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Pharmacokinetics and Biodistribution of Paclitaxel-loaded Pluronic P105/L101 Mixed Polymeric Micelles

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(Received October 26, 2007; Accepted February 12, 2008)

A mixed polymeric micelle formulation of paclitaxel (PTX) has been developed with the purpose of improving the solubility and prolonging the time of blood circulation of PTX in comparison to current Taxol injection. The mixed micelles were prepared by thin-film method using a nonionic surfactant Pluronic P105, L101 and PTX. The mean size of PTX-loaded mixed micelles was 185 nm with narrow size distribution shown by a dynamic light scattering sizer and a transmission electron microscopy. The *in vitro* release profiles indicated that PTX release from the mixed 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 mixed micelle formulation were 5.5 and 4.9-fold higher than that of Taxol injection. The biodistribution study in mice showed that the PTX-loaded mixed micelles not only decreased drug uptake by liver, but also prolonged drug retention in blood, and increased distribution of the drug in lung, spleen and kidney. These results suggested that the mixed 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 intravenous administration of PTX.

Key words——paclitaxel; mixed micelle; pharmacokinetics; biodistribution; pluronic block copolymer

INTRODUCTION

Polymeric micelles possess several advantages compared with micelles formed from low molecular weight surfactants, including good structural stability, slow dissociation rate, low critical micelle concentration (CMC), easy control of particle size, good solubilization of hydrophobic drugs, *etc.*[1,2] They can accumulate anticancer drugs in tumors, release drugs for an extended time, and prevent rapid clearance by the reticular endothelial system (RES) because of their size and surface characteristics.[3] They have been applied in the field of drug targeting because of the unique characteristics in the body. Generally, amphiphilic block or graft copolymers composed of hydrophilic and hydrophobic segments will self-assemble into polymeric micelles when their concentration is above their CMC. So far, many types of block copolymers have been used to deliver anticancer drug into various tumors, including poly(ether)-*block*-poly(ester), poly(ether)-*block*-poly(L-amino acid), poly(ether)-*block*-polycation, poly(ether)-*block*-poly(ether), *etc.*[4,5]

Among these block copolymers, Pluronics, triblock poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) based copolymers, have been commonly used as micellar nanocontainers to solubilize hydrophobic drugs.[6] Recently, it was found that Pluronics as a biological response modifier could interact with multidrug resistant (MDR) cancer cells resulting in drastic sensitization of these tumors with respect to doxorubicin[7] and other anticancer agents, such as paclitaxel (PTX).[8] 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 alter the *in vitro* interaction with isolated macrophages and the biodistribution of model polystyrene nanospheres after coating the particle surface with a Pluronic.[9,10] Additionally, we also reported that PTX-loaded Pluronic P123 micelles could effectively prolong blood circulation time and modify the biodistribution of PTX *in vivo*[11] and exert higher cytotoxicity against resistant human ovarian tumor cell line, SKOV-3/PTX (unpublished data).

Previously, we have reported on micellar PTX formulation employing Pluronic P105, and have demonstrated that the micellar PTX could increase the cytotoxicity against MCF-7/ADR, resistant breast tumor

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cell line. However, the drug-loading contents and encapsulation ratio of the 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 Pluronic micellar PTX, a small percentage of hydrophobic, lamella-forming L101 was added into the hydrophilic P105 micellar system to form a mixed micelle. The results has demonstrated that the mixed micellar system (P105/L101=8/1, w/w) increased the volume of the hydrophobic region of the micelle and particle size, and exhibited a higher solubilization capacity for the hydrophobic PTX, as well as higher cytotoxicity against MCF-7/ADR compared to PTX-loaded P105 micelles.[8)]

The aim of this study was to evaluate the influence of the mixed block copolymer micelle carrier (P105/L101) on the pharmacokinetics and biodistribution of anticancer drug, PTX, in rats and mice. These preliminary results will provide the basis for evaluating *in vivo* antitumor efficacy of the new copolymer micelle, PTX-loaded P105/L101 mixed micelle.

MATERIALS AND METHODS

Drugs and Reagents Pluronic P105 and L101 were kindly supplied by BASF China Ltd. (Shanghai, China) and used without further treatment. 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 & Co. Ltd (Melbourne, Australia). Diazepam was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All reagents for HPLC analysis, including acetonitrile and methanol were of HPLC grade. Other reagents were of analytical grade.

Animals Sprague-Dawley rats (220±30 g) and female Kunming strain mice (20±5 g) were supplied by 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 a temperature of 25±2℃ and a relative humidity of 70±5% under natural light/dark conditions for at least 24 h before dosing.

Preparation of PTX-loaded Mixed Micelles PTX-loaded mixed micelle was prepared by thin film method.[12)] Briefly, 6 mg PTX, 266 mg P105 and 34 mg L101 samples were dissolved in 10 ml acetonitrile in a round-bottom flask. The solvent was removed by rotary evaporation at 60℃ for 1 h to obtain a solid PTX/copolymer matrix, and then the solid PTX/copolymer matrix was hydrated with 5 ml H_2O at 70℃ to obtain a clear micellar solution. The unincorporated drug aggregates were removed by passing through 0.22 μm filters (Millipore), followed by lyophilization.

Characterization of Mixed Micelles Drug-loading coefficient (DL%) and encapsulation ratio (ER%) were calculated as described earlier.[13,14)] Firstly, PTX was extracted from the mixed micelles with acetonitrile, and then the extract solution was properly diluted prior to HPLC analysis. The content of PTX in the mixed micelles was determined by HPLC method described below. Then, DL% and ER% were calculated according to Eqs. (1) and (2):

$$DL\% = W_M/(W_P + W_M) \times 100 \quad (1)$$

$$ER\% = W_M/W_F \times 100 \quad (2)$$

Where W_P is the weight of initial feeding polymer, W_M is the weight of drug incorporated in the mixed micelles, and W_F is the weight of initial feeding drug.

Particle size distribution and mean diameter of the prepared mixed micelles were determined by dynamic light scattering using a NICOMP 380 Submicron Particle Sizer (Santa Barbara, CA, USA) equipped with a 5 mW heliumneon laser at 632.8 nm. Sample solutions filtered through a 0.22 μm filter membrane were transferred into the light scattering cells. The intensity autocorrelation was measured at a scattering angle of 90° at room temperature.. Data were analyzed in terms of intensity-weighted NICOMP distributions. Each reported experimental result is the average of at least three $\bar{d}_h$ values obtained from analysis of the autocorrelation function accumulated for at least 20 min. The morphological examination of the mixed micelles was performed using a transmission electron microscope (Philips CM120, Netherlands). A drop of micellar solution containing 0.1% (w: v) phosphotungstic acids was placed on a carbon film coated on a copper grid and observed at 80 kV in the electron microscope.

***In Vitro* Release of PTX from Mixed Micelles** The *in vitro* release properties of PTX from the mixed micelles were investigated in an aqueous medium containing 1 mol/l sodium salicylate by a dialysis method.[15)] 1 ml of micellar PTX solution (0.1 mg/ml PTX) was introduced into a dialysis bag (MWCO=14 kDa, Greenbird Inc., Shanghai, China). The end-

sealed dialysis bag was immersed into 50 ml 1 mol/l sodium salicylate solution at 37℃. The release medium was stirred at the speed of 75 rpm for 24 h. Samples of 0.5 ml 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 release medium. The concentration of PTX in the samples was determined by the HPLC method described below. PTX release from the PTX stock solution and Taxol injection placed in a dialysis bag was conducted under the same conditions as controls. The PTX stock solution was prepared using 0.1% dimethyl sulfoxide (DMSO) and phosphate buffered saline (PBS) as a solvent, and the concentration of PTX stock solution used as a control is 0.1 mg/ml PTX.

Pharmacokinetic Studies Twelve Sprague-Dawley rats were used to investigate the effect of formulation on the pharmacokinetics of PTX after intravenous administration. Rats were divided into 2 groups at random, and given a single 6 mg/kg dose of Taxol injection or PTX-loaded mixed micelles by tail-vein injection. The concentration of PTX in the mixed micellar solution for the pharmacokinetic study was 1000 μg/ml. As the control, Taxol injection was diluted 6-fold to 1 mg/ml with 5% glucose solution shortly before administration.

Blood samples (0.5 ml) were collected into heparinized tubes from the femoral artery at 0 (predose), 5, 15, 30 min, 1, 2, 3, 4, 6, 8 and 12 h after intravenous administration. Blood was immediately processed for plasma by centrifugation at 100×g for 10 min. Plasma samples were frozen and maintained at −70℃ until analysis.

Biodistribution Studies Seventy-two female Kunming strain mice were used in the experiment to assess the effect of formulation on the biodistribution of PTX after intravenous administration. All mice were divided into 2 groups at random and given a single 3 mg/kg dose of either Taxol injection or PTX-loaded mixed micelles by tail-vein injection. At 5, 15, 30 min, 1, 2, 3, 4, 6, and 8 h after drug injection, each animal (n=4 for each time point) was killed and heart, spleen, lung, liver, kidney, uterus/ovaries, 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℃ until assessed for drug concentration by HPLC.

HPLC Analysis The analysis of PTX levels *in vitro* and *in vivo* were carried out using a reversed-phase HPLC method on a system equipped with an LC-10ATVP pump, a SPD-10AVP UV-Vis detector (Shimadzu, Kyoto, Japan) and a 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. Samples of 20 μl were injected into the HPLC column for all the analysis. The retention time of PTX was approximately 8.2 min with a flow rate of 1.0 ml/min for the mobile phase.

Tissue samples were homogenized in the mixed solution of acetonitrile and water (50 : 50, v/v). Diazepam (1 μg/ml, 50 μl) as 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 diethyl ether by vortex mixing for 2 min. After centrifugation at 6000×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×g for 5 min before HPLC analysis.

Statistical Analysis The compartment of model was simulated by a 3p97 program (Practical Pharmacokinetic Program 1997, China), whereby the parameters of pharmacokinetics were calculated. The calculation of AUC (area under the plasma concentration-time curve) 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 α=0.05. In the biodistribution studies, the AUC could not be determined in individual mice because of the destructive study design.

RESULTS

Preparation and Characterization of PTX-loaded Mixed Micelles The Pluronic block copolymers used in this work and their characteristics are presented in Table 1. The CMC values of P105 and L101 determined using iodine incorporation UV spectroscopy technique[16] were found to be 0.006% and 0.0008%, respectively. It was consistent with previous reports that the CMC values of the Pluronic P105 and L101,

Table 1. The Selected Characteristic Parameters of Pluronic P105 and L101 Block Copolymer and PTX-loaded Mixed Micelles (P105/L101, 8/1)

Block copolymer		Pluronic P105	Pluronic L101
Physicochemical characteristics	CMC[a], %wt	0.006	0.0008
	Mw[b]	6500	3800
	HLB[b]	15	1
Optimum PTX-loaded mixed micelles (P105/L101, 8/1)	DL (%)	1.7	
	ER (%)	83.3	
	Particle size (nm)	185.3±21.0 nm	

Note: DL, Drug loading coefficient; ER, encapsulation ratio; [a] CMC values were determined using iodine incorporation UV spectroscopy method. [b] Mw and HLB of the copolymers were determined by the manufacturer.

determined using pyrene solubilization technique, was 0.004% (6.2×10^{-6}M) and 0.0008% at 37°C.[17] Amphiphilic Pluronic diblock copolymers can form spherical micelles when their concentration is at or above their CMC in an aqueous environment. Hydrophobic drugs can be physically incorporated within the cores of the polymeric micelles by hydrophobic interactions. In this work, the diameter of the PTX-loaded mixed micelles (P105/L101=8 : 1, w/w) was 185 nm, and a narrow size distribution was confirmed by dynamic light scattering (DLS) measurement (Fig. 1). In addition, the mean diameter of PTX-unloaded mixed micelles was 176.8 nm, which was not significantly different compared to the PTX-loaded mixed micelles ($p>0.05$). The drug loading coefficient (DL%) and the encapsulation ratio (ER%) were 1.7% and 83.3%, respectively (Table 1). Furthermore, the morphology of PTX-loaded mixed micelle observed with transmission electron microscope (TEM) revealed that the mixed micelles had a spherical shape and no drug crystal was visible (Data not shown).

***In Vitro* Release of PTX from Mixed Micelles** 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 mixed micelles, while the solubility of PTX in this release medium is about 28.1 μg/ml.[15] Therefore, this system could maintain a good sink condition for the *in vitro* release studies of PTX by using the aqueous release medium containing 1 mol/l sodium salicylate which solubilized more than 10 times the total amount of PTX incorporated in the mixed micelles.

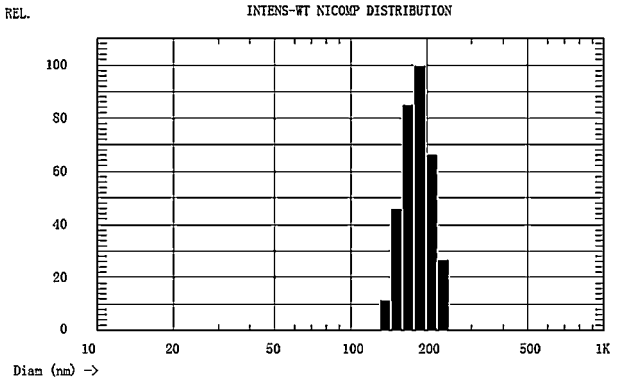

Fig. 1. Representative Size Distribution Analysis of PTX-loaded Mixed Micelle (P105/L101, 8/1): $\frac{d}{h}$=185.3±21.0 nm

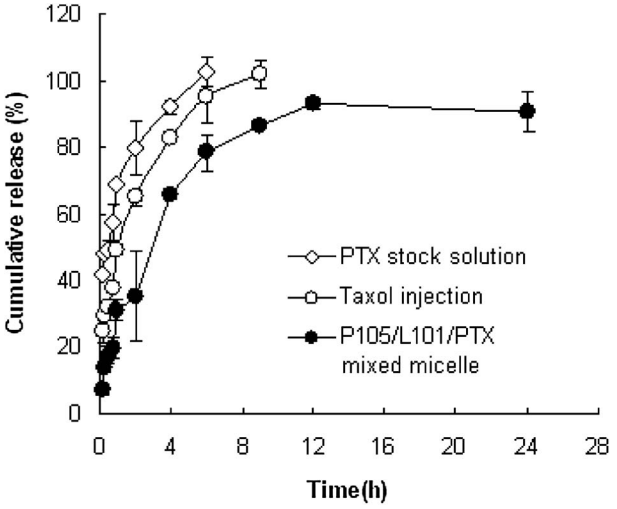

Fig. 2. A Release Profile of PTX from Pluronic Mixed Micelles (P105/L101, 8/1) in 1.0 mol/l Sodium Salicylate at 37°C

PTX was continuously released from the mixed micelles in this aqueous release medium for 24 h at 37°C. PTX release from the PTX stock solution and Taxol injection were investigated as controls. The results of the cumulative PTX release profile from the mixed micelle are shown in Fig. 2. During a 6-h period, 100% PTX was released form the PTX stock solution, and 95.2% PTX from Taxol injection. There was not significantly different in the PTX release from the Taxol injection and the stock solution ($p>0.05$). During the same period, only 78.4% PTX was released from the mixed micelles, which showed significant difference compared with Taxol injection ($p<0.05$). During a 24-h period, the mixed micelle cumulatively released 90.7% PTX.

Pharmacokinetics of PTX-loaded Mixed Micelle The HPLC method for analysis has been validated.

Linearity in the standard curves was demonstrated over the concentration range studied, and endogenous components had no interference in the chromatograms. The LOD (limit of detection) for PTX defined as a minimum signal-to-noise of three was 10 ng/ml. The intra-day accuracy (deviation) was within 5%, and the intra-day precision (R.S.D.) was less than 4%. The inter-day accuracy and precision were within 11% and 10%, respectively. Figure 3 shows the mean plasma concentration-time profiles of PTX in blood after intravenous administration of the PTX-loaded mixed micelles and Taxol injection at a single dose of 6 mg/kg, respectively. A two-compartment open model with 1/C weight was fitted to the plasma PTX concentration using a computer program 3p97. The related pharmacokinetic parameters are listed in Table 2, and analyzed for statistical significance by unpaired Student's *t*-test. The plasma concentration of PTX delivered by the mixed micelles was higher than Taxol injection during all experimental hours. The former provided significantly higher (4.9 fold) AUC compared to the latter ($p<0.01$). The concentrations showed a rapid decline in distribution phase for the first 0.4 h after dosing with two preparations. The $t_{1/2\alpha}$ (distribution half-life) showed significant difference ($p<0.05$) between two groups, and the corresponding values were 0.33 h and 0.11 h for the mixed micelles and Taxol injection, respectively. The $t_{1/2\beta}$ (elimination half-life). of the mixed micelles and Taxol injection was 6.99 h and 1.27 h, respectively. The statistical analysis of the $t_{1/2\beta}$, Cl (clearance), k_{10} (central compartment elimination rate constant) ($p<0.01$) and k_{21} (elimination rate constant from peripheral to central compartment) ($p<0.05$) showed significant differences between two groups. These data indicated that the mixed micelles increased the systemic circulation time of PTX, which was in agreement with the *in vitro* release of PTX from the mixed micelles.

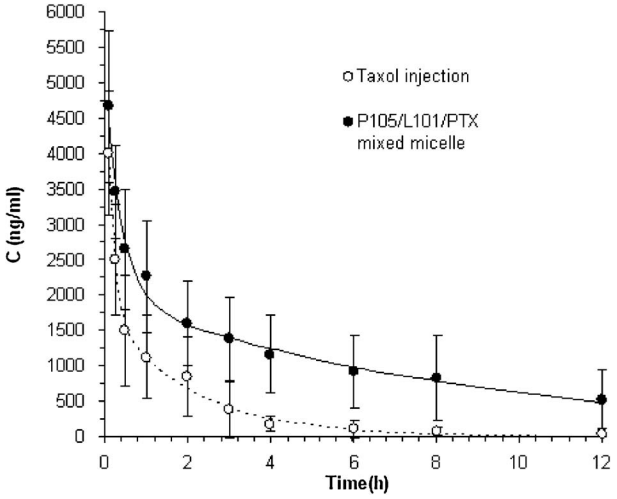

Fig. 3. Mean Plasma Concentration-time Profiles of PTX after Intravenous Administration of a Single 6 mg/kg Dose of Taxol Injection and PTX-loaded Mixed Micelles (P105/L101, 8/1) to Rats

The solid and dash lines represent model simulations of the two-compartment model for the PTX-loaded mixed micelles and Taxol injection, respectively. The open or filled circles represent the mean±SD of 6 rats.

Biodistribution of PTX-loaded Mixed Micelle

In vivo behavior of PTX after intravenous administration of the mixed micelles to mice was investigated with Taxol injection as a control (Fig. 4). The amounts of drug distributed in unit mass of plasma, heart, spleen, lung, liver, kidney, ovary and uterus of mice at various times were then measured, and the total amount of drug accumulated in each organ within 8 h (AUC_{0-8}) was calculated, and the results are shown in Table 3. The blood PTX concentration-time

Table 2. Pharmacokinetic Parameters of PTX after Intravenous Administration of Taxol Injection and PTX-loaded Mixed Micelles (P105/L101, 8/1) to Rats ($n=6$)

Parameters	Unit	Taxol injection	P105/L101/PTX mixed micelle
$t_{1/2\alpha}$	h	0.11±0.03	0.33±0.26**
$t_{1/2\beta}$	h	1.27±0.23	6.99±4.12*
k_{21}	h^{-1}	2.49±0.72	1.28±0.84**
k_{10}	h^{-1}	1.62±0.59	0.37±0.19*
k_{12}	h^{-1}	3.22±1.07	3.03±4.29
$AUC_{0\sim12h}$	$\mu mg\cdot l^{-1}\cdot h$	4215.15±2375.50	20720.64±12382.97*
Cl	$l\cdot h^{-1}\,kg^{-1}$	1.73±0.74	0.38±0.22*
V	$l\cdot kg^{-1}$	1.06±0.22	1.10±0.47

Note : ** $p<0.05$, * $p<0.01$, denotes significant difference between the mixed micelle group and the Taxol injection (reference formulation).

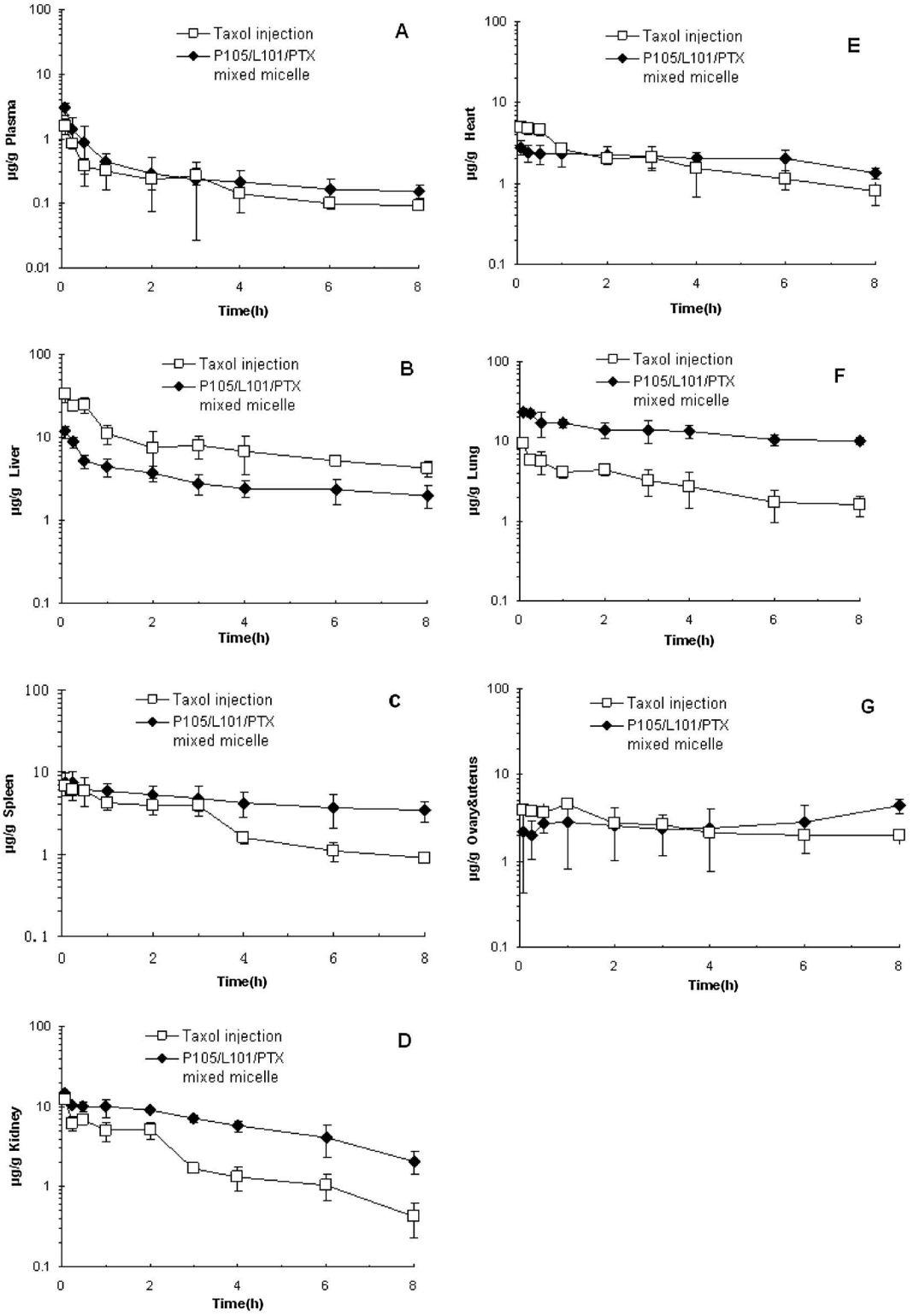

Fig. 4. Mean Concentration-time Profiles of PTX in (A) Plasma, (B) Liver, (C) Spleen, (D) Kidney, (E) Heart, (F) Lung and (G) Ovary/uterus following Intravenous Administration of a Single 3 mg/kg Dose of Taxol Injection and PTX-loaded Mixed Micelles (P105/L101, 8/1) to Mice
Each point represents the mean ± SD of 4 mice.

profiles observed in mice were similar to the pharmacokinetic study in rats. The time of distribution phase was short and the concentration decreased quickly in this phase. The PTX AUC_{0-8} of the mixed micelles was lower in liver, and higher in plasma, lung, spleen, and kidney compared to Taxol injec-

Table 3. The AUC[a] of PTX in Plasma and Tissues after Intravenous Administration of Taxol Injection, PTX-loaded Mixed Micelles (P105/L101, 8/1) to Mice ($n=4$)

	Plasma	Liver	Spleen	Lung	Kidney	Heart	Ovary/uterus
Taxol injectin ($\mu g \cdot h \cdot g^{-1}$)	1.82	68.62	21.06	24.7	20.99	15.07	20.77
P105/L101/PTX mixed micelles ($\mu g \cdot h \cdot g^{-1}$)	2.79	25.81	36.67	106.82	51.01	16.48	22.44
Ratio[b]	1.53*	0.38*	1.74*	4.32*	2.43*	1.09	1.08

Note: [a] AUC of the tissues, 0–8 h; [b] The ratio was AUC (micelle)/AUC (Taxol); * $p<0.05$: micellar PTX *vs* Taxol injection.

tion. There were statistically significant differences in plasma, liver, spleen, kidney and lung between the mixed micelles and Taxol injection ($p<0.05$). However, no significant difference was observed in heart, ovary and uterus. The AUC_{0-8h} of these tissues for Taxol injection in a descending order was liver> lung>spleen>kidney>ovary and uterus>heart> plasma. In contrast, the corresponding order for the mixed micelle was lung>kidney>spleen>liver>ovary and uterus>heart>plasma.

DISCUSSION

Like the Pluronic P105 micelles, the PTX-loaded mixed micelles (P105/L101=8 : 1, w/w) were prepared by a dialysis method, by which PTX was effectively encapsulated into the mixed P105/L101 micelles. It was earlier reported that the average particle size of the PTX-loaded P105 micelles was 23.9± 3.0 nm with a narrow particle size distribution, and the drug-loading coefficient (DL%) and encapsulation ratio (ER%) of PTX in the P105 micelles were 1.0% and 54.5%, respectively.[8] In comparison with the P105 micelles, an increased particle size of the mixed micelles (185 nm) was observed when a small percentage of hydrophobic L101 was inserted into the P105 micellar system, and at the same time, the drug-loading coefficient (DL%) and encapsulation ratio (ER%) of PTX in the mixed micelles were increased to 1.7% and 83.3%, respectively (Table 1). Pluronic P105 block copolymer has an intermediate hydrophile-lipophile balance (HLB) value of 15, which can form sphere micelles in aqueous solution with a small particle size. Pluronic L101 is a hydrophobic Pluronic (HLB≈1) with long poly (propylene oxide) (PPO) chains and short poly (ethylene oxide) (PEO) chains and can usually form lamellar aggregates with larger sizes (1000 nm) in aqueous solution which exhibit a higher solubilization capacity than spherical micelles formed by hydrophilic Pluronic.[18] These results indicate that insertion of a small percentage of hydrophobic L101 into the P105 micellar system can improve the solubilization capacity of Pluronic P105 for hydrophobic PTX, although PTX is a lipophilic drug whose aqueous solubility at different pH conditions was around 1.0 μg/ml.[19]

Inclusion of surfactants in release media is the most popular method for *in vitro* PTX release, but the addition of surfactants in release media might have a significant effect on the micellar structure and distort the release profiles. Cho *et al.*[15] reported that 1.0 mol/l sodium salicylate solution could solubilize PTX and maintain the sink condition for PTX in aqueous release medium, which did not significantly affect the physical stability of polymeric micelles. In the *in vitro* release experiment, 1 mol/l sodium salicylate solution was used as a release medium. It was shown that almost all PTX was rapidly released from the PTX stock solution over a 6-h period in this release medium, which indicated that the hindrance effect of the dialysis bag was minimal. The cumulative release amount of PTX from Taxol injection in 6 h was around 95%, which was similar to the PTX stock solution. However, there was a sustained PTX release observed from the mixed micelles. The percentage of PTX released in 6 h and in 24 h were only 78.4% PTX and 90.7% PTX, respectively, which was much slower than Taxol injection. The cumulative release of PTX from the mixed micelles in 6 h was reduced 1.2-fold compared to that of Taxol injection. In contrast, 45.4% and 87.8% PTX were cumulatively released from the PTX-loaded P105 micelles in 6 h and 24 h, respectively (unpublished data). The cumulative release of PTX from the P105 micelles in 6 h were reduced 2.1-fold compared to that of Taxol injection. This indicated that, compared to the P105 micelles, the drug release from the mixed micelles showed much faster during a 6-h period, but remained the same level over a 24-h period, which was presumably related to the different *in vitro* stability of the polymeric micelles.

The reticuloendothelial system (RES), especially in the liver, spleen and lung, seems to be responsible for the accumulation of stealth nanoparticles in these organs. Some stealth particles whose surface is decorated with PEG are known to be invisible to macrophages and have prolonged half-lives in the blood compartment. This property endows the particles higher probability to extravasate in pathological sites, like tumors or inflamed regions where a leaky vascular structure is developed.[20–22] The *in vivo* pharmacokinetics and biodistribution studies of the PTX-loaded mixed micelles in rats indicated the hydrophilic shell of Pluronic P105 of the mixed micelles could make the micellar PTX avoid the recognition and uptake by the reticuloendothelial systems (RES) and prolong the time of blood circulation of PTX. This effect of the mixed micelles resulted in a slower clearance (0.22-fold), as well as higher $t_{1/2\beta}$ (5.50-fold) and AUC (4.92-fold) than Taxol injection ($p<0.01$) in the pharmacokinetics study. The higher AUC (1.53-fold) in plasma of the PTX-loaded mixed micelles than Taxol injection in biodistribution study was also a result of this effect. In one aspect, although there is a discrepancy of blood concentrations of PTX between rats and mice which is probably due to species differences or administration of different doses of the PTX mixed micelles, the *in vivo* prolonged retention of PTX in plasma is consistent with the *in vitro* sustained release behaviors of this drug from the mixed micelles. In other aspect, this phenomenon is probably related to a significant decrease of hepatic distribution of PTX. In mouse tissue distribution study, a lower AUC of the mixed micelles was seen in liver. The Kp (tissue to plasma concentration ratio) values of PTX in liver are 9.25 and 37.7 after the mixed micelle and Taxol injection administration, respectively. This 4-fold decrease in the hepatic distribution of the PTX-loaded mixed micelle causes reduction of hepatic clearance of PTX, which has a great impact on its plasma concentration because PTX elimination is primarily by the hepatic metabolism by cytochromes P450 2C8 and 3A4.[23]

In tissue distribution studies in mice, compared to Taxol injection, higher AUC was found in other RES-containing organs including spleen and lung following intravenous administration of the mixed micelles. However, the Kp values in spleen are almost same between Taxol injection (11.6-fold) and the mixed micelles (13.1-fold), indicating higher concentration in the spleen after the mixed micelle administration is dependent on its higher plasma concentration, not increasing the uptake into the spleen. But the Kp values in lung are significantly different between Taxol injection (13.6-fold) and the mixed micelle (38.3-fold). The mechanism or cause of this 2.8-fold increase in the lung distribution of the PTX-loaded mixed micelle is not clear. On the one hand, this phenomenon is presumably related to the intermediately hydrophilic shell formed by PEO segments of the mixed micelles. On the other hand, it is presumably related to the reduced *in vivo* stability of the mixed micelles, especially in the presence of endogenous components, such as some lung surfactant components, which probably induces aggregation of the mixed micelles in the lung.

Tissue distribution studies also showed that the higher AUC were found in kidney in mice. Generally, amphiphilic polymeric micelles can reduce PTX uptake by the kidney. However, the AUC of PTX of the mixed micelles in kidney was higher than Taxol injection in our research. This may be explained by the biological property of Pluronic P105. It was earlier reported that Pluronics were excreted primarily through the kidneys.[24] The clearance in rats, dogs, and humans was shown to be almost entirely by renal excretion. Some studies showed that the formation of micelles had no effect on the elimination clearance of the block copolymer.[25] The biodistribution study of Pluronic P105 micelles also showed that the concentration of this material accumulated in kidney cells was higher than the other organs in mice after i.p. injections.[26] Therefore, the mixed micelles comprised of P105 and L101 (8 : 1, w : w) leading to an increment in AUC of PTX in kidney could be induced. In addition, there is not significantly different in the biodistribution profiles of the mixed micelles and Taxol injection in mouse brain, which were so low that most of these samples could not be accurately measured because the concentrations were below the quantitation limit. This phenomenon is consistent with the previous literature reported by Rowinsky and Donehower.[27]

In contrast with the P105 micelles, the mixed micelles showed the similar pharmacokinetics and biodistribution properties. The $t_{1/2\beta}$ and AUC of the P105 micelles in rats in the pharmacokinetics studies were 4.9-fold and 5.3-fold higher compared to Taxol injection, respectively, and its AUC in plasma in mice

in the biodistribution studies was 1.6-fold higher than that of Taxol injection. The PTX AUC of the P105 micelles was also lower in liver, and higher in plasma, spleen, lung, and kidney in mice in the biodistribution studies compared to Taxol injection (unpublished data). These data indicate that insertion of a small percentage of hydrophobic L101 (1/9, w/w) into the spherical P105 micelles can significantly improve the solubilization strength for hydrophobic PTX, but a small percentage of hydrophobic L101 in the P105 micellar system can not significantly change its *in vivo* behavior.

On the basis of these preliminary results, a study on evaluating *in vivo* antitumor efficacy of the PTX-loaded mixed micelles (P105/L101, 8/1) on BALB/c nude mice bearing s.c. human resistant ovarian SKOV-3/PTX carcinoma is under investigation in our laboratory.

CONCLUSION

In summary, *in vitro* characteristics of the PTX-loaded mixed micelles (P105/L101, 8/1) and *in vivo* pharmacokinetics and biodistribution of PTX delivered by the mixed micelle carriers were demonstrated. The mixed micelles may efficiently load, protect and retain PTX in both *in vitro* and *in vivo* environments. *In vitro* release profiles indicated that PTX release from the mixed micelles exhibited a sustained release behavior. A similar phenomenon was also observed in plasma in rats. Incorporating PTX in the mixed micelles not only decreased drug uptake by liver, but also prolonged drug retention in the blood, and increased the distribution of drug in spleen, lung, and kidney in mice. These results suggested that the mixed P105/L101 micellar formulation can provide a useful alternative dosage forms for intravenous administration of PTX.

Acknowledgements This work was financially supported by National Natural Science Foundation of P. R. China (No. 30472094). The authors are also thankful to Innovative Research Team of 211 Project in Anhui University, P. R. China (No. 02203109), for support of this study.

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