1247 and 708 cm^{-1} were also detected in the physical mixtures (Figure 3c). It was clear that some of the IR absorption peaks in the solid dispersion formulated using the supercritical fluid process (Figure 3d) differed from those of the corresponding physical mixtures, particularly the shape and location of the bands in the regions of 3100–3800, 2800–3100, 1600–1700 and 1000–1100 cm^{-1}.[22]

The carbonyl stretching peak of paclitaxel was shifted from 1732 cm^{-1} towards lower frequencies, and the peak of the tetra-substituted benzene ring was shifted from 708 cm^{-1} towards higher frequencies in the solid dispersions. This suggested hydrogen bonding interactions between paclitaxel and HP-β-CD. IR spectra did not show any dramatic changes in the characteristic peaks of paclitaxel frequency, suggesting the absence of chemical interactions between paclitaxel and the excipients, as also reported by Ford (1986).[23] The spectral changes were all related to the C–OH, C=O and C–O–C groups of paclitaxel and cyclodextrins, suggesting that the host–guest interactions were dominated by hydrogen bonds among the groups mentioned above. These findings are in full agreement with those of other authors.[24,25]

XRD provides information on crystallinity and crystal orientation, and the shape of the XRD patterns reflects the regular arrangement of molecules inside the crystals. Figure 4 shows the results of the XRD analysis. Many diffraction peaks of high intensity were observed in the diffraction pattern of paclitaxel raw material due to its crystallinity (Figure 4a). The particles prepared by the SAS process showed a significantly different pattern in which none of the peaks for crystalline paclitaxel was observed (Figure 4b). This result was comparable with that from the DSC thermogram of the paclitaxel raw material, indicating that the amorphous state of paclitaxel was formed in this processing condition.

Wherever possible, parenteral products should be isotonic. Typically, an osmolarity of 280–290 mOsm/l is targeted during formulation. This is essential for large volume

Table 2 Stability test of paclitaxel solid dispersion in accelerated conditions and stress conditions

Items tested	Limits (%)	Initial value	Accelerated condition (months)			Stress conditions (weeks)		
			1	2	6	1	2	4
Content of paclitaxel[a]	90.0–110.0	99.0 ± 0.8	98.7 ± 0.5	98.6 ± 0.6	98.2 ± 0.6	99.0 ± 0.3	99.4 ± 0.3	97.9 ± 0.3
pH (0.6 mg/ml in water)[b]	3.0–7.0	4.4	4.3	4.3	4.3	4.3	4.4	4.4
Related compounds								
Baccatin III[b]	≤0.8	0.002	0.002	ND	0.032	0.002	0.002	0.003
Ethyl ester side chain[b]	≤0.4	0.003	0.003	0.060	0.054	0.008	0.008	0.014
10-Deacetylpaclitaxel[b]	≤0.8	0.042	0.042	0.070	0.031	0.074	0.141	0.125
10-Deacetyl-7-epipaclitaxel[b]	≤0.5	0.005	0.005	ND	ND	0.005	0.005	0.005
7-Epipaclitaxel[b]	≤0.6	ND	ND	ND	ND	ND	0.003	0.003

Accelerated conditions: temperature, 0 ± 2°C; RH: 75 ± 5%, Stress conditions: 60°C . [a]Data are expressed as mean ± SD ($n = 3$). [b]Data are expressed as mean value ($n = 3$). ND, not detectable.

parenterals (LVPs) since either rapid dilution with blood will occur after injection, or the product itself will be diluted with an LVP before administration. In the case of the paclitaxel solid dispersion, when treated with 0.9% sodium chloride, the osmolality of solution containing the formulation (300 μg/ml paclitaxel) was 308 mOsmol/kg. When the paclitaxel concentration was increased up to 1000 μg/ml, the osmolality of the solution changed to 365 mOsm/kg, although the difference was not significant. These results indicated that the osmolality of the solution containing the paclitaxel solid dispersion was not affected greatly by paclitaxel concentration and that the prepared formulation can be administered at a high concentration.

The physical and chemical stability of the prepared paclitaxel solid dispersion was evaluated under accelerated and stress conditions. As shown in Table 2, the stability profiles of the paclitaxel solid dispersion did not significantly differ from the initial profile. The percentage of paclitaxel from the solid dispersion remaining after six months was 98.2%. When the solid dispersion was dissolved, the pH value of resulting solution maintained a value of 4.3–4.4 for the storage period, with no significant change during any interval. In the case of degraded compounds, although the amounts of baccatin and ethyl ester side chains increased, their levels remained low. These results led to the prediction that the formulation would also be stable at room temperature, with no change in the tested items.

For the stress stability study, the paclitaxel solid dispersion alone was investigated after storage for 0, 1, 2 and 4 weeks at 60°C. The results are shown in Table 2. After 4 weeks, each test item such as the remaining drug content and degradation compounds was comparable with the initial value. The amount of paclitaxel remaining was 97.9% and the pH value was 4.3–4.4. The degradation compounds of paclitaxel had not increased significantly. Thus, based on these data, the paclitaxel solid dispersion is thermally stable under high temperatures of 60°C for up to four weeks.

In accordance with previous results, paclitaxel processed by the supercritical fluid method changed from a crystalline form to an amorphous form. As the amorphous form of paclitaxel is thermodynamically unstable, it has a natural tendency to form the crystal form, which is more stable. Therefore, the physical stability of the paclitaxel solid dispersion was investigated in each storage condition using DSC. Our results showed that the peak of paclitaxel was not observed at 40°C, 75% RH after six months or at 60°C after four weeks. In addition, the glass transition temperature (Tg) of the paclitaxel solid dispersion remained at around 85°C without significant change during the storage periods (data not shown).These results indicate the acceptable physical stability of the paclitaxel solid dispersion.

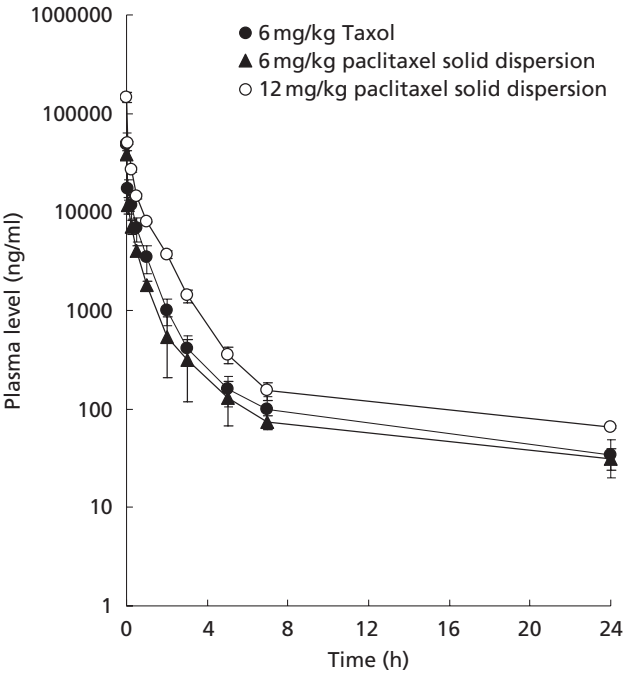


Figure 5 Time courses of paclitaxel level in rat plasma after intravenous administration of 6 mg/kg of Taxol and 6 mg/kg or 12 mg/kg of paclitaxel solid dispersion in male SD rats. Data are expressed as mean ± SD ($n = 5$).

An animal study was performed to evaluate the bioavailability of the paclitaxel solid dispersion. The plasma profile of paclitaxel in rats following intravenous administration of the reference injection (Taxol) was compared with that of the prepared paclitaxel solid dispersion. Figure 5 shows the time course of paclitaxel levels after intravenous administration of the paclitaxel solid dispersion at doses of 6 mg/kg and

Table 3 Average non-compartmental pharmacokinetic parameters of paclitaxel obtained after intravenous administration of paclitaxel solid dispersion and Taxol in male SD rats

Parameters	Taxol (6 mg/kg)	Paclitaxel solid dispersion	
		(6 mg/kg)	(12 mg/kg)
λ_z (h^{-1})	0.076 ± 0.007	0.062 ± 0.010*	0.073 ± 0.019
$t^1/_2$ (h)	9.2 ± 0.9	11.4 ± 1.8*	9.9 ± 2.5
T_{max} (h)	0.017 ± 0.000	0.017 ± 0.000	0.017 ± 0.000
C_{max} (ng/ml)	49 273.0 ± 14 784.8	39 577.7 ± 9 363.2	146 999.8 ± 26 960.0**
AUC_{all} (ng·h/ml)	15 330.1 ± 4 515.5	10 524.7 ± 3 272.2	39 095.8 ± 1 780.7**

All values are expressed as mean ± SD. *$P < 0.05$ and **$P < 0.001$, compared with Taxol (Kruskal-Wallis test).

12 mg/kg compared with Taxol injection (6 mg/kg) in male SD rats.

The pharmacokinetic parameters, calculated from the time profiles, revealed a nonlinear disposition of the drug, which may have significant implications in that greater-than-expected increases in systemic exposure may result from a given increase in dose. For example, at a dose of 6 mg/kg the paclitaxel solid dispersion resulted in a mean C_{max} of 39 577.7 ng/ml and a mean AUC_{all} of 10 524.7 ng·h/ml, whereas at 12 mg/kg, the mean C_{max} and AUC_{all} values were 146 999.8 ng/ml and 39 095.8 ng·h/ml, respectively (Table 3, Figure 5). Thus, a 100% increase in dose resulted in a 371% increase in the C_{max} and a 372% increase in the AUC_{all}. Based on these results, the pharmacokinetic values of paclitaxel solid dispersion increased disproportionately with doses increasing from 6 to 12 mg/kg in rats. However, the relative bioavailability of the paclitaxel solid dispersion at 6 and 12 mg/kg compared with the reference (Taxol) was 68.7% and 126.7%, respectively.

The paclitaxel concentrations in various organs after intravenous administration are presented in Figure 6. The paclitaxel levels in the liver, lung and kidney after administration of 6 mg and 12 mg (paclitaxel)/kg paclitaxel solid dispersion were 8190, 3810 and 5470, and 15 670, 8230 and 9220 ng/g, respectively. After administration of a 6 mg/kg dose of Taxol, the paclitaxel levels in the same organs were 7630, 3400 and 4710 ng/g. However, the paclitaxel level in the brain was negligible because it shows poor penetration into the central nervous system.[26] These findings seemed to be consistent with the literature,[27] which also reported an over-proportionate increase in plasma level of paclitaxel upon dosage escalation with doses of paclitaxel solid dispersion increasing from 6 to 12 mg/ kg, although the paclitaxel levels in organs increased proportionately.

Our previous study suggested that paclitaxel solid dispersion free of Cremophor EL caused less toxicity than Taxol in mice treated with a single intravenous administration of drug. Furthermore, the lower toxicity of paclitaxel solid dispersion might allow paclitaxel to be given at a higher dose.[28] Thus, judging by the results from the pharmacokinetic study, this injectable solid dispersion of paclitaxel might increase the maximum efficacy of paclitaxel for treatment of cancer.

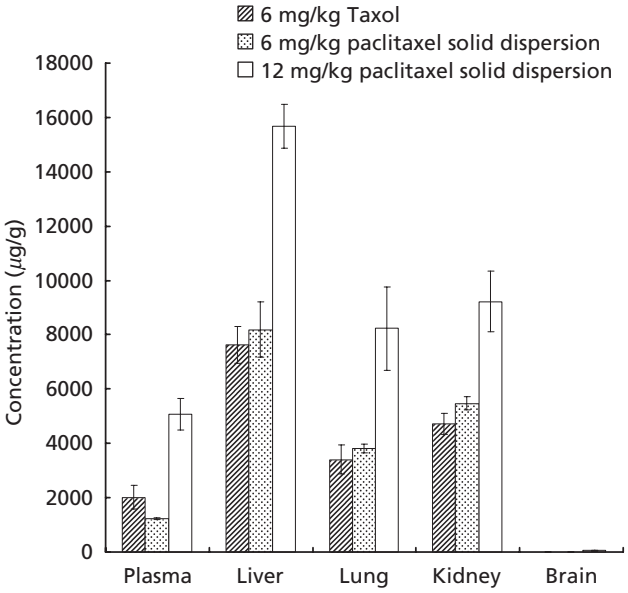


Figure 6 Paclitaxel level in rat plasma and organs at 1 h after intravenous administration of 6 mg/kg of Taxol and 6 mg/kg or 12 mg/kg of paclitaxel solid dispersion. Data are expressed as mean ± SD ($n = 3$).

Conclusion

In this study, a Cremophor-free, injectable paclitaxel solid dispersion formulation was developed by using a supercritical antisolvent process. The optimized solid dispersion system containing paclitaxel/HP-β-CD/HCO-40 at a weight ratio of 1 : 20 : 40 provided 10 000-fold increase in paclitaxel solubility. This system was found to be physically stable for at least six months or four weeks in accelerated conditions (40 ± 2°C; RH: 75 ± 5%) and stress conditions (60°C), respectively. The precipitation time of paclitaxel solid dispersion in 0.9% sodium chloride injection at a concentration of 1000 μg/ml was above 70 h at room temperature, showing a physical stability after reconstitution. After intravenous administration of paclitaxel solid dispersion to rats at a dose of 6 mg/kg, the pharmacokinetic parameters were not significantly different from those of commercial product, but the levels of paclitaxel in various organs in the solid dispersion group were higher with comparison with those in the control group. As the dose was increased from 6 mg/kg to 12 mg/kg, both plasma C_{max} and AUC_{all} were over-proportionally increased by 3.7 times, whereas the paclitaxel levels in organs increased proportionately. The novel injectable paclitaxel solid dispersion appears to offer advantages of better physicochemical properties and safety, as well as improved

convenience (shorter infusion time) and pharmacokinetic characteristics in rats.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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