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Pharmacokinetics and biodistribution of polymeric micelles of paclitaxel with Pluronic P105/poly(caprolactone) copolymers

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A novel polymeric micellar formulation of paclitaxel (PTX) with Pluronic/poly(caprolactone) (P105/PCL_{50}) has been developed with the purpose of improving *in vitro* release and *in vivo* circulating time of PTX in comparison to the current Taxol injection. This study was designed to investigate the preparation, *in vitro* release, *in vivo* pharmacokinetics and tissue distribution of the PTX-loaded, biodegradable, polymeric, P105/PCL_{50} micelle system. The drug-loaded micelles were prepared by dialysis using the hydrophobic drug, PTX, and the nonionic surfactant Pluronic P105 modified with a low molecular weight PCL. The results of dynamic light scattering (DLS) experiment indicated that the PTX-loaded micelles had a mean size of approximately 150 nm with narrow size distribution (polydispersity index < 0.3). The *in vitro* release study showed that the release of PTX from the micelles exhibited a sustained release behavior. A similar phenomenon was also observed in a pharmacokinetic assessment in rats, in which $t_{1/2\beta}$ and AUC of the PTX micelle formulation were 4.0 and 2.2-fold higher than that of Taxol injection. The biodistribution study in mice showed that the PTX micelle formulation not only decreased drug uptake by the liver, but also prolonged drug retention in the blood, and increased the distribution of drug in kidney, spleen, ovaries and uterus. These results suggested that the P105/PCL_{50} polymeric micelles may efficiently load, protect and retain PTX in both *in vitro* and *in vivo* environments, and could be a useful drug carrier for i.v. administration of PTX.

1. Introduction

Polymeric micelles possess several advantages compared to micelles formed from low molecular weight surfactants, including better structural stability, slower dissociation rate, lower critical micelle concentration (CMC), easier control of particle size and improved solubilization of hydrophobic drugs (Ha et al. 1999; Lin et al. 2006). They can accumulate anticancer drugs in tumors, release drugs for an extended time, and prevent rapid clearance by the reticular endothelial system (RES) due to their size and surface characteristics (Liu et al. 2000). They have been applied in the field of drug targeting because of their unique characteristics in the body. In general, amphiphilic block or graft copolymers composed of hydrophilic and hydrophobic segments will self-assemble into polymeric micelles when their concentration is above the CMC. Currently, many types of block copolymers are used to deliver anticancer drugs into various tumors, including poly(ether)-*b*-poly(ester), poly(ether)-*b*-poly(L-amino acid), poly(ether)-*b*-polycation, poly(ether)-*b*-poly(ether), etc (Jones and Leroux 1999; Kumar et al. 2001).

Among these block copolymers, Pluronics, a triblock, non-ionic, macromolecular, surface active agent consisting of poly(ethylene glycol) and poly(propylene glycol) (PEO-*b*-PPO-*b*-PEO), have commonly been used as micellar nanocontainers to solubilize hydrophobic drugs (Kabanov and Alakhov 2002). Recently, it was found that Pluronics, as a biological response modifier, could interact with multi-drug resistant cancer cells resulting in drastic sensitization of these tumors with respect to doxorubicin (Alakhov et al. 1999) or other anticancer agents including paclitaxel (PTX) (Wang et al. 2007). Pluronics have been used in pharmaceutics for the controlled release of drugs, which can prolong blood circulation time and change the biodistribution of drugs. Illum et al. demonstrated that it was possible to significantly change the *in vitro* interaction with isolated macrophages and the biodistribution of polystyrene nanospheres after coating the particle surface with Pluronic block copolymers (Dunn et al. 1997; Illum et al. 1987). In addition, we also reported that PTX-loaded Pluronic P123 micelles could effectively prolong blood circulation time and modify the biodistribution of PTX *in vivo* (Han et al. 2006), and exert higher cytotoxicity against a resistant human ovarian tumor cell line, SKOV-3/PTX (unpublished work). Previously, we reported on a PTX micelle formulation employing Pluronic P105, and have demonstrated that the PTX micelles could increase the cytotoxicity against MCF-7/ADR, a resistant breast tumor cell line (Wang et al. 2007). However, the drug-loading contents and encapsulation ratio of PTX-loaded Pluronic P105 micelles are relatively low compared with other

PTX-loaded polymeric micelles reported elsewhere. In order to improve the loading characteristics of PTX-loaded Pluronic P105 micelles, the hydroxyl functional groups at the chain ends of Pluronic P105 were modified with poly(epsilon-caprolactone) (PCL), and a new triblock copolymer, PCL-P105-PCL, was synthesized. It was demonstrated that the PTX-loaded P105/PCL micelles with higher drug-loading contents and encapsulation ratio showed higher cytotoxicity against MCF-7/ADR compared with PTX-loaded P105 micelles (unpublished work).

The aim of this study was to evaluate the influence of the new triblock polymeric micelle carrier (PCL-P105-PCL) on the pharmacokinetics and biodistribution of anticancer drug, PTX, in rats and mice. These preliminary results will provide the basis for evaluating the *in vivo* antitumor efficacy of the new polymeric, PTX-loaded, P105/PCL micelle.

2. Investigations and results

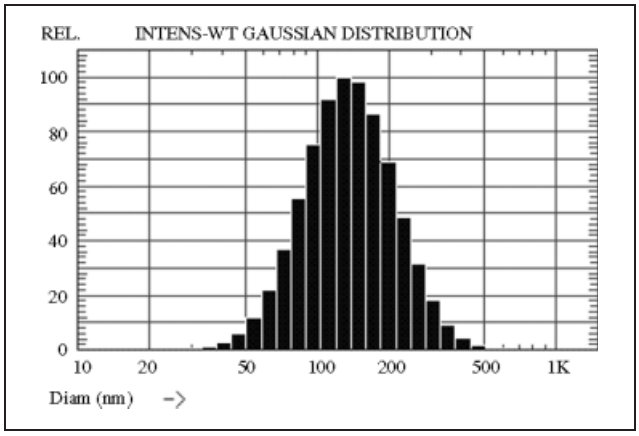
2.1. Preparation and characterization of PTX-loaded P105/PCL micelles

The physicochemical characteristics of the P105/PCL_{50}, one of three P105/PCL block copolymers, used are presented in Table 1. The molecular weight of the Pluronic P105/PCL_{50} block copolymers determined by ^{1}H NMR spectroscopy and GPC measurement exhibited similar values. Both measurements showed that Mn (number average molecular weight) of the copolymer was ca. 14478 Da, and the results of GPC measurement indicated that the copolymer had a low molecular weight distribution with polydispersity index of 1.39. The CMC value of the copolymer determined using pyrene fluorescence spectroscopy method was found to be ca. 0.0007%. Amphiphilic copolymers can form spherical micelles when their concentration is at or above their CMC in an aqueous environment. Hydrophobic drugs can physically be incorporated within the core of the polymeric micelles by hydrophobic interactions. In this work, the PTX-loaded P105/PCL_{50} micelles were prepared by a dialysis method in an aqueous environment. The mean diameter and particle size distribution of the P105/PCL_{50} micelles were determined by dynamic light scattering (DLS) measurement. The mean diameter of the PTX-loaded micelles was 149.0 ± 65.4 nm, and the micelles showed a narrow size distribution with polydispersity index of 0.26. Figure 1 shows a typical size distribution of the PTX-loaded micelles. In addition, the mean size of the PTX-unloaded micelles was ca. 145.0 ± 53.8 nm, which was not significantly different from the PTX-loaded micelles ($P > 0.05$). The drug loading coefficient (DL%) and encapsulation ratio (ER%) of the PTX-loaded micelles were 5.43% and 76.53%, respectively. Furthermore, the morphology of the PTX-loaded micelles observed with TEM (transmission electron microscope) revealed the micelles had a spherical shape and no drug crystal was visible (Data not shown).

Table 1: Selected characteristic parameters of Pluronic P105/PCL_{50} block copolymer and PTX polymeric micelles

Block copolymer		P105/PCL_{50}
Physicochemical characteristics	CMC[a], %wt	0.0007
	Mn[b]	14478
	Polydispersity (Mw/Mn)[c]	1.39
Optimum micellar PTX	DL%	5.43
	ER%	76.53
	Particle size (nm)	149.0 ± 65.4

Note: Drug loading coefficient, DL; Encapsulation ratio, ER; Critical micelle concentration, CMC; Number Average Molecular Weight, Mn.
[a] CMC values were determined using pyrene fluorescence spectroscopy method.
[b] Determined by ^{1}H-NMR spectroscopy ($CDCl_3$).
[c] Measured by GPC analysis.



2.2. In vitro release of PTX from the polymeric micelles

PTX was continuously released from the P105/PCL_{50} micelles in the release medium containing 1 mol/L sodium salicylate for 24 h at 37 °C, and PTX release from PTX stock solution and Taxol injection were used as controls in this release experiment. The theoretical maximum concentration of PTX in this aqueous medium is about 2.0 μg/mL assuming complete release of the PTX incorporated in the polymeric micelles, while the solubility of PTX in this release medium is about 28.1 μg/mL (Cho et al. 2004). Therefore, this system provides a good sink condition for PTX. As shown in Fig. 2, the in *vitro* cumulative release profile of PTX illustrates that only 66.8% PTX was released from the micelles, while 95.2% PTX was released from Taxol injection over a 6 h period. During the period, the PTX release from the micelles showed a significant difference compared with Taxol injections ($P < 0.05$). However, 100% PTX was released from the PTX stock solution during the same period, and showed no significant difference compared with Taxol injections ($P > 0.05$).

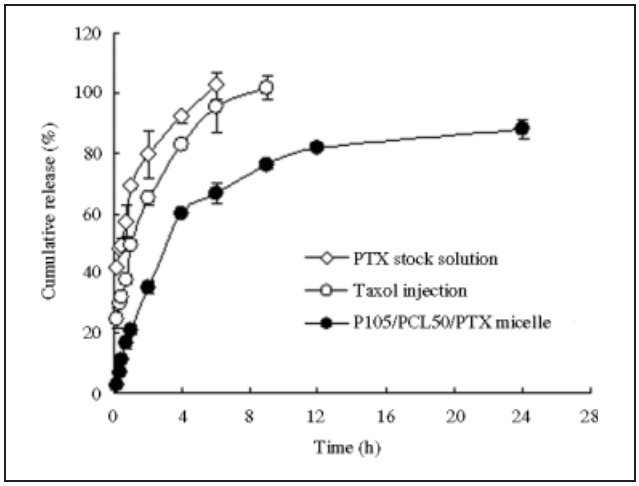


Table 2: Pharmacokinetic parameters of PTX after i.v. administration of Taxol injection and P105/PCL_{50}/PTX micelles to rats (n = 6)

Parameters	Unit	Taxol injection	P105/PCL_{50}/PTX Micelle
$t_{1/2\alpha}$	h	0.11 ± 0.03	$0.49 \pm 0.18^{*}$
$t_{1/2\beta}$	h	1.27 ± 0.23	$5.14 \pm 0.87^{*}$
K_{21}	h^{-1}	2.49 ± 0.72	$0.55 \pm 0.29^{*}$
K_{10}	h^{-1}	1.62 ± 0.59	$0.42 \pm 0.10^{*}$
K_{12}	h^{-1}	3.22 ± 1.07	$0.76 \pm 0.35^{*}$
$AUC_{0\sim12h}$	$\mu g \cdot L^{-1} \cdot h$	4215.15 ± 2375.50	$9217.34 \pm 2333.70^{*}$
Cl	$L \cdot h^{-1} Kg^{-1}$	1.73 ± 0.74	$0.70 \pm 0.23^{*}$
V	$L \cdot Kg^{-1}$	1.06 ± 0.22	$1.72 \pm 0.62^{**}$

Note: ** $P < 0.05$, * $P < 0.01$, denotes significant difference between the PTX micelle formulation and the Taxol injection (reference formulation)

During a 24 h period, the micelles cumulatively released ca. 88.2% PTX.

2.3. Pharmacokinetics of PTX-loaded polymeric micelles

A validated HPLC method was used for the quantitative analysis of PTX in the analytical samples. Linearity in the standard curves was demonstrated over the concentration range studied, and endogenous components had no interference in the chromatograms. Pharmacokinetic parameters were calculated with a 3P97 computer program from plasma concentration-time data using a single-dose, two-compartment model with the 1/C weight. Figure 3 shows the mean plasma concentration-time profiles of PTX in blood after i.v. administration of PTX-loaded micelles and Taxol injection at a single dose of 6 mg/kg, respectively. The concentration of PTX delivered by the micelles was higher than that of Taxol injection during the time of the assay, and the concentration levels showed a rapid decline in the distribution phase for both preparations. The related pharmacokinetic parameters are listed in Table 2. The $t_{1/2\alpha}$ (distribution half-life) showed significant difference ($P < 0.01$) between two groups, and the corresponding values were 0.11 h and 0.49 h for the micelles and Taxol injection, respectively. The $t_{1/2\beta}$ (elimination half-life) of the micelles and Taxol injection was 5.14 h and 1.27 h, respectively. The statistical analysis of the $t_{1/2\beta}$, K_{10} (central compartment elimination rate constant), K_{12} (elimination rate constant from central to peripheral compartment), K_{21} (elimination rate constant from peripheral to central compartment), Cl (clearance) showed significant differences between two groups ($P < 0.01$). The data indicated that the PTX micelle formulation increased the systemic circulation time of PTX, which was in agreement with the *in vitro* release of PTX from the micelles. Meanwhile, the AUC (area under the plasma concentration-time curve) of PTX in the micelles was higher than that in Taxol injection. The former provided significantly higher (2.2 fold) AUC compared to the latter ($P < 0.01$).

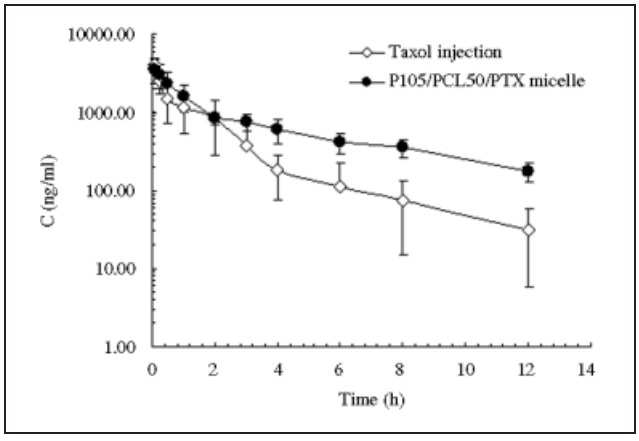


Fig. 3: Mean plasma concentration-time profiles of PTX after i.v. administration of a single 6 mg/kg dose of Taxol injection and P105/PCL_{50}/PTX micelles to rats (Each point represents the mean ± SD of 6 rats)

2.4. Biodistribution of PTX-loaded polymeric micelles

In vivo behavior of PTX after i.v. administration of the polymeric micelles to mice was investigated with Taxol injection as a control. Figure 4 presents the mean concentration-time profiles of PTX in unit mass of plasma, heart, spleen, lung, liver, kidney, ovary and uterus of mice. The total amount of drug accumulated in each organ within 8 h (AUC_{0-8h}) was then calculated and is shown in Table 3. The PTX AUC_{0-8h} of the polymeric micelles was lower in liver, and higher in plasma, ovary and uterus, spleen, and kidney compared to the control. There were statistically significant differences in plasma, liver, spleen, kidney and ovary/uterus between the PTX micelle formulation and Taxol injection ($p < 0.05$). No significant difference was observed in heart and lung. The AUC_{0-8h} of the tissues for Taxol injection in a descending order was liver > lung > spleen > kidney > ovary/uterus > heart > plasma. In contrast, the corresponding order for the PTX-loaded micelles was liver > ovary/uterus > lung > kidney > spleen > heart > plasma.

Table 3: AUC_{0-8h} of PTX in plasma and tissues after i.v. administration of Taxol injection and P105/PCL_{50}/PTX micelles to mice (n = 4)

	Plasma	Liver	Spleen	Lung	Kidney	Heart	Ovary/uterus
Taxol injection ($\mu g \cdot h \cdot g^{-1}$)	1.82	68.62	21.06	24.7	20.99	15.07	20.77
P105/PCL_{50}/PTX Micelles ($\mu g \cdot h \cdot g^{-1}$)	3.26	58.41	25.87	28.72	25.94	14.74	31.47
Ratio[a]	1.79*	0.85*	1.23*	1.16	1.24*	0.98	1.52*

Note: [a] The ratio was AUC (micelle)/AUC (Taxol); * $P < 0.05$: PTX micelle vs. Taxol injection

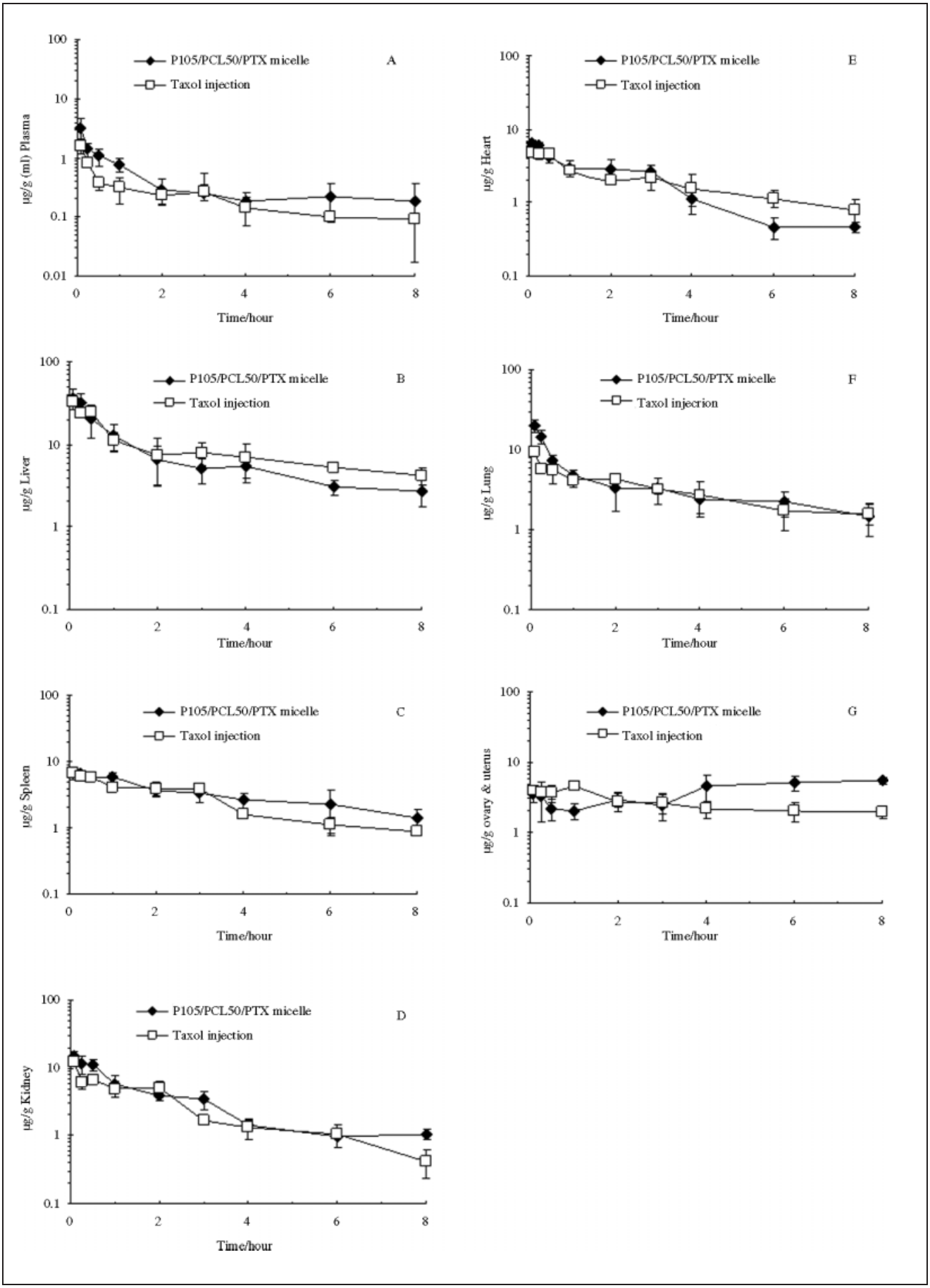


Fig. 4: Mean concentration-time profiles of PTX in (A) plasma, (B) liver, (C) spleen, (D) kidney, (E) heart, (F) lung and (G) ovary/uterus following i.v. administration of a single 3 mg/kg dose of Taxol injection and P105/PCL_{50}/PTX micelles to mice (each point represents the mean ± SD of 4 mice)

3. Discussion

PTX is a lipophilic drug. The solubility of PTX in aqueous medium at different pH conditions is about 1.0 μg/ml (Zhang et al. 2005). PTX-loaded P105/PCL_{50} micelles were prepared by a dialysis method, by which PTX was effectively encapsulated into the P105/PCL_{50} micelles. The average particle size of the micelles was about 150 nm with a narrow particle size distribution. The entrapment efficiency of PTX in the P105/PCL_{50} micelles was about 77% (Table 1), which is higher than that of single Pluronic P105 micelles (only 54.5%) (Wang et al. 2007). This may be partially due to increased hydrophobic interaction between PTX and the PCL inner cores of the micelles. It was reported that Pluronic P105 micelles have 'liquid-like' cores, which easily dissociate into unimers upon dilutions below the CMC (Gao et al. 2005), and its solubilization strength for PTX is quite low (Wang et al. 2007). In contrast, the P105/PCL_{50} micelles have 'solid-like' PCL inner cores, which could easily provide structural integrity to micelle-encapsulated hydrophobic drugs.

It is a challenge to maintain a good sink condition for PTX in designing *in vitro* release experiments of its polymeric micelles. Inclusion of surfactants in release media is the most popular method for *in vitro* PTX release (Cho et al. 2004; Suh et al. 1998), however, the addition of surfactants in release media might have an adverse effect on the micellar structure and distort the release profiles. Cho et al. (2004) reported that 1.0 mol/L sodium salicylate solution could solubilize PTX and maintain the sink condition for PTX in aqueous release media, which did not significantly affect the physical stability of micelles. Therefore, in the *in vitro* release experiment, we used 1 mol/L sodium salicylate solution for drug release. Almost all PTX in the PTX stock solution was rapidly released in 6 h, which indicates that the hindrance effect of the dialysis bag is minimal for *in vitro* release of PTX. The release profile of PTX from Taxol injection was similar to the PTX stock solution. The cumulative release amount of PTX from Taxol injection in 6 h was around 95%. However, there was a sustained release observed from the PTX-loaded P105/PCL_{50} micelles. The percentages of drug released in 6 h and in 24 h were only 66.8% PTX and 88.2% PTX, respectively, which was much slower than with Taxol injection. The cumulative release amount of the micelles was reduced 1.4-fold in 6 h due to micelle carriers compared with the Taxol injection. This indicates that the micellar carrier can not only solubilize the water-insoluble drug but also sustain its release *in vitro*.

In comparison with the *in vitro* sustained release behavior of PTX from the PTX-loaded P105/PCL_{50} micelles, a similar phenomenon was also observed in the *in vivo* pharmacokinetic assessment in rats. The PTX micelles had a slower clearance (Cl), as well as higher $t_{1/2\beta}$ and AUC than the Taxol injection ($p < 0.05$). The $t_{1/2\beta}$ and AUC of the former were 4.0 and 2.2-fold higher than that of the latter. This result indicates that the hydrophilic shell of Pluronic P105 could make the PTX micelles avoid the recognition and uptake by the reticuloendothelial systems (RES) and prolong the time of blood circulation of PTX. The higher AUC in plasma, and lower AUC in liver of the PTX micelles than Taxol injection in biodistribution investigation in mice were also a result of this effect. However, compared to Taxol injection, a slightly higher concentration of PTX was found in the spleen following i.v. administration of the micelles. Presumably, the PTX micelles can only partly avoid the recognition and uptake by the RES system because of the intermediately hydrophilic shell formed by Pluronic P105 segments (HLB = 15) of the P105/PCL_{50} copolymers. Generally, amphiphilic polymeric micelles can reduce PTX uptake by the kidney. However, the AUC of the PTX micelles in kidney was higher than the Taxol injection in our research, which may be explained by the biological properties of Pluronic P105. It was reported earlier that Pluronics were excreted primarily through the kidneys. Their clearance in rats, dogs, and humans were shown to be almost entirely by renal excretion (Grindel et al. 2002). Other studies have also shown that the formation of micelles do not have an effect on the elimination clearance of the block copolymers (Batrakova et al. 2004). The biodistribution study of Pluronic P105 micelles has shown that the concentration of this material accumulated in kidney cells is higher than the other organs in mice after i.p. injections (Gao et al. 2004). Therefore, Pluronic P105/PCL_{50} micelles leading to an increment in AUC of PTX in kidney could be induced.

In biodistribution studies, both the ovaries and uterus are too small to analyze individually, therefore, the results have been combined. Interestingly, PTX concentrations in the ovaries and uterus were relatively high after i.v. injection of the micelles compared with Taxol injection ($P < 0.05$). This may be beneficial in treatment of ovarian carcinoma because PTX had been proved to be active to ovarian tumor (Rowinsky and Donehower 1995). However, the drug concentrations in the brain were so low that most of these samples could not be accurately measured because the concentrations were below the LLOQ (lower limit of quantitation) of 50 ng/ml in our work. This phenomenon was consistent with the previous work reported by other reseachers (Rowinsky and Donehower 1993).

In summary, the *in vitro* characteristics of the PTX-loaded P105/PCL_{50} micelles and the *in vivo* pharmacokinetics and biodistribution of PTX delivered by the micelle carriers has been demonstrated. The micelle carriers may efficiently load, protect and retain PTX in both *in vitro* and *in vivo* environments. *In vitro* release profiles indicated that the release of PTX from the micelles exhibited a sustained release behavior. A similar phenomenon was also observed in the *in vivo* pharmacokinetic study of rats. Incorporating PTX in the P105/PCL_{50} polymeric micelles not only decreased drug uptake by the liver, but also prolonged the drug retention in the blood as well as increased the distribution of drug in kidney, spleen, ovaries and uterus. These results suggest that the polymeric micelle formulation can provide a useful alternative dosage form for i.v. administration of PTX.

On the basis of these preliminary results, a study on evaluating *in vivo* antitumor efficacy of PTX-loaded P105/PCL_{50} micelles on BALB/c nude mice bearing s.c. human resistant ovarian SKOV-3/PTX carcinoma is in progress in our laboratory.

4. Experimental

4.1. Drugs and reagents

Pluronic P105 was kindly supplied by BASF China Ltd. (Shanghai, China) and used without further treatment. Stannous octoate [$Sn(OOCC_7H_{15})_2$] and ε-caprolactone monomer were obtained from Sigma Chemical Co. (St. Louis, MO, USA). PTX was purchased from Xi'an Sanjiang Bio-Engineering Co. Ltd. (Xi'an, China). Taxol injection (Anzatax injection concentrate, 30 mg/5 mL) was produced by FH Faulding trading (Australia). Diazepam was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All reagents for HPLC, including

acetonitrile and methanol, were of HPLC grade. Other reagents were of analytical grade.

4.2. Animals

Sprague-Dawley rats (220 ± 30 g) and female Kunming strain mice (20 ± 5 g) were supplied by the Laboratory Animal Center of Fudan University, Shanghai, China. The animals were used following the guideline of the Ethical Committee for Animal Experiments of Fudan University. The animals were acclimatized at 25 ± 2 °C and 70% ± 5% relative humidity under natural light/dark conditions for at least 24 h before dosing.

4.3. Preparation of PTX-loaded P105/PCL micelles

Amphiphilic block copolymers (P105/PCL) were synthesized by ring opening polymerization of epsilon-caprolactone monomer in the presence of PEO-*b*-PPO-*b*-PEO triblock copolymer, Pluronic P105, using stannous octoate as a catalyst (Ha et al. 1999; Kim et al. 2000). Three kinds of Pluronic P105/PCL block copolymers were synthesized by varying the feed conditions of two components. The structures of block copolymers, P105/PCL, were confirmed by Fourier transform infrared spectroscopy (FT-IR). The molecular weight and molecular weight distribution of the block copolymers were determined by gel permeation chromatography (GPC) and ^{1}H NMR. The details will be reported elsewhere.

One of three P105/PCL block copolymers, designated as P105/PCL_{50}, was used in this work. PTX-loaded P105/PCL_{50} micelles were prepared by a dialysis method (Kim et al. 2000; Kim and Lee 2001). Briefly, 240 mg of the P105/PCL_{50} block copolymer were dissolved in 5 ml of dimethylformamide (DMF) followed by the addition of 18 mg of PTX, and stirred at room temperature. In order to form PTX-loaded polymeric micelles and to remove free PTX and organic solvent, the solution was dialyzed against 3 L of ultrapure water for 24 h using a cellulose dialysis bag (MWCO: 12,000–14,000, size: 21/35). It was then centrifuged to eliminate unloaded PTX and aggregated particles. The supernatant, micellar solution, obtained in this process, was collected as the final PTX micelle formulation.

4.4. Characterization of the micelles

The CMC value of the P105/PCL_{50} copolymer was determined using pyrene fluorescence spectroscopy (Ge et al. 2002). The mean diameter and particle size distributions of the polymeric micelles were determined by DLS measurement using a NICOMP 380 ZLS Zeta Potential/Particle Sizer (PSS Nicomp, Santa Barbara, CA, USA) equipped with a 5 mW helium-neon laser at 632.8 nm. The morphological examination of the micelles was performed using a TEM (Philips CM120, Netherlands) (Han et al. 2006).

4.5. In vitro release of PTX from the micelles

The *in vitro* release properties of PTX from the micelles were investigated in an aqueous medium containing 1 mol/L sodium salicylate by a dialysis method (Cho et al. 2004). One mL of freshly prepared PTX micelle solution (0.1 mg/mL PTX) was introduced into a dialysis bag (MWCO: 14 kDa, Greenbird, Shanghai, China). The end-sealed dialysis bag was immersed into 50 mL of 1 mol/L sodium salicylate solution at 37 °C. The release medium was stirred at the speed of 75 rpm for 24 h. 0.5 mL samples were withdrawn at different time intervals (0, 10, 20, 30, 45 min, and 1, 2, 4, 6, 9, 12, 24 h), and replaced with an equal volume of fresh medium. The concentration of PTX in the samples was determined by the HPLC method described below. PTX release from PTX stock solution and Taxol injection placed in a dialysis bag was conducted under the same conditions as controls.

4.6. Pharmacokinetic studies

Twelve Sprague-Dawley rats were used to investigate the effect of micellar formulation on the pharmacokinetics of PTX after i.v. administration. Rats were divided into 2 groups at random, and given a single 6 mg/kg dose of the PTX micelle formulation or Taxol injection by tail-vein injection. As a control, Taxol injection was diluted 6-fold to 1 mg/mL with 5% glucose solution shortly before administration. The concentration of PTX in the P105/PCL_{50} micelles for the pharmacokinetic study was about 1 mg/mL. Blood samples (0.5 mL) were collected into heparinized tubes from the femoral artery at 0, 5, 15, 30 min, 1, 2, 3, 4, 6, and 8 h after i.v. administration. Blood was immediately processed for plasma by centrifugation at $1000 \times g$ for 10 min. Plasma samples were frozen and maintained at −20 °C until analysis.

4.7. Biodistribution studies

Seventy-two female Kunming strain mice were used in the experiment to assess the effect of micellar formulation on the biodistribution of PTX after i.v. administration. The mice were divided into 2 groups at random and given a single 3 mg/kg dose of either the PTX micelle formulation or Taxol injection by tail-vein injection. At 5, 15, 30 min, 1, 2, 3, 4, 6, and 8 h after injection, subject animals ($n = 4$ for each time point) were euthanized, and heart, spleen, lung, liver, kidney, ovary/uterus, brain as well as blood samples were collected. Tissue samples were washed in ice-cold saline, blotted with paper towel to remove excess fluid, weighed and stored at −70 °C until analysis by HPLC.

4.8. HPLC analysis

The analysis of PTX *in vitro* and *in vivo* was carried out using an RP-HPLC method on a system equipped with a LC-10ATVP pump, a SPD-10AVP UV-Vis detector (Shimadzu, Kyoto, Japan), and HS2000 interface (Empire Science & Tech, Hangzhou, China) operated at 230 nm. A reversed-phase column (Gemini 5 μm C18, 150×4.6 mm, Phenomenex, California, USA) was used at room temperature. The mobile phase consisted of acetonitrile and ammonium acetate buffer solution (10 mmol/L, pH 5.0) (50 : 45, v/v) was freshly prepared for each run and degassed before use. 20 μL of samples were injected into the HPLC column for all the analyses. The flow rate for mobile phase was 1.0 mL/min, and the retention time of PTX was about 8.2 min.

Tissue samples were homogenized in a mixed solution of acetonitrile and water (50 : 50, v/v). Diazepam (1 μg/mL, 50 μL) as an internal standard was added into 200 μL of plasma or tissue samples, and vortexed for 1 min. The drug and internal standard were then extracted into 3 mL of anhydrous diethyl ether by vortex mixing for 2 min. After centrifugation at $6000 \times g$ for 10 min, the clear supernatant was removed and evaporated under a gentle stream of nitrogen. The residue was then dissolved by 100 μL acetonitrile and centrifuged at $1500 \times g$ for 5 min before HPLC analysis.

4.9. Statistical analysis

The compartment of model was simulated by a 3P97 program (Practical Pharmacokinetic Program 1997, China), whereby the pharmacokinetic parameters were calculated. The calculation of AUC was based on statistical moment theory. The pharmacokinetic parameters were analyzed for statistical significance by unpaired Student's t-test. For this purpose the level of significance was set at $\alpha = 0.05$. In the biodistribution studies, the AUC could not be determined in individual mice because of the destructive sampling approach of the study.

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