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Polysorbate 80 coated poly (ε-caprolactone)–poly (ethylene glycol)–poly (ε-caprolactone) micelles for paclitaxel delivery

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ABSTRACT

In this article, polysorbate 80 coated poly (ε-caprolactone)–poly (ethylene glycol)–poly (ε-caprolactone) (PCEC) micelles were successfully prepared for paclitaxel (PTX) delivery. The particle size distribution, morphology, drug loading, encapsulation efficiency and sustained release profile of the micelles were studied in detail. The safety of the micelle formulation was evaluated by MTT assay on HEK293 cells. And the encapsulated PTX in the micelles remained potent antitumor effect on C6 glioma cells. The pharmacokinetic study showed that the PCEC micelles coated with polysorbate 80 altered the biodistribution pattern and increased PTX concentration in the brain significantly compared to the uncoated micelles and the free drug after intravenous injection. The results indicated that polysorbate 80 coated PCEC micelles might be a candidate for PTX delivery for brain tumor chemotherapy.

1. Introduction

With the rapid development of nanotechnology in the past decades, nanobiomaterials showed promising application in the field of nanomedicine (Caldorera-Moore and Peppas, 2009; Sakamoto et al., 2010; Wagner et al., 2006). Recently, drug delivery system (DDS) based on biodegradable polymeric micelles is highlighted, especially as vehicles of chemotherapeutics (Jadhav et al., 2010; Licciardi et al., 2010; Liu et al., 2010; Na et al., 2010). Many previous investigations indicated that the novel nano-drug-carriers can not only overcome the poor water solubility of hydrophobic drugs (Sezgin et al., 2007; Shin et al., 2009; Zheng et al., 2010), but also promote the pharmacokinetic profiles of the chemotherapeutic agents, which may include sustained delivery, targeted delivery and delivery drugs across the physiological drug barriers: the gastrointestinal barrier and the blood–brain barrier (BBB) (Liu et al., 2007; Min et al., 2010; Shao et al., 2010; Yang et al., 2010). To date, an increasing number of studies focused on micelles with anticancer drugs encapsulated in have been performed in the preclinical and clinical research (Armstrong et al., 2006; Hamaguchi et al., 2007; Lee et al., 2008; Nakanishi et al., 2001). For example, the formulation of PTX-loaded lyophilized polymeric micelle (Genexol-PM) has already reached phase II stage (Lee et al., 2008), while the polymeric delivery system for doxorubicin based on Pluronic L61 and F127 (SP1049C) has been studied in phase III clinical trials (Armstrong et al., 2006).

Previously, we have successfully synthesized a series of amphiphilic poly (ε-caprolactone)/poly (ethylene glycol) (PCL/PEG) copolymers (Dong et al., 2010; Gong et al., 2009; Gou et al., 2008; Li et al., 2010). Due to their good biodegradability and great biocompatibility, the self-assembled micelles based on the triblock PCL/PEG/PCL copolymer have been proved as a promising candidate for DDS (Ge et al., 2002; Gou et al., 2009a,b; Wei et al., 2009). In micellar geometry, the hydrophobic PCL blocks incorporate the poor water-soluble drugs, while the hydrophilic PEG blocks serve as a protective shell. Therefore, the novel formulation may increase the solubility of lipophilic chemotherapeutics for clinical application.

Paclitaxel (PTX) is one of the best antineoplastic drugs widely used in clinical trials. It demonstrates significant activity against a variety of tumors, including breast, ovarian, colon, bladder, lung, and head and neck cancers (Belani and Ramanathan, 1998; Hortobagyi et al., 1994; Rowinsky and Donehower, 1995; Singla et al., 2002). Since PTX is a hydrophobic drug with poor aqueous solubility (approximately 1 μg/ml) (Kim et al., 2006), the commercial preparation in clinical is formulated in a mixture of Cremophor EL and dehydrated alcohol (1:1, v/v). Unfortunately, this kind of adjuvant is associated with severe adverse effects, such as hypersensitivity reactions, neurotoxicity and nephrotoxicity (Gelderblom et al., 2001; Szebeni et al., 2001). During the last years, many efforts have been undertaken to search for an optimal PTX formulation void of problems caused by Cremophor EL

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(Danhier et al., 2009b; Park et al., 2005; Yang et al., 2007). Micelles prepared from biodegradable copolymers are widely applied as PTX carrier, which have the potential advantages of drug controlled release and target delivery (Wei et al., 2010; Zhang et al., 2008). The self-assembled PTX micelles based on triblock PCEC copolymer were developed in the present study.

Moreover, malignant brain tumors are insensitive to chemotherapeutic drugs, presumably because the BBB restricts the drug flow into brain tissue. Some studies have found that nanoparticles coated with polysorbate 80 have the ability to cross BBB after intravenous injection (Alyautdin et al., 1997; Kreuter et al., 1997; Wilson et al., 2008a, 2010). They have been used to deliver drugs for Alzheimer's diseases to brain, such as rivastigmine and tacrine, resulting better effects (Wilson et al., 2008a, 2010). This suggests that polysorbate 80-coated micelles may help agent cross BBB in the treatment of brain tumor. Therefore, polysorbate 80-coated PCEC micelles were developed for PTX delivery to brain in this work. We investigated the drug release profile, the anticancer activity on the C6 glioma cell line in vitro and the effect of polysorbate 80 on pharmacokinetic behavior in vivo. Compared with our previous reports about PCEC micelles, the polysorbate 80-coated PTX micelle is a novel formulation that might be an anticancer agent for brain cancer therapy.

2. Experimental

2.1. Materials

PCEC triblock copolymer was synthesized by our group. Other materials included paclitaxel (PTX, Shanxi Senfu Biotechnology Co., Ltd., Shanxi, China), polysorbate 80 (Rejinte Chemicals, Tianjin, China), dehydrated alcohol (Haixing Chemicals, Chengdu, China), acetonitrile (Fisher Scientific, Loughborough, U.K.), methanol (Fisher Scientific, Loughborough, U.K.), dimethyl sulfoxide (DMSO, KeLong Chemicals, Chengdu, China), ethyl acetate (Kemio Chemicals, Tianjin, China), normal hexane (Rejinte Chemicals, Tianjin, China) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA). Acetonitrile, methanol, ethyl acetate and normal hexane were HPLC grade, all other chemicals used in this work were analytical grade. Water was deionized by the Milli-Q Plus system (Millipore).

HEK293 cell line and C6 rat glioma cell line were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and grown in DMEM media. The cell culture was maintained in a 37 °C incubator with a humidified 5% CO_2 atmosphere.

Male Sprague-Dawley (SD) rats at weight of 200 ± 20 g were used for the pharmacokinetic study. Kunming mice at the weight of 20 ± 2 g, were used for the investigation of tissue distribution study. The animals were purchased from the Laboratory Animal Center of Sichuan University (Chengdu, China). Throughout the experiment, the animals were housed at a temperature of 20 ± 2 °C, relative humidity of 50–60%, and 12 h light–dark cycles. Free access to food and water was allowed. All the animals were in quarantine for at least 1 week before treatment and fasted for 24 h prior to the experiment. All animal care and experimental procedures were conducted according to Institutional Animal Care and Use Guidelines.

2.2. Preparation of blank or polysorbate 80 coated PTX-loaded micelles (PTX–PCEC–P80)

The PCEC copolymer was synthesized by ring-opening polymerization of ε-caprolactone initiated by PEG (Mn = 2000) according to our previous reports (Wei et al., 2010). The molecular weight of the obtained triblock was about 3700 calculated from ^{1}H NMR spectrum.

Blank PCEC micelles or PTX-loaded PCEC micelles (PTX–PCEC) were prepared by thin-film hydration method. Briefly, 100 mg of PCEC and calculated amount of PTX was co-dissolved in 2 mL dehydrated alcohol in a round-bottomed flask. For blank micelles, the PTX was omitted. The solvent was evaporated under reduced pressure at 60 °C to obtain a thin layer of uniform film on the wall of the flask. And then, the residual film was hydrated with 5 mL water under moderate shaking. In this condition, amphiphilic PCEC copolymer could self-assemble into micelles and the PTX was encapsulated in the micelles.

The coating of the PCEC micelles was performed according to the procedure described by Kreuter et al. (2003). The polysorbate 80 was added to the micellar solution to get the final polysorbate 80 concentration of 1% (w/v), and the mixture was incubated for 30 min. Finally, the suspension was filtered with a syringe filter (pore size: 220 nm) (Millex-LG, Millipore Co., USA) and lyophilized for further application.

2.3. Physicochemical characterization of PTX–PCEC–P80

The particle size distribution of prepared PTX–PCEC–P80 micelles was determined by laser diffraction particle sizer (Nano-ZS, Malvern Instrument, UK). The measurements were performed at 25 °C after equilibration for 2 min. All results were the mean of 3 test runs.

The morphology of the self-assembled PTX–PCEC–P80 micelles was observed under a transmission electron microscopy (TEM, H-6009IV, Hitachi, Japan). Before observation, the PTX–PCEC–P80 micelles were prepared as following: samples were diluted with distilled water and placed on a copper grid covered with nitrocellulose. Then they were negatively stained with phosphotungstic acid and dried at room temperature.

The concentration of PTX in the prepared micelles was determined by high performance liquid chromatography (HPLC) Instrument (Shimadzu LC-20AD, Japan). Solvent delivery system equipped with a column oven (CTO-20A) and a plus autosampler (SIL-20AC). Detection was taken on a diode array detector (SPD-M20A). Chromatographic separations were performed on a reversed phase C18 column (4.6 mm × 150 mm, 5 μm, zorbax eclipse XDB, Agilent, US). And the column temperature was kept at 30 °C. Acetonitrile/water (43/57, v/v) was used as eluent at a flow rate of 1 mL/min. Detection wavelength was 227 nm.

Drug loading (DL) and encapsulation efficiency (EE) of PTX–PCEC and PTX–PCEC–P80 micelles were determined as follows. Briefly, 0.5 mL of drug loaded micelles slurry was introduced into pre-weighed EP tube and was lyophilized to constant weigh. Afterwards, the dried deposit was dissolved and diluted by methanol. The amount of PTX in the solution was determined by HPLC. At last, DL and EE of drug loaded micelles were calculated according to Eqs. (1) and (2):

$$\mathrm{DL} = \frac{\text{amount of drug}}{\text{amount of polymer} + \text{drug}} \times 100\% \quad (1)$$

$$\mathrm{EE} = \frac{\text{amount of drug in micelles}}{\text{amount of the feeding drug}} \times 100\% \quad (2)$$

2.4. In vitro drug release behavior

In vitro release profiles of PTX from PCEC micelles and polysorbate 80-coated PCEC micelles were performed using the dialysis method. In this study, 0.5 mL of PTX–PCEC and PTX–PCEC–P80 micelles solution (1 mg/mL) was placed in a dialysis bag (molecular mass cutoff 8–1.4 kDa), while 0.5 mL of PTX solution in dimethyl sulfoxide (DMSO) at the same concentration was used as control.

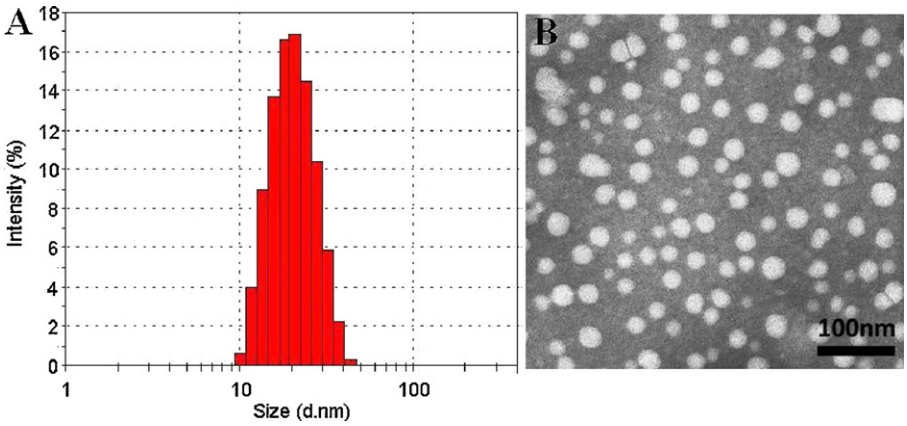

Fig. 1. Characterization of PTX–PCEC–P80 micelles. (A) Size distribution spectrum determined by laser diffraction size detector; (B) the TEM image of prepared PTX–PCEC–P80 micelles.

The dialysis bags were incubated in 40 mL of phosphate buffer (PBS, pH = 7.4) containing 0.5% polysorbate 80 at 37 °C with gentle shaking. The media was displaced by pre-warmed fresh PBS at predetermined time points. The released drug was quantified with the HPLC method described before, and the cumulative release profile with time was demonstrated.

2.5. Analysis of cytotoxicity

The cytotoxicity of blank polysorbate 80-coated PCEC micelles was evaluated by cell viability assay on HEK293 cell line. The anticancer activity of prepared PTX–PCEC–P80 micelles was evaluated by proliferation assay on C6 rat glioma cell line in vitro.

In the tests, both of the cells were seeded in 96-well plates at a density of 3×10^3 cells/well in 100 μL of medium and incubated for 24 h. HEK293 cells were exposed to a series of blank micelles at different concentrations. And C6 cells were exposed to a series of micelle-encapsulated PTX and free PTX at different concentrations, respectively. Each kind of concentration has six complex wells, and untreated cells were added to equivalent volumes of media used as control groups. After incubation for another 24 h, the viability of cells was measured using the methylthiazoletetrazolium (MTT) method.

Briefly, each well was added in 20 μL MTT solution (5 mg/mL) and treated for 4 h. Then, the supernatant was fully removed and the DMSO was added to dissolve the blue crystals which was produced by viable cell. The absorbance of the formazan was measured at 570 nm and the cell survival was calculated from it.

2.6. Pharmacokinetic profile

Pharmacokinetics studies of PTX were performed in male SD rats. The rats were randomly divided into three groups ($n = 6$ for each group) and injected intravenously with 4 mg/kg of PTX–PCEC–P80, PTX–PCEC and Taxol® (paclitaxel in Cremophor® EL and ethanol mixture), respectively. Blood samples were collected into heparinized tubes from the femoral vein at appropriate time intervals for 24 h after administration. Plasma samples were obtained by immediately centrifuging the blood at 8000 rpm for 5 min and stored at −20 °C until analyzed by HPLC.

For plasma samples, 100 μL of plasma was spiked with 20 μL of levonorgestrel as an internal standard, and was extracted with the mixture of ethyl acetate and *n*-hexane (1:1). After centrifugation, the organic layer was collected and evaporated under nitrogen gas at 40 °C. The residue was then reconstituted in methanol and centrifuged at 15,000 rpm for 10 min. 20 μL of the supernatant was injected into the HPLC system. The pharmacokinetic parameters of PTX after intravenous administration were calculated using a non-compartmental model by the drug and statistics (DAS) software (version 2.1.1, Mathematical Pharmacology Professional Committee, China).

2.7. Tissue distribution

Male Kunming mice were administered with PTX–PCEC–P80, PTX–PCEC and Taxol® (4 mg/kg) via tail vein. At predetermined time points after injection, the mice were sacrificed by cervical dislocation. The organs (heart, liver, spleen, lung, kidney and brain) were removed and washed with precooled normal saline. Then the tissue samples were weighed and stored at −20 °C until analyzed by HPLC. Tissue distribution was expressed as the amount of PTX per gram of the tissues.

Briefly, 50 mg of the tissue samples were homogenized with 5 times of normal saline, using the tissue homogenizer (Ultra-Turrax T10 basic, IKA, Germany) for 5 min. The tissue homogenate was spiked with 20 μL of internal standard (levonorgestrel), and extracted with the mixture of ethyl acetate and *n*-hexane (2:3). The organic fraction was collected and dried under nitrogen gas, as described for the plasma sample. The residue was then dissolved with methanol and 20 μL of the supernatant was injected into the HPLC system.

3. Results

3.1. Preparation and characterization of PTX–PCEC–P80 micelles

In the present study, the PTX micelles were prepared by thin-film hydration method. The triblock PCEC copolymer could self-assemble into micelles with PTX encapsulated in the PCL core in distilled water at 60 °C. With the intention of finding a suitable drug loading and encapsulation efficiency of PTX, we fed various weight ratio of PTX/PCEC during the preparation of micelles. The results are shown in Table 1. According to M1–M4, the drug loading was increased with an increase in PTX/PCEC ratio (w/w) from 2/98 to 12/88. Meanwhile, except for M4, the encapsulating efficacies were all higher than 90%. The lower encapsulation efficiency of M4 might due to a saturation effect of PTX in the PCEC micelles. Thereby, PTX micelles with PTX/PCEC weight ratio in feed of 8/92 were chosen for further application and characterized in detail.

The PTX–PCEC–P80 micelles (PTX/PCEC = 8/92) had the drug loading of ca. 4.8% and encapsulation efficiency of ca. 90.4%. The mean particle size of the micelles was about 20.5 nm with the polydisperse index (PDI) of 0.209, as shown in Fig. 1(A). The morphology of the PTX–PCEC–P80 micelles observed by TEM is described in

Table 1
Drug loading and encapsulation efficiency of the PTX-loaded micelles.

Sample codes	PTX/PCEC (w/w)	PTX–PCEC		PTX–PCEC–P80	
		DL (%)	EE (%)	DL (%)	EE (%)
M1	2/98	1.86 ± 0.07	93.22 ± 3.60	1.20 ± 0.08	89.80 ± 6.12
M2	4/96	3.67 ± 0.17	91.74 ± 4.34	2.42 ± 0.11	90.87 ± 3.94
M3	8/92	7.37 ± 0.39	92.07 ± 4.91	4.82 ± 0.31	90.39 ± 5.82
M4	12/88	8.61 ± 1.12	71.73 ± 9.29	5.55 ± 0.59	69.35 ± 7.38

DL, drug loading; EE, encapsulation efficiency.

Fig. 2. Morphology of PTX-loaded PCEC micelles: (A) water; (B) PTX–PCEC micelles; (C) PTX–PCEC–P80 micelles; (D) freeze-dried powder of PTX–PCEC–P80 micelles; (E) the re-dissolved PTX–PCEC–P80 micelles in normal saline.

Fig. 1(B), which revealed that the self-assembled micelles are uniform mono-dispersed spheres and did not form aggregates in aqueous solution. Meanwhile, the mean diameters of the micelles were in good agreement with the determination using laser particle size analyzer method. Fig. 2 presents the appearance of the suspension and powder form of PTX in the micelles. The obtained PTX–PCEC micelles suspension was homogeneous and transparent, and the appearance did not change when the micelles were coated with polysorbate 80. Moreover, the lyophilized powder was easy to dissolve in water to form clarified micelles solution.

The release profile of free PTX and micelle-encapsulated PTX is shown in Fig. 3. In comparison to free PTX, the release of PTX from PTX–PCEC and PTX–PCEC–P80 micelles represented a sustained manner. It was clearly observed that the release profile of PTX exhibited a biphasic pattern: the initial burst release within the first 24 h and a following slower and sustained release over a long

Table 2
Release kinetics of the PTX-loaded micelles.

Formulation	First order	Higuchi	Korsmeyer–Peppas		Weibull
	r^2	r^2	r^2	n	r^2
Free PTX	0.9678	0.9286	0.9400	0.411	0.9882
PTX–PCEC	0.9714	0.9964	0.9909	0.465	0.9911
PTX–PCEC–P80	0.9317	0.9756	0.9835	0.473	0.9928

time. That might be ascribed to the instantaneous diffusion of the drug entrapped in the surface layer of the micelles in the first stage, while the drug inside the micelles slowly dissolved into the media with the erosion of the copolymers in the second stage. After coated with polysorbate 80, the PTX micelles remain the function of sustained release, and a faster release of PTX was visibly observed than the uncoated micelles. This might be the result of the solubilization of hydrophobic drug caused by polysorbate 80. To predict the release kinetics, several drug release models (including first order, Higuchi, Korsmeyer–Peppas and Weibull distribution model) were adopted, and the results are presented in Table 2. The PTX release profile from uncoated PCEC micelle was best fitted with the Higuchi model (r^2 = 0.9964), while Weibull distribution model gave the best fit for polysorbate 80 coated micelles (r^2 = 0.9928). Otherwise, the n values obtained from the Korsmeyer–Peppas equation indicated that the mechanism of drug release from both coated and uncoated micelles was the coalition of diffusion and erosion.

3.2. Safety evaluation of blank micelles in vitro

The cytotoxicity of blank polysorbate 80 coated PCEC micelles was evaluated by cell viability assay on HEK293 cell line, as shown in Fig. 4. Cell proliferation was not suppressed by the blank micelles at a concentration lower than 1 mg/mL. The blank micelles (>1 mg/mL) displayed some concentration-dependent cytotoxicity on HEK293 cells. It was implied that the polysorbate 80 coated PCEC

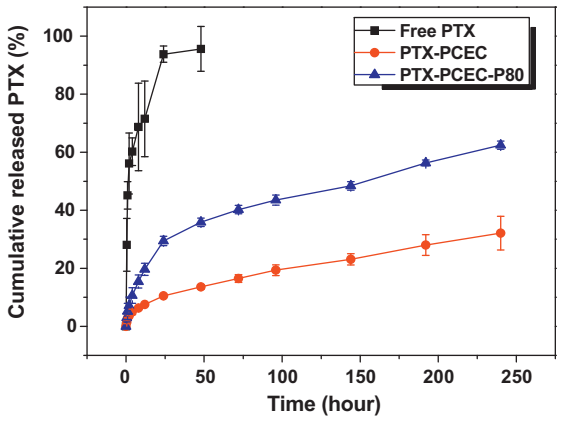

Fig. 3. The release profiles of PTX from uncoated and coated PCEC micelles with polysorbate 80 in vitro.

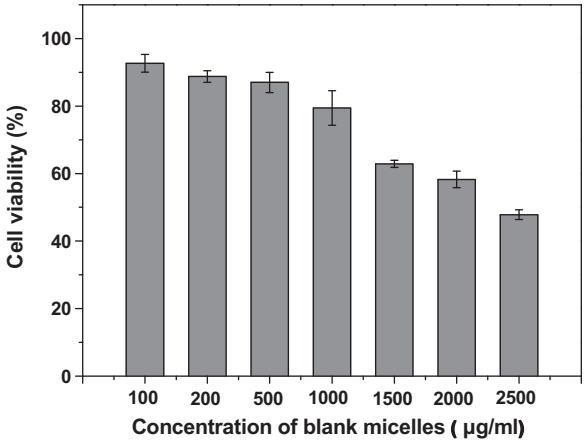

Fig. 4. Cytotoxicity of blank polysorbate 80-coated PCEC micelles against HEK293 cells in vitro.

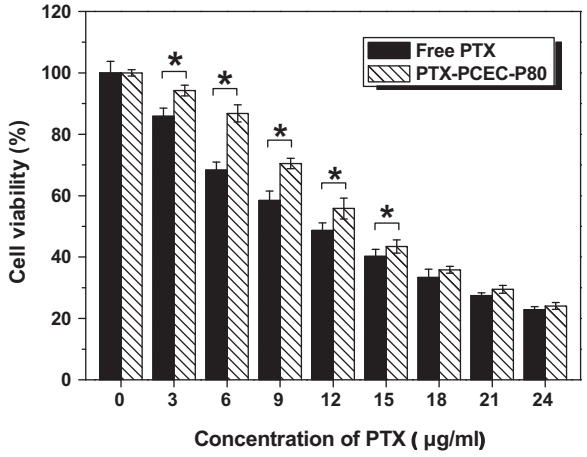

Fig. 5. Anticancer activity of free PTX and PTX–PCEC–P80 micelles on C6 cells in vitro. *: Statistical difference at the same concentration ($p < 0.05$).

micelles were low-toxic according to the evaluation on HEK293 cell line in vitro.

3.3. Anticancer activity in vitro of PTX–PCEC–P80 micelles

The cytotoxicity of PTX–PCEC–P80 micelles and free PTX was compared in the C6 glioma cell line in vitro. The PTX-loaded micelles maintained the anticancer activity of PTX. According to Fig. 5, both of the free PTX and PTX-loaded micelles significantly decreased the viability of C6 cells with the increase of drug concentration. But it was shown that the anticancer effect of PTX micelles was a little weaker than free PTX at the low concentrations below 15 μg/mL ($p < 0.05$). That might because there was a slow releasing process of drug from the micelles, which is also shown in Fig. 3. The results indicated that the encapsulation of PTX in micelles did not lose its anticancer activity, although the toxicity was somewhat reduced in comparison with free PTX. In summary, The PTX–PCEC–P80 micelles had efficient anticancer effect on C6 glioma cell line in vitro.

3.4. Pharmacokinetic profile and tissue distribution

The plasma concentration–time profiles of PTX after intravenous injection of the PTX–PCEC, PTX–PCEC–P80 micelles and Taxol® in rats are shown in Fig. 6. The pharmacokinetic parameters of PTX in the formulations are shown in Table 3. The

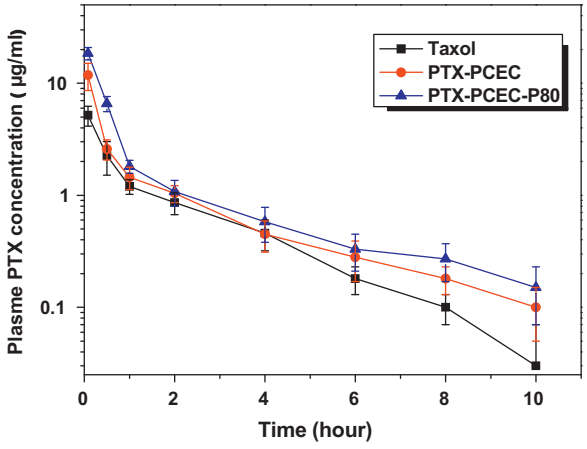

Fig. 6. Plasma concentrations–time profiles of PTX after intravenous injection of PTX formulations in rats.

Table 3
Pharmacokinetic parameters of the PTX formulations after intravenous administration in rats.

Parameters	Taxol	PTX–PCEC	PTX–PCEC–P80
AUC (mg/L h)	5.758 ± 0.975	7.088 ± 1.525	11.563 ± 2.018
AUMC (mg/L h^2)	14.952 ± 2.629	22.432 ± 9.190	29.510 ± 13.192
MRT (h)	2.642 ± 0.587	3.093 ± 0.674	2.476 ± 0.635
VRT (h^2)	10.255 ± 6.562	14.943 ± 8.564	13.432 ± 6.626
$t_{1/2}$ (h)	2.287 ± 0.870	2.726 ± 0.794	2.785 ± 0.652
CL (L/h/kg)	0.711 ± 0.120	0.588 ± 0.136	0.354 ± 0.056
V (L/kg)	2.420 ± 1.318	2.249 ± 0.494	1.399 ± 0.278

values of area under the plasma concentration–time curve (AUC) for Taxol, PTX–PCEC and PTX–PCEC–P80 were 5.758, 7.088 and 11.563 mg/L h, respectively; whereas the corresponding area under the moment curve (AUMC) 14.952, 22.432 and 29.510 mg/L h^2, respectively. The terminal half life ($t_{1/2}$) of PTX-loaded micelles increased to 2.726 h and 2.785 h, respectively, compared to Taxol (2.287 h). The corresponding total body clearance (CL) decreased from 0.711 to 0.588 and 0.354 L/h/kg, respectively. Though it was not clear whether the coated polysorbate 80 was a result of reduced RES uptake of PTX micelles, the higher AUC and decreased CL of PTX-loaded micelles were observed in comparison to Taxol.

The concentration of PTX in brain, heart, liver, spleen, lungs and kidneys after intravenous injection of Taxol®, PTX–PCEC and PTX–PCEC–P80 is shown in Fig. 7. The AUC values of each organ are shown in Table 4. The result indicated that only the PTX–PCEC–P80 micelles were able to deliver PTX in the brain, with the concentration of 0.36 ± 0.10 and 0.24 ± 0.09 μg/g at 0.5 and 2 h after administration, respectively. No drug was detected in the brain tissue in the Taxol group and uncoated micelles group. In lung tissue, PTX–PCEC micelles showed a significant high concentration of 6.5 and 8.1-fold when compared to PTX–PCEC–P80 micelles and Taxol at 0.5 h after administration, respectively. It might because the increased size of PCEC micelles owing to aggregation enhanced the uptake of the lung tissue. At 12 and 24 h after administration, the PTX concentrations of micelles group in kidney were markedly lower than that of Taxol group based on Fig. 7. Moreover, the AUC values of kidney in Taxol group (70.72 ± 3.98 mg/g h) was much larger than that of PTX–PCEC–P80 micelles group (29.77 ± 4.07 mg/g h). The reduced PTX concentration in kidney of micelles group might decrease the renal toxicity and it indicated no security risk. The concentration of PTX in the heart, liver and lungs were not significantly different between the groups of Taxol. In a word, the PTX–PCEC–P80 micelles significantly increased the uptake of PTX into the brain without any execrable accumulation in the other organs in comparison with Taxol and the uncoated micelles.

Table 4
AUC values of each organ after intravenous administration of the three PTX preparations.

Organ (μg/g h)	Taxol	PTX–PCEC	PTX–PCEC–P80
Brain	–	–	0.93 ± 0.31
Heart	14.80 ± 1.37	9.43 ± 1.48	19.77 ± 7.94**
Liver	97.50 ± 20.59	207.97 ± 17.48*	144.14 ± 15.75**
Spleen	15.51 ± 1.49	15.18 ± 0.57	21.72 ± 0.88*,**
Lung	10.77 ± 1.25	155.13 ± 17.86*	29.72 ± 0.98*,**
Kidney	70.72 ± 3.98	38.13 ± 6.78*	29.77 ± 4.07*

– Under the limit of detection of the technique.
* Statistical difference from Taxol group ($p < 0.05$).
** Statistical difference from PTX–PCEC group ($p < 0.05$).

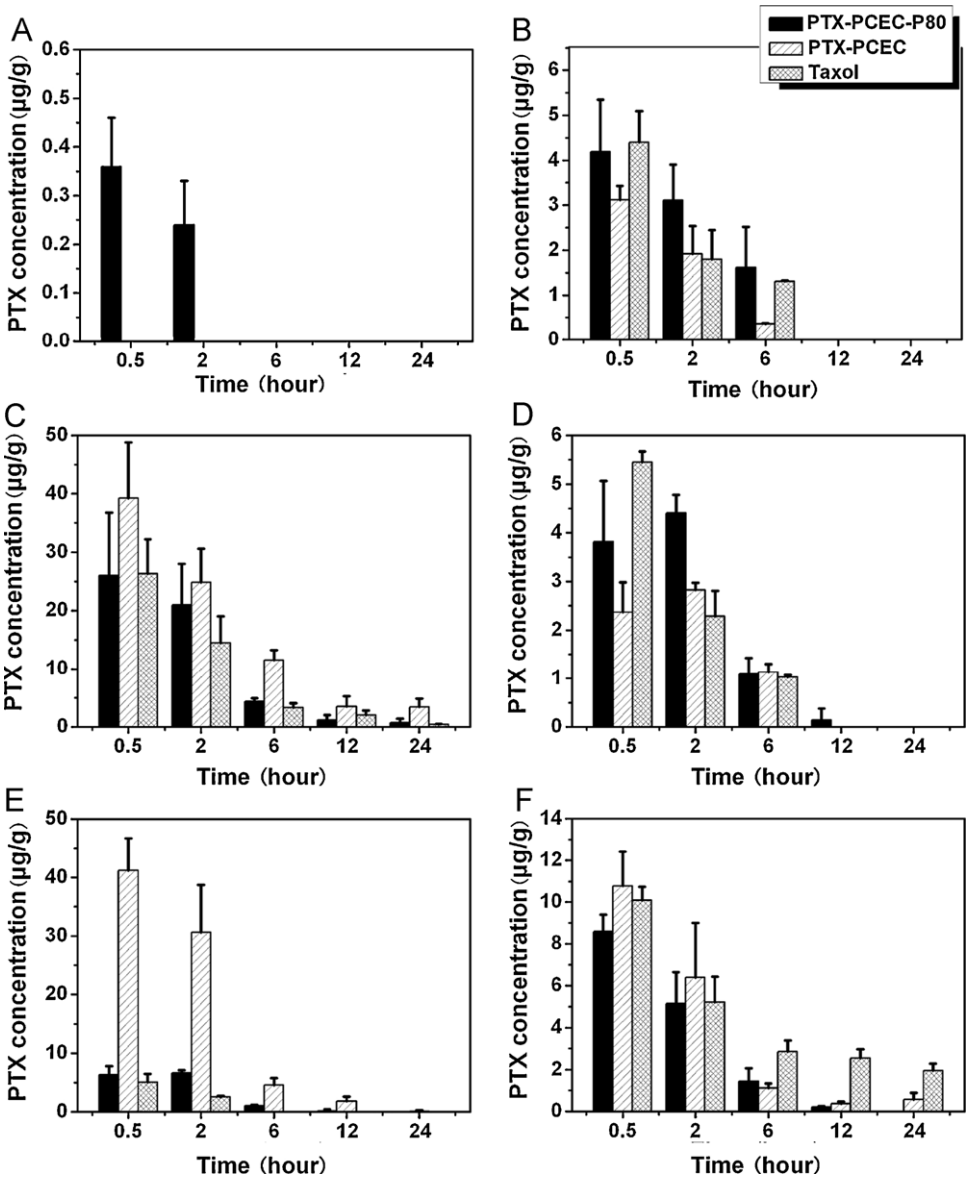

Fig. 7. PTX concentrations in different organs after intravenous injection of PTX formulations: (A) brain, (B) heart, (C) liver, (D) spleen, (E) lung, (F) kidney.

4. Discussion

Because of the hydrophobicity of PTX, lots of work has been done to improve its water solubility. Loading PTX in the polymeric micelles is an effective way to obtain injectable PTX formulation (Singla et al., 2002). Zhang et al. also chose PCL–PEG–PCL as PTX delivery system (Zhang et al., 2011a,b). In Zhang's work, PTX was loaded in PCEC (4000-8000-4000). However, the molecular mass of PCEC (850-2000-850) was lower in our work considering that the degradation product PEG (Mn < 5000) could be excreted from kidney. Previously, toxic organic solvents (methanol (Hu et al., 2006), acetonitrile (Cho et al., 2004; Liggins and Burt, 2002), tetrahydrofuran (THF) (Lee et al., 2003) or dimethyl formamide (DMF) (Du et al., 2010)) were introduced to dissolve PTX and polymeric materials in the preparation of the micelles, and ultimately they were removed through the dialysis method (Huh et al., 2005; Lee et al., 2003) or the solvent evaporation method (Danhier et al., 2009a; Liggins and Burt, 2002). Though there was no report related on the quantity of residual solvents, high attention must be drawn to the great potential threats of these insecure raw materials. Recently, Qu et al. developed PTX micelles by dialysis method, using dehydrated ethanol and water as the solvents of PTX and polymers, respectively (Qu et al., 2009; Zhang et al., 2008). It was implied that the toxic solvents of PTX mentioned above could be replaced by non-toxic dehydrated ethanol during the preparation. In the present work, both PTX and PCEC were dissolved in dehydrated ethanol, and drug-loaded micelles were achieved by thin-film hydration method. So, we have successfully established a safe, simple and rapid method for the construction of PTX-loaded PCEC micelles to increase the solubility of the hydrophobic chemotherapeutics in water. The prepared micelles had the advantages of high encapsulation efficiency, uniform size, sustained drug release behavior and the maintained anticancer activity. More important, the results in the safety studies demonstrated that the formulation of PTX micelles might be a safe carrier for intravascular administration.

The results of animal testing suggested that polysorbate 80 coated PCEC micelles were able to deliver the antitumor agent PTX in the brain significantly in comparison to Taxol, but the PCEC micelles without coating could not enhance the drug uptake by brain tissue. It was concluded that the micelles coated with

polysorbate 80 had the ability to help PTX cross BBB, in good agreement with the previous reports of other drugs, such as 5-fluorouracil, amphotericin B and loperamide (Alyautdin et al., 1997; Lemke et al., 2010; Soni et al., 2006). To the best of our knowledge, BBB is a physiological barrier of tight junctions between the blood system and the brain tissue. It can prevent most venenous foreign substances and maintain the normal physiological functions of brain. However, because it is difficult for therapeutic drugs to permeate BBB, the treatment of central nervous system diseases is unsatisfactory. With the development of nanotechnology in the past decade, polysorbate 80 coated nanoparticles has been explored to transport drugs across the BBB into brain (Tröster et al., 1990). Though the mechanism is still unclear until now, several points of view are supported by previous studies: (1) promotion of endocytosis by the capillary endothelial cells; (2) inhibition of the P-glycoprotein (P-gp) expressed on choroid plexus epithelial cells. It is proposed that after administration of polysorbate 80 coated nanoparticles intravenously, polysorbate 80 cause specific adsorption patterns of apolipoprotein E onto the surface of nanoparticles (Goppert and Muller, 2005; Kreuter et al., 2002). The apolipoprotein-overcoated nanoparticles mimics low density lipoprotein (LDL), leading to the uptake by endothelial cells on brain blood vessels via LDL receptor-mediated endocytosis (Kreuter, 2004; Zensi et al., 2009). Besides, P-gp, a kind of membrane transport proteins, is a potent efflux pump expressed on the endothelial cells of brain capillary blood vessels (Fricker, 2005; Terasaki and Hosoya, 1999). The inhibition of P-gp with the aid of polysorbate 80 may increase the drug concentration of brain tissue (Kreuter, 2001; Tamai and Tsuji, 1996). The result in the present study demonstrated that coating PCEC micelles with polysorbate 80 is an effective way to transport drug across BBB, in accordance with the widely reported poly (butylcyanoacrylate) nanoparticles coated with polysorbate 80 (Alyautdin et al., 1998; Sun et al., 2010; Wahab et al., 2005; Wilson et al., 2008b).

According to the results of proliferation assay on C6 rat glioma cell line in vitro, PTX showed efficient anticancer activity. The polysorbate 80 coated PCEC micelles designed in the present study was a novel PTX vehicle which could deliver the chemotherapeutic agent to brain tissue.

5. Conclusion

In this study, an excellent PTX formulation based on polysorbate 80 coated PCEC micelles was successfully prepared. It was demonstrated as a safe and effective dosage form with good water solubility. This novel PTX delivery system might open a new page of PTX application in brain tumor treatment.

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