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Research Paper

Preparation and the in-vivo evaluation of paclitaxel liposomes for lung targeting delivery in dogs

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Abstract

Objectives The aim of this study was to develop paclitaxel liposomes for a lung targeting delivery system.

Methods The liposomes composed of Tween-80/HSPC/cholesterol (0.03 : 3.84 : 3.84, mol/mol), containing paclitaxel and lipids (1 : 40, mol/mol), were prepared by a combination of solid dispersion and effervescent techniques, and then subjected to ultrasonication. The pharmacokinetics and biodistribution of liposomal and injectable formulation of paclitaxel in dogs were studied after intravenous administration.

Key findings The mean diameter, polydispersity index, zeta-potential and entrapment efficiency of the liposomes were 501.60 ± 15.43 nm, 0.28 ± 0.02, –20.93 ± 0.06 mV and 95.17 ± 0.32%, respectively. The liposomal formulation kept stable for at least 3 months at 6 ± 2°C and didn't cause haemolysis. The liposome carrier decreased the area under the curve and terminal half-life of paclitaxel compared with paclitaxel injection ranging from 0.352 ± 0.031 mg/l*h and 0.0671 ± 0.144 h to 0.748 ± 0.062 mg/l*h and 1.978 ± 0.518 h, respectively. The paclitaxel liposomes produced a drug concentration in the lung that was markedly higher than that in other organs or tissues and was about 15-fold of that of paclitaxel injection at 2 h.

Conclusions To sum up, these results demonstrated that the paclitaxel liposomes are an effective lung targeted carrier in the treatment of lung cancer.

Keywords distribution; liposomes; lung targeting drug delivery; paclitaxel; preparation

Introduction

Paclitaxel belongs to the taxane class (Figure 1) of antitumoral drugs that has shown substantial anti-tumour effect against non-small cell lung cancers (NSCLC) and ovarian, breast, colon, head and neck cancers.[1–4] However, the strong hydrophobicity of paclitaxel drastically limits its use in the natural form. Paclitaxel must be dissolved in a 1 : 1 mixture of Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol for clinical use.[5] The two problems with this injectable formulation still haven't been resolved: in clinical use, this preparation must be diluted with 5% of dextrose solution or 0.9% NaCl of saline (5–20 fold), but paclitaxel crystal precipitation is often observed;[6,7] also, Cremophor EL used in this formulation has been associated with considerable side effects, such as hypersensitivity and neurotoxicity.[6,8,9] To overcome these difficulties, many new dosage forms and technologies have been developed to improve its aqueous solubility, such as liposomes,[7,10–12] emulsions,[13] cyclodextrins[8] and mixed-micellar formulations.[14] In addition, water-soluble prodrug synthesis has also been extensively investigated.[15]

In the treatment of lung cancer, a lung-targeting delivery system is an ideal strategy for enhancing the therapeutic index and reducing the side effects of many anti-tumour drugs. In a previous study, we developed docetaxel loaded liposomes with a diameter of about 1 μm by a combination of solid dispersion and effervescent dispersion techniques.[16] Interestingly, it was found that this liposome carrier could greatly enhance the drug concentration in the lung rather than in the heart, kidney, stomach or brain after intravenous administration of liposomal formulation of docetaxel in a rabbit model.

Therefore, the aim of this study was to investigate whether the smaller-size liposome carrier with a size of about 500 nm could also produce a lung-targeting effect. In this study, paclitaxel liposomes were developed and evaluated *in vitro*, and then a comparison was

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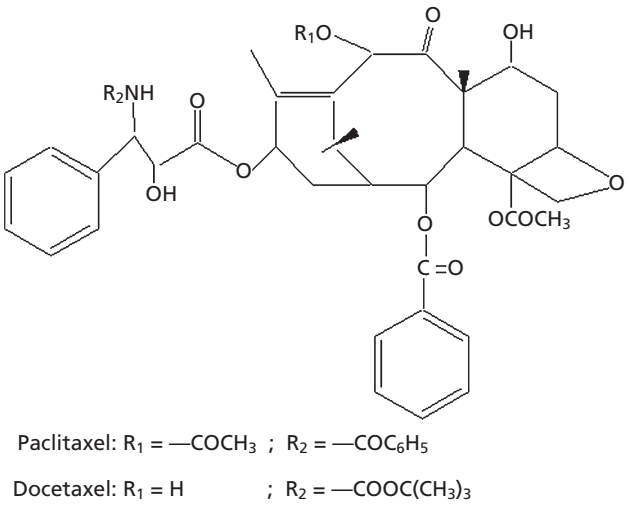

Figure 1 The chemical structures of paclitaxel and docetaxel.

made between the pharmacokinetics and biodistribution of the liposomal and injectable formulation of paclitaxel in dogs.

Materials and Methods

Animals and reagents

Twenty-five dogs (6.0 ± 1.0 kg) were obtained from the Laboratory Animal Center of Luzhou Medical College. The dogs used in this study were provided with standard care and nutrition. All the experimental procedures were approved by the Luzhou Medical College animal ethical experimentation committee (Approve number: LZMC1052-09021).

Paclitaxel was purchased from Chongqing Meilian Pharmaceutical Co., Ltd (Chongqing, P.R. China). Cremophor EL was obtained from Shanghai Yunhong Chemical Co., Ltd (Shanghai, P.R. China). Cholesterol was provided by Nanjing Xinbai Medicine Co., Ltd (Jiangsu, P.R. China). Phosopholipon 90 H (HSPC) was purchased from Nattermann-Phospholipid (Cologne, Germany). Citric acid was obtained from Hunan Dongting Citric acid Co., Ltd (Hunan, P.R. China). Carbonic acid monosodium salt (NaHCO3) was purchased from Hebei Huachen Medicine Co., Ltd (Hebei, P.R. China). Injection-grade Tween-80 was from Beijin Huiyou chemical Co., Ltd (Beijing, P.R. China). Dialysis membrane bag with molecular of 8000–10 000 and 8000–12 000, analytical-grade ethanol and other reagents were purchased from Chengdu Chemical Regent Co., Ltd (Chengdu, P.R. China). Methanol and acetonitrile used (HPLC grade) were purchased from Jiangsu Hanbang Co., Ltd (Jiangsu, P.R. China).

Liposome preparation

Paclitaxel liposomes were prepared by a modified method as described in our previous research.[16] Briefly, the paclitaxel proliposomes composed of Tween-80/HSPC/cholesterol/citric acid (0.03 : 3.84 : 3.84 : 92.29, mol/mol), containing paclitaxel and lipids (1 : 40, mol/mol), were prepared by solid dispersion technique. These components were dissolved in ethanol and transferred to a round-bottom flask and continually stirred at 400 rev/min at 50°C until the organic solvent was completely removed to obtain paclitaxel solid granules (paclitaxel proliposomes). The granules were solidified at 6 ± 2°C under vacuum for 24 h and placed in sealed desiccators under nitrogen gas at 6 ± 2°C in the dark.

These proliposomes were hydrated with $NaHCO_3$ solution (5.0%, w/v) and rapidly converted into liposome suspension under constant shaking. To obtain small and homogeneous particles, the liposome suspension was performed by ultrasonication for 5 min followed by five extrusion cycles through a 0.5-μm filter.

The zeta-potential, particle size and polydispersity index (PDI) of liposomes were measured by Malvern ZEN3600 (Malvern Instruments, UK) after dilution of liposome suspension with distilled water (30 fold). The paclitaxel concentration of the liposomal preparation and biosamples was analysed by reverse-phase HPLC using Phenomenex LUNA C18 (250 mm × 4.6 mm, 5 μm particle size) (Phenomenex, USA) and a mobile phase containing methanol/acetonitrile/0.02 M ammonium acetate buffer (pH 5) (50 : 20 : 30, v/v/v) with ultraviolet (UV) detection at 227 nm, running at a flow rate of 1 ml/min.

The entrapment efficiency was measured by using a dialysis method (dialysis membrane with molecular weight cut-off of 8000–10 000). The liposome sample prepared by diluting liposome suspension with distilled water (5 fold, v/v) was placed in a dialysis membrane bag and dialysed against phosphate-buffered saline (pH 7.4) for 5 h by gentle shaking. After dialysis, the free drug content (W_f) was determined by HPLC described above. Acetonitrile (2 ml) was mixed with the liposome suspension (1 ml) by vortex for 5 min to destroy the liposome structure followed by filtration through a 0.22-μm hydrophobic Millipore membrane to determine the total amount of paclitaxel (W_{total}) by HPLC. The entrapment efficiency was calculated according to the equation as follows.

$$\text{Entrapment efficiency}(\text{EE}\%) = (W_{total} - W_f)/W_{total} \times 100\% \quad (1)$$

Examination of stability of liposomes

An accelerated stability test and long-term stability test were conducted according to the Technical Standard of Drug Stability Test (Chinese Pharmacopoeia 2005, appendix XIX C). For the accelerated stability test, liposome sample was placed at 25 ± 2°C, RH 60% ± 10% for 3 months and sampled at months 0, 1, 2 and 3. For the long-term stability test, liposome samples were stored at 6 ± 2°C for 3 months and sampled at months 0, 1, 2 and 3. The entrapment efficiency, particle size, PDI and zeta-potential of paclitaxel liposomes in both stability tests were determined by using the methods described above.

Haemolysis test

Blood sample (10 ml) obtained from the heart of a healthy albino rabbit approved by the Luzhou Medical College animal ethical experimentation committee (Approve number: LZMC1052-09013) was stirred to remove fibrinogen by glass stick. Then 0.9% saline (10 ml) was added and the supernatant was removed by centrifugation. Additional 0.9% saline (10 ml) was added to the precipitation followed by gently shaking and centrifuging to remove the supernatant again. The

Table 1 Haemolysis test for paclitaxel liposomes for injection

No. of test tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Paclitaxel liposomes (ml)	0.1	0.1	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.5	0	0	0	0
Saline (ml)	2.4	2.4	2.3	2.3	2.2	2.2	2.1	2.1	2.0	2.0	2.5	2.5	0	0
Distilled water (ml)	0	0	0	0	0	0	0	0	0	0	0	0	2.5	2.5
2% Haematocyte (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Result of haemolysis test[a]	–	–	–	–	–	–	–	–	–	–	–	–	+	+

[a]–, no haemolytic reaction; +, haemolytic reaction.

same course was repeatedly performed until a colourless supernatant resulted. The obtained haematocyte (2 ml) was used and diluted with 0.9% saline (2%, v/v) for the haemolysis test described in Table 1. Of 14 test tubes, tubes 11 and 12 were chosen as blank control without adding paclitaxel liposome sample. Tubes 13 and 14 were chosen as positive control by adding distilled water instead of 0.9% saline. Every test tube was placed in a water bath (37°C) for 3 h with gentle shaking for measurements.

Pharmacokinetics and distribution

Twenty-five dogs were divided into five groups (five dogs per group). Group 1 (No. 1–5) and group 2 (No. 6–10) were treated with paclitaxel-loaded liposomes at 2 mg/kg body weight via the forelimb by intravenous injection. Group 3 (No. 11–15) and group 4 (No. 16–20) were treated with paclitaxel injection (2 mg/kg) prepared by using the same commercial formulation, in which free paclitaxel was dissolved in a mixture of Cremophor EL and ethanol (1 : 1) followed by dilution with 0.9% NaCl saline to the desired concentration. Group 5 (No. 21–25), treated with 0.9% NaCl saline at the same volume, was used to collect blank plasma and tissues.

For group 1 and 3, blood samples (2.5 ml) were taken from the hind limb vein at 5, 10, 15 and 30 min, and 1, 2, 4, 6, 8 and 12 h after drug administration. Blood samples were centrifuged at 5000 rev/min for 5 min to separate the plasma. After the last (12 h) blood sample was obtained, these dogs were treated with 3.0% pentobarbital sodium solution anaesthesia and killed immediately. In addition, dogs in groups 2 and 4 were sacrificed by the same method at 2 h after drug administration. The dogs were dissected and various tissues (heart, liver, spleen, lung, kidney, stomach and brain) were extracted, weighed and homogenized with 0.9% saline (4 ml/g).

The obtained biosamples were pretreated by a modified method as described previously.[16] Briefly, plasma (1 ml) or tissue homogenates (1 ml) were mixed with 50 μl of docetaxel methanol (40 μg/ml) as the internal standard for the determination of paclitaxel. The mixture was vortexed with 3 ml protein precipitation agent (acetonitrile–10 M ammonium acetate buffer (pH 5.0) (1 : 1 v/v)) for 5 min followed by ultrasonication at 50°C for 5 min and centrifugation at 12 000 rev/min for 5 min. The clear supernatant was diluted (5 fold, v/v) with distilled water. The samples were loaded into an individual solid-phase extraction column that was first activated with 2 ml of methanol followed by 2 ml of 0.01 M ammonium acetate (pH 5.0). After loading the sample solution, each column was washed in turn with 2 ml of 0.01 M ammonium acetate (pH 5.0), methanol–0.01 M ammonium acetate (pH 5.0) (1 : 9 v/v) and methanol–0.01 M ammonium acetate (pH 5.0) (2 : 8 v/v), and dried under vacuum for about 1 min. Paclitaxel was eluted from the columns using 2 ml of acetonitrile and was dried under a stream of nitrogen at 40°C. The residues were dissolved in 0.2 ml of mobile phase and injected into HPLC system (20 μl each sample) as described above.

Statistical analysis

In this study statistical analysis of the effect of time on the entrapment efficiency, particle size, PDI and zeta-potential of paclitaxel loaded liposomes was performed by using a Kruskal–Wallis test. Individual differences between times were then analysed by using a post-hoc test (Dunn's test). The statistical difference between means of the two formulations was identified by using Mann–Whitney *U*-test. In all cases a significance level of less than 0.05 was considered statistically significant.

Results

Analysis method validation

The representative HPLC-UV chromatograms for determination of paclitaxel in plasma and lung tissues were shown in Figures 2 and 3, respectively. Paclitaxel and docetaxel as internal standard were well separated and no interference was detected from endogenous substances or metabolites.

With this analysis method the limit of quantification (LOQ) in plasma was 12.5 ng/ml, in lung tissue 50 ng/ml and in other tissues 25 ng/ml. The precision and accuracy of this method were determined by adding known amounts of paclitaxel to blank plasma and tissues and the results are summarized in Table 2. It was believed from these results that this analysis method was accurate and precise with coefficients of variation with intra-and inter-day R.S.D. below 10% for all the biosamples. The relative recoveries of paclitaxel in plasma and tissues ranged from 95.5% to 97.6% and 80.8% to 108.2%, respectively. According to the requirements of Chinese Pharmacopoeia (2010 edition, part II), these results were within the acceptable range.

The extraction efficiency for paclitaxel biosamples was calculated by comparing peak area with QC samples at low, medium and high concentration levels. The mean extraction efficiency of paclitaxel in dog plasma and tissues was in the range 82.5–90.1% and 69.3–81.2%, respectively.

Preparation and characterization of liposomes

The liposomal formulation of paclitaxel used in this study was prepared by a combination of solid dispersion and

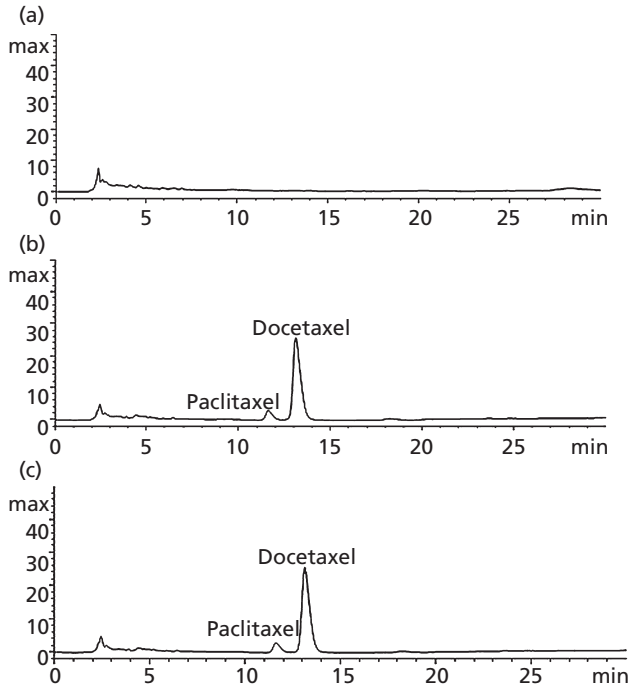

Figure 2 HPLC-UV chromatograms obtained for blank plasma (a), blank plasma spiked with paclitaxel + docetaxel as the internal standard (b), plasma sample collected from a dog at 0.5 h after intravenous administration of paclitaxel at a dosage of 2 mg/kg + docetaxel as the internal standard (c).

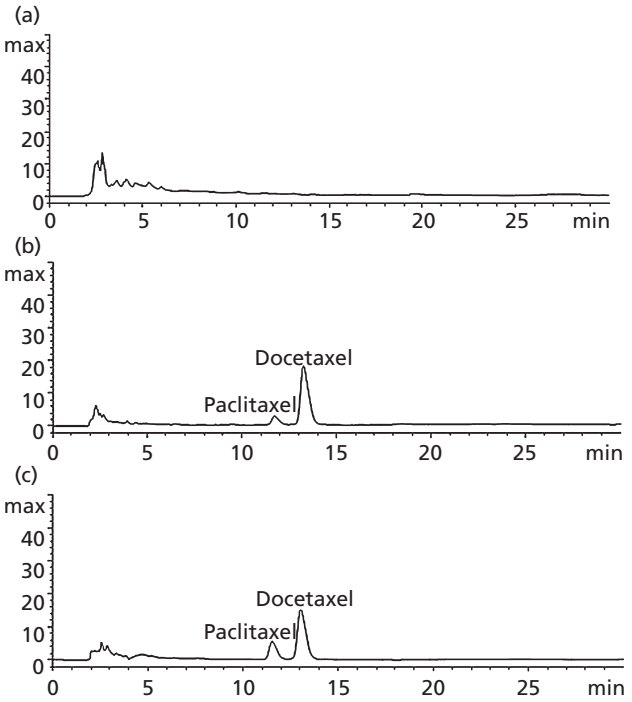

Figure 3 HPLC-UV chromatograms for determination of paclitaxel in a dog lung. Blank lung tissue (a), blank lung tissue spiked with paclitaxel + docetaxel as the internal standard (b), lung tissue sample collected from a dog at 12 h after intravenous administration of paclitaxel at a dosage of 2 mg/kg + docetaxel as the internal standard (c).

effervescent techniques, and ultrasonication method. According to the zeta-potential, entrapment efficiency, mean diameter and PDI as markers, the formulation and technology was optimized to obtain the optimal formulation composed of Tween-80/HSPC/cholesterol/citric acid at a molar ratio of 0.03 : 3.84 : 3.84 : 92.29, containing paclitaxel and lipids (1 : 40, mol/mol). To obtain desirable liposomes with a diameter of about 0.5 μm, the liposome suspension was subjected to ultrasonication for 5 min. The zeta-potential, entrapment efficiency, mean diameter and PDI of liposomes were 20.93 ± 0.06 mV, 95.17 ± 0.32%, 501.60 ± 15.43 nm and 0.28 ± 0.02, respectively (Table 3).

Stability of liposomes

The results of the accelerated stability and long-term stability tests are shown in Table 3. The accelerated testing results showed marked change in particle size, PDI, zeta-potential and entrapment efficiency ($n = 3$; $P < 0.05$). However, no significant difference in particle size, PDI, zeta-potential and entrapment efficiency was observed in long-term stability testing ($n = 3$; $P > 0.05$). Therefore, the liposomal formulation of paclitaxel kept stable for at least 3 months at 6 ± 2°C.

Haemolysis test

As shown in Table 1, paclitaxel liposomes did not cause haemolysis. In addition, no haemolysis was observed in plasma collected from pharmacokinetics and biodistribution study. Therefore, to some extent, it was believed that this liposomal formulation did not result in haemolysis in rabbits and dogs.

Pharmacokinetics

After intravenous administration of injectable and liposomal formulation containing paclitaxel in dogs at a dose of 2 mg/kg, the plasma pharmacokinetic profile of paclitaxel was assessed with DAS software. The plasma paclitaxel concentration vs time curve and pharmacokinetic parameters of the two preparations of paclitaxel are shown in Figure 4 and Table 4. As shown in Figure 4, the paclitaxel concentration on the plasma was markedly lower at each time point, except for 0.083 h, for the liposomal formulation than for the injectable formulation. The tested formulations of paclitaxel presented biphasic clearance kinetics with a rapid distribution phase ($t^1/_2\alpha$ 0.122 ± 0.011 h and 0.060 ± 0.006 h, respectively). The liposome carrier decreased the terminal half-life of paclitaxel ranging from 1.978 ± 0.518 h to 0.0671 ± 0.144 h ($P < 0.05$). The clearance rate of the injectable and liposomal formulation of paclitaxel was 2.388 ± 0.201 l/h/kg and 4.363 ± 0.471 l/h/kg, respectively ($P < 0.05$). Furthermore, it was found that the area under the plasma drug concentration vs time curve (AUC) presented a marked decrease with the liposomal formulation compared with that of paclitaxel injection ($P < 0.05$).

Biodistribution

To further evaluate the in-vivo lung-targeting behaviour of the liposome carrier, the biodistribution in dogs was studied after

Table 2 Intra-day and inter-day accuracy and precision of paclitaxel in dog plasma and tissues homogenous quality control samples (means ± SD, $n = 3$)

Added concentration (μg/ml or μg/g)[a]	Intra-day			Inter-day		
	Measured concentration (μg/ml or μg/g)[a]	Accuracy (%)	(R.S.D.) (%)	Measured concentration (μg/ml or μg/g)[a]	Accuracy (%)	(R.S.D.) (%)
Plasma						
0.0125	0.01442	115.5	8.55	0.0141	112.4	9.87
0.125	0.1296	103.7	6.78	0.1315	105.2	8.34
1.00	0.9760	97.6	5.55	0.9810	98.1	4.53
Heart						
0.025	0.02123	84.9	8.56	0.02208	88.3	9.25
0.25	0.2313	92.5	7.51	0.2273	90.9	6.94
2.00	2.0220	101.1	5.58	1.9520	97.6	5.78
Liver						
0.05	0.0458	91.6	7.65	0.0442	88.3	9.82
1.00	1.0050	100.5	6.09	0.9210	92.1	6.98
4.00	4.1800	104.5	2.68	3.8200	95.5	5.32
Spleen						
0.05	0.0443	88.5	6.68	0.0431	86.2	7.55
0.50	0.5075	101.5	4.53	0.4515	90.3	6.32
2.00	1.9900	99.5	2.33	1.978	98.9	4.12
Lung						
0.10	0.0955	95.5	7.46	0.0987	98.7	6.75
2.00	2.1080	105.4	4.19	2.1640	108.2	4.78
20.00	20.5000	102.5	2.09	20.2600	101.3	2.35
Kidney						
0.05	0.0413	82.5	8.90	0.0404	80.8	9.38
0.50	0.4680	93.6	6.09	0.4625	92.5	7.87
2.00	1.8800	94	3.89	1.9220	96.1	5.67
Stomach						
0.025	0.0202	80.9	9.18	0.0209	83.6	8.98
0.50	0.4175	83.5	8.31	0.4020	80.4	8.12
2.00	1.7080	85.4	5.75	1.6360	81.8	6.29
Brain						
0.0125	0.0108	85.9	8.56	0.0110	88.0	6.88
0.125	0.1219	97.5	5.08	0.1199	95.9	5.21
1.00	1.0120	101.2	2.45	0.9890	98.9	3.34

[a]The unit of drug concentration in plasma and tissues is μg/mL and μg/g, respectively.

Table 3 Accelerated and long-term stability test

Parameters		Accelerated test			Long-term test		
	0 (month)	1 (month)	2 (month)	3 (month)	1 (month)	2 (month)	3 (month)
Particle size (nm)	501.60 ± 15.43	531.87 ± 27.05[a]	593.83 ± 36.08[ab]	683.50 ± 60.21[abc]	502.93 ± 11.97	504.67 ± 23.08	507.07 ± 16.06
PDI	0.28 ± 0.02	0.32 ± 0.01[a]	0.49 ± 0.02[ab]	0.68 ± 0.02[abc]	0.25 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
Zeta-potential (mV)	−20.93 ± 0.06	−18.56 ± 0.0.25[a]	−18.10 ± 0.38[ab]	−17.90 ± 0.17[ab]	−20.63 ± 0.21	−20.43 ± 0.53	−21.03 ± 0.13
Entrapment efficiency (%)	95.17 ± 0.32	91.16 ± 3.57[a]	87.17 ± 1.22[ab]	81.63 ± 1.24[abc]	95.80 ± 0.52	95.40 ± 0.19	95.63 ± 0.49

Results are means ± SD, $n = 3$. [a]$P < 0.05$, compared with 0 month; [b]$P < 0.05$, compared with 1 month; [c]$P < 0.05$, compared with 2 month.

intravenous administration of the injectable and liposomal formulation of paclitaxel at a dose of 2 mg/kg and the results are shown in Figure 5. For the paclitaxel injection, the drug concentration was the highest in kidney (2.244 ± 0.335 μg/g) and the next highest in liver (1.951 ± 0.112 μg/g), while it was only 1.07 ± 0.299 μg/g in lung. However, compared with paclitaxel injection, drug concentrations in lung at 2 h and 12 h after intravenous administration of liposomal formulation were enhanced from 1.07 ± 0.299 μg/g to 15.119 ± 1.040 μg/g (about 15 fold) and from 0.196 ± 0.069 μg/g to 2.793 ± 0.295 μg/g (about 14 fold), respectively ($P < 0.05$). Therefore, the drug distribution pattern *in vivo* in dogs was very similar during the experiment.

Discussion

For the treatment of lung disease, lung-targeted drug delivery systems have become an attractive strategy. Considering

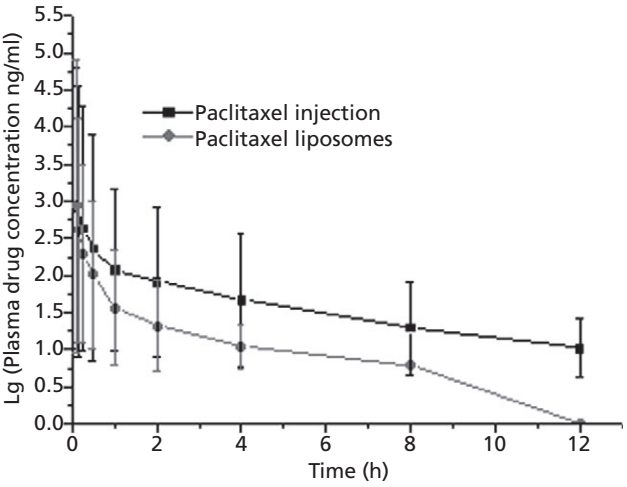

Figure 4 Pharmacokinetic behaviour of paclitaxel in Cremophor EL and ethanol and paclitaxel-loaded liposomes in dogs. Paclitaxel formulations were administrated via the forelimb intravenous injection at a dose of 2 mg/kg paclitaxel. Paclitaxel concentrations in plasma were determined after solid-phase extraction by reverse phase HPLC. The values are the means of five animals and standard deviation.

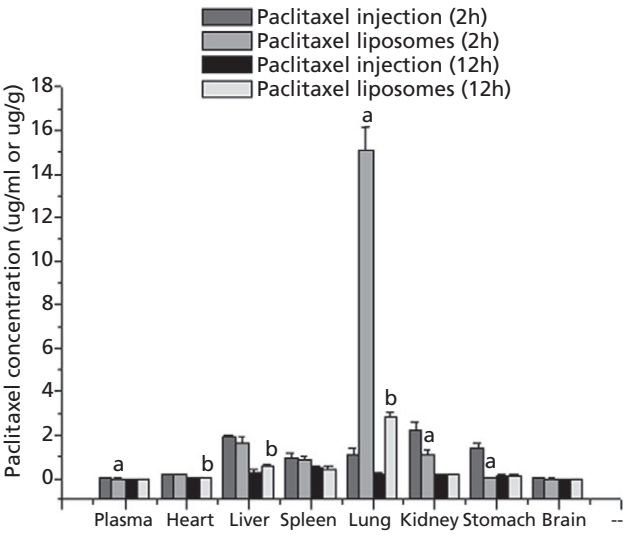

Figure 5 Biodistribution in dogs of paclitaxel injection and liposomes by the forelimb intravenous injection at a dose of 2 mg/kg paclitaxel. The values are the means of five animals and standard deviation. The units of paclitaxel concentration in plasma and in tissues are μg/ml and μg/g. $^{a,b}P < 0.05$, compared with paclitaxel injection.

Table 4 Pharmacokinetic parameters of paclitaxel formulations in dogs

Formulation	AUC(0-t) (mg/l*h)	t1/2á (h)	t1/2β (h)	CL (l/h/kg)	V (l/kg)	Cmax (mg/l)
Paclitaxel injection	0.748 ± 0.062	0.122 ± 0.011	1.978 ± 0.518	2.388 ± 0.201	0.992 ± 0.219	0.817 ± 0.078
Paclitaxel liposomes	0.352 ± 0.031[a]	0.060 ± 0.006[a]	0.0671 ± 0.144[a]	4.363 ± 0.471[a]	1.695 ± 0.165[a]	0.858 ± 0.094

Results are means ± SD, $n = 5$, $^{a}P < 0.05$, compared with paclitaxel injection.

safety in the clinic, great attention has been concentrated on lung-targeted liposomal formulations.[16–18] In our previous study,[16] docetaxel, as a sort of taxane, was chosen as a model drug. We prepared negatively charged docetaxel liposomes with a diameter of about 1 μm by solid dispersion and effervescent dispersion techniques. After intravenous administration of the liposomal formulation of docetaxel in rabbits, the drug concentration in lung was higher than that in other organs or tissues. These results indicated that the liposome carrier was associated with a lung-targeting effect. Therefore, in the present study, Paclitaxel liposomes were prepared and characterized, then the pharmacokinetic behaviour and biodistribution of liposomal and injectable formulation of paclitaxel were investigated to further study the lung-targeting property of the liposome carrier.

Preliminary experiments showed that the particle size was similar (about 1 μm) to all liposomal formulations of paclitaxel with different compositions after hydration by effervescent dispersion techniques. To obtain smaller-sized paclitaxel liposomes, these liposomes were further subjected to ultrasonication. Interestingly, the formulation described in this study was not composed of negatively charged lipids; however, the surface charge of paclitaxel liposomes prepared by the formulation reached about −21 mV (Table 3). It was concluded from the result that the negative charges on the surface of liposomes might be ascribed to the carboxylic acid ion in citric acid molecular structure after hydration with $NaHCO_3$ solution. Indeed, it was found that the negative charged value of particles in suspension tended to decrease with decrease in $NaHCO_3$ content. However, when the pH value of the suspension solution was less than 3.0, these paclitaxel liposomes were not stable. In general, the higher the absolute value of the zeta-potential (more than 15 mV), the stronger the electrostatic repulsion between particles achieved, which made it more difficult for these particles to congregate, and they remained more stable in the disperse system.[19] Therefore, the liposomal formulation of paclitaxel could maintain excellent stability over 3 months at 6 ± 2°C.

The plasma pharmacokinetics of paclitaxel liposomes showed a biphasic curve with a higher rate of clearance, lower AUC and shorter $t^1/_2\alpha$ and $t^1/_2\beta$ compared with those of paclitaxel injection ($P < 0.05$). As seen from previous literature,[10,20] the kinetic behaviour in some way reflects the profile and difference presented by general liposomes containing paclitaxel with respect to the paclitaxel injection. This result does not fit in with previous experimental findings. However, for lung-targeting preparations such as microspheres, liposomes and solid lipid nanoparticles containing other drugs, their pharmacokinetic results were similar to those of our experiment.[17,21,22] It is possible that the paclitaxel was delivered to the lung site by liposome carrier via the blood route,

which resulted in different dynamic parameters in comparison with paclitaxel injection.

As shown in Figure 5, the liposome carrier markedly altered the tissue distribution profile of paclitaxel in dogs compared with the paclitaxel injection ($P < 0.05$). After intravenous administration of paclitaxel liposomes in dogs, the drug concentration in the lungs was higher than in the other organs or tissues. As we know, the particle size, surface charge and lipid composition of the liposome carrier can influence the drug distribution *in vivo*.[23–25] At present, many researchers have demonstrated that a liposome carrier of ≥ 4 μm particle size exhibits improved localization in lung,[17,26] which may be explained by simple physical trapping of the liposomes in the capillary bed of the lungs. However, although the mean diameter of paclitaxel liposomes prepared in this study was only 503.37 ± 18.22 nm, the carrier showed the highest distribution of paclitaxel in lung. Therefore, the specific lung-targeted mechanism needs to be further studied.

Conclusions

In this study, we successfully prepared negatively charged liposomes containing paclitaxel with good stability and high entrapment efficiency. The paclitaxel liposomes showed excellent lung targeting properties in comparison with paclitaxel injection. In conclusion, the liposomes are a promising carrier for a lung-targeting drug delivery system for the treatment of lung diseases, such as lung cancer and tuberculosis.

Declarations

Conflict of interest

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

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References

1. Celikoglu F *et al*. Intratumoural chemotherapy of lung cancer for diagnosis and treatment of draining lymph node metastasis. *J Pharm Pharmacol* 2010; 62: 287–295.
2. Rowinsky EK, Donehower RC. Paclitaxel (taxol). *New Engl J Med* 1995; 332: 1004–1014.
3. Spencer CM, Faulds D. Paclitaxel – a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer. *Drugs* 1994; 48: 794–847.
4. Tanino T *et al*. Organic anion transporting polypeptide 2-mediated uptake of paclitaxel and 2'-ethylcarbonate-linked paclitaxel in freshly isolated rat hepatocytes. *J Pharm Pharmacol* 2009; 61: 1029–1035.
5. Henningsson A *et al*. Mechanism-based pharmacokinetic model for paclitaxel. *J Clin Oncol* 2001; 19: 4065–4073.
6. Balasubramanian SV, Straubinger RM. Taxol-lipid interactions: taxol-dependent effects on the physical properties of model membranes. *Biochemistry* 1994; 33: 8941–8947.
7. Straubinger RM *et al*. Novel taxol formulations: taxol-containing liposomes. *J Natl Cancer Inst Monogr* 1993; 15: 69–78.
8. Sharma US *et al*. Pharmaceutical and physical properties of paclitaxel (Taxol) complexes with cyclodextrins. *J Pharm Sci* 1995; 84: 1223–1230.
9. Sharma A *et al*. Activity of paclitaxel liposome formulations against human ovarian tumor xenografts. *Int J Cancer* 1997; 71: 103–107.
10. Crosasso P *et al*. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. *J Control Release* 2006; 63: 19–30.
11. Fetterly GJ, Straubinger RM. Pharmacokinetics of paclitaxel-containing liposomes in rats. *AAPS Pharm Sci* 2003; 5: 1–11.
12. Schmitt-Sody M *et al*. Neovascular targeting therapy: paclitaxel encapsulated in cationic liposomes improves antitumoral efficacy. *Clin Cancer Res* 2003; 9: 2335–2341.
13. Kan P *et al*. Development of nonionic surfactant/phospholipid o/w emulsion as a paclitaxel delivery system. *J Control Release* 1999; 58: 271–278.
14. Sznitowska M *et al*. Paclitaxel solubility in aqueous dispersions and mixed micellar solutions of lecithin. *Chem Pharm Bull* 2008; 56: 70–74.
15. Choi J, Jo BW. Enhanced paclitaxel bioavailability after oral administration of pegylated paclitaxel prodrug for oral delivery in rats. *Int J Pharm* 2004; 280: 221–227.
16. Zhao L *et al*. PK and tissue distribution of docetaxel in rabbits after i.v. administration of liposomal and injectable formulations. *J Pharm Biomed Anal* 2009; 49: 989–996.
17. Cheng J *et al*. Characterization, lung targeting profile and therapeutic efficiency of dipyridamole liposomes. *J Drug Target* 2006; 14: 717–724.
18. Wang JS *et al*. Preparation of lung targeting azithromycin liposomes and its tissue distribution in mice. *Acta Pharma Sinica* 2005; 40: 274–278.
19. Dong YC, Feng SS. Methoxy poly (ethylene glycol)-poly (lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. *Biomaterials* 2004; 25: 2843–2849.
20. Wu J *et al*. A folate receptor-targeted liposomal formulation for paclitaxel. *Int J Pharm* 2006; 316: 148–153.
21. Tang S *et al*. Pharmacokinetics and lung-targeting characterization of a newly formulated enrofloxacin preparation. *J Vet Pharmacol Ther* 2007; 30: 443–450.
22. Xiang QY *et al*. Lung-targeting delivery of dexamethasone acetate loaded solid lipid nanoparticles. *Arch Pharm Res* 2007; 30: 519–525.
23. Hirano K, Hunt CA. Lymphatic transport of liposome-encapsulated agents: effects of liposome size following intraperitoneal administration. *J Pharm Sci* 1985; 74: 915–921.
24. Kirby C *et al*. Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in vitro. *Biochem J* 1980; 186: 591–598.
25. Sada E *et al*. Effects of surface charges and cholesterol content on amino acid permeabilities of small unilamllar vesicles. *J Pharm Sci* 1990; 79: 232–235.
26. Sree H *et al*. Ofloxacin targeting to lungs by way of microspheres. *Int J Pharm* 2009; 380: 127–132.