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In vitro and in vivo evaluation of mPEG-PLA modified liposomes loaded glycyrrhetinic acid

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Abstract

In order to prolong the in vivo residence of glycyrrhetinic acid, a liposomes with surface modified by methoxy poly(ethyleneglycol) (mPEG)-PLA was prepared for the first time. The liposomes (C-LP) and long-circulating liposomes (LC-LP) were prepared by film-dispersion method using soybean phospholipid/cholesterol mixture (1:0.5 mol/mol), containing 5% (w/w) sodium deoxycholate, and 2% (w/w) mPEG-PLA (only for LC-LP). The diameter of LC-LP was 243.3 ± 17.1 nm. The pharmacokinetics behaviors of the conventional injection and liposomes (C-LP and LC-LP) were compared after a single intravenous injection to rats. Pharmacokinetic parameters were calculated based on a two-compartment open model analysis. LC-CP had a 1.7-fold longer residence time (MRT), a 2.75-fold larger AUC and a 0.4-fold lower clearance compared with conventional injection, respectively. These results combined suggested that the LC-CP had a well-improved residence in rats.

Keywords: Glycyrrhetinic acid; mPEG-PLA; Long-circulating; Physicochemical characteristics; Pharmacokinetics

1. Introduction

Glycyrrhetinic acid is an active principal aglycone of glycyrrhizin which has been shown to be hydrolyzed by glucuronidase in intestinal bacteria after oral administration (Takeda et al., 1996). Glycyrrhetinic acid is a lipophilic drug with a very low solubility in water (<0.01 mg/ml), which may result in its poor bioavailability. After absorption in gut, glycyrrhetinic acid is eliminated fast in plasma and enriched in hepar. In the liver, it is metabolized into glycyrrhetinic acid monoglucuronide, which is predominantly excreted in the faeces. Glycyrrhetinic acid has been shown to possess several beneficial pharmacological activities, such as antiulcerative effect (Doll et al., 1962), anti-inflammatory activity (Ohuchi et al., 1981), direct and indirect antiviralactivity (Ito et al., 1988), interferon inducibility (Abe et al., 1982), and an antihepatitis effect (Kiso et al., 1984). Glycyrrhetinic acid may cause sodium retention and potassium loss, which are associated with hypertension (Edwards et al., 1988) while the adverse effects of glycyrrhetinic acid seem to be dose-dependent.

To avoid the side effects and maintain effective concentration of glycyrrhetinic acid, a liposomal injection system is under development in our laboratory, which is a suitable strategy to enhance the bioavailability of administered drugs. Liposomes have contributed significantly to drug delivery for a diverse array of therapeutic agents due to their relatively high stability in the blood and intrinsic ability to extravasate into tissues with increased vascular permeability, such as solid tumors and sites of inflammation (Harrington et al., 2001, 2002). Glycyrrhetinic acid liposomes can avoid organic solvent addition, but conventional liposomes will promote capture and retention in the liver that impair a lot of pharmacological activities of glycyrrhetinic acid. In clinic, more drugs would be given in order to maintain effective in vivo drug concentration, but the side effects would be enhanced too. PEG was used to modify the liposome to improve the stability of the complexes for systemic in vivo delivery. Nanoparticles (NP) with methoxy poly(ethyleneglycol) (mPEG) at their surface have been shown to reduced interaction with cells of the mononuclearphagocytic system (MPS) and to remain in the systemic circulation for a prolonged period (Gref et al., 1994;

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Dong and Feng, 2004, 2007). Biodegradable A and B block copolymers such as methoxy poly(ethylene glycol)-poly(D,L-lactic acid) can be used to prepare micelle or particles in which the mPEG chains are covalently bound to the core of the NP (Zhang et al., 2006; Trimaille et al., 2006). Long poly(D,L-lactic acid) chains are necessary.

In this study, a new kind of surface active material, the block copolymers with longer hydrophilic chain of mPEG2000-PLA was synthesized. The information about the long-circulating liposomes was obtained by differential scanning calorimetry. The long-circulating liposomes modified by the mPEG-PLA copolymers were prepared using a novel film-dispersion method (Yang et al., 2007). Also, glycyrrhetinic acid conventional injections and liposomes (C-LP) were prepared as the reference preparations. The pharmacokinetic of glycyrrhetinic acid conventional injections, conventional liposomes and long-circulating liposomes (modified by mPEG-PLA) post-intravenous administration on rats were characterized and compared.

2. Materials and methods

2.1. Materials

mPEG with molecular weight of 2000 was obtained from Aldrich. Prior to use, and was dried by an azeotropic distillation in toluene. The D,L-lactide was prepared from D,L-lactic acid in the laboratory. Soybean phospholipid (SP) was a gift sample from Degussa (China) Co., Ltd., Cholesterol (CH) and sodium deoxycholate (SD) were purchased from Sigma Chemical Co., China. Methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA 80/20 by weight ratio) was synthesized by ring-opening polymerization of D,L-lactide in the presence of mPEG (M_W of 2000 Da) as described in a pervious report (Nguyen et al., 2003). Glycyrrhetinic acid was purchased from Xi'an Fujie Pharmaceutical Co., Ltd., China. Acetonitrile, methanol, water and acetic acid were of HPLC grade (Qualigens, China) and all other reagents were of analytical grade.

2.2. Synthesis and characterization of mPEG-PLA

In a flame-dried and argon-purged flask, 8 g mPEG (M_w of 2000 Da), 2 g lactide, 20 ml toluene and $Sn(Oct)_2$ were added under Ar stream, and the sealed flask was maintained at 110 °C for 24 h, The synthesized polymer was recovered by dissolving in methylene chloride followed by precipitation in ice-cooled diethyl ether. The resultant precipitate was filtered and dried at room temperature in vacuum (Fig. 1).

The structure and the properties of copolymers were characterized by ^{1}H NMR, IR, DSC, respectively.

2.3. Preparation of liposomes

Preparation of methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA) containing liposomes was based upon film-dispersion method. The required amount of glycyrrhetinic acid, soybean phospholipid, cholesterol and sodium deoxycholate was dissolved by mixed solvent of dichlormethane:acetone (1:1) in a clean, dry round bottom flask. Organic solvent was later removed by rotary vacuum evaporation under lipid transition temperature (Rotary Evaporator-RE52CS, Yarong, China); traces of solvent were removed from the deposited lipid films under vaccum overnight. Lipid films were then hydrated with small amount of 5% lactose solution (using 0.5 M phosphate buffer adjust to pH 6.5) with mPEG-PLA by rotation (100 r/min, 10 min) at the corresponding temperature. The surplus lactose solutions were then added with rotation (100 r/min, 20 min). The modified proliposome powder was obtained by lyophilization of hydrated suspension.

The conventional liposomes were prepared similarly. The required amount of glycyrrhetinic acid and adjuvants was dissolved, lipid films were then hydrated with full dosage of 5% lactose solution (using 0.5 M phosphate buffer adjusted to pH 6.5). The conventional proliposome powder was also obtained by lyophilization of hydrated suspension.

2.4. Characterization of liposomes

2.4.1. Appearance

The liposome samples were translucent ivory white solutions with visible sky-blue opalescence. Spherical particles could be detected under electronic microscope.

2.4.2. Particle size and distribution

An aliquot of lyophilized glycyrrhetinic acid proliposome (C-LP) and long-circulating proliposome powders (LC-LP) was resuspended in deionized water. Measurements were performed at a fixed angle of 90° to the incident light and data were collected over a period of 3 min. The mean particle size and particle size distribution were analyzed by photon correlation spectroscopy (PCS) employing a Zetasizer (Malvern Instruments, Master sizer 2000, UK) (Takeda et al., 1996).

2.4.3. Transmission electron microscopy

Liposome was formed automatically by dropping distilled water to glycyrrhetinic acid C-LP or LC-LP powders and shaking the mixture manually for 5 min. A drop of the resultant liposome suspension was placed onto a carbon-coated copper grid, forming a thin liquid film. The films on the grid were negatively stained by adding immediately a drop of 2% (w/w) ammonium molybdate in 2% (w/v) ammonium acetate buffer (pH 6.8), removing the excess staining solution with a filter paper, and followed by a thorough air-drying. The stained films were then viewed on a transmission electron microscope (Hitachi H27000, Japan) and photographed.

2.4.4. Determination of glycyrrhetinic acid in liopsomes (loading efficiency)

The prepared liposome sample was filtered by micropore film (0.22 μm). A fixed amount of filtered liposomes was diluted into suitable concentration with methanol. Then 20 μl was injected into HPLC system. For the quantitative determination of glycyrrhetinic acid, a reverse-phase HPLC

method was used (SHIMADZU LC-10AD pump liquid chromatograph; Diamonsil® C_{-18} column, 250 mm × 4.6 mm, 5 μm, Dikma Technology Company, China). The mobile phase was methanol:water:acetic acid (85:14.6:0.4, v/v) (Palakurthia et al., 2005). The analysis was performed at the flow rate of 1 ml min^{-1} with the UV detector at 250 nm and the sensitivity was 0.01 AUFS.

2.4.5. In vitro drug release study

Release of glycyrrhetinic acid from LC-LP or C-LP was evaluated through dialysis, and was compared with that durg solution. Each reconstituted liposomal glycyrrhetinic acid sample (2 ml) was placed in a dialysispocket (MWCO 8000–10,000, Sigma). Then, the pocket was immersed in 200 ml of release medium, PBS (adjusting 0.01 mol/l NaOH to pH 10.5, 37 °C) to maintain sinking condition. While stirring the release medium using the magnetic stirrer at 200 rpm, samples (0.5 ml) were taken at predetermined time intervals from the release medium over a 48 h period and refilled with the same amount of the fresh medium. Concentration of glycyrrhetinic acid was determined by HPLC.

2.5. Determination of glycyrrhetinic acid in plasma

2.5.1. Determination of plasma samples

An aliquot of 100 μl plasma sample was placed into a centrifuge tube and then 100 μl acetonitrile was added. After vortexed for 1 min, the mixture was centrifuged at 10,000 r/min for 10 min. The 50 μl supernatant was placed into another centrifuge tube and was centrifuged at 10,000 r/min for 10 min again. 20 μl was injected into HPLC system. The peak area of glycyrrhetinic acid was recorded and the content of glycyrrhetinic acid was determined as the peak area. The HPLC condition was: the mobile phase composed of methanol:water:acetic acid (86:13.6:0.4, v/v), flow rate of 1.0 ml min^{-1}, detecting wave length of 250 nm and sensitivity of 0.01 AUFS (Wu et al., 2005).

2.5.2. Preparation of the standard curve

One hundred microliter blank plasma each was placed into centrifuge tubes. Then glycyrrhetinic acid was added so that the final glycyrrhetinic acid concentrations were 200, 400, 600, 800, 1000, 2000, 3000 and 4000 ng/ml. These concentrations were then treated as in Section 2.5.1 and the peak area of glycyrrhetinic acid was recorded. The linear regression of glycyrrhetinic acid peak area (A) versus concentration (C) served as the standard curve of glycyrrhetinic acid concentration in plasma.

2.5.3. The determination of relative recovery and precision

One hundred microliter blank plasma each was placed into centrifuge tubes. Then glycyrrhetinic acid of different concentrations were added to make the final glycyrrhetinic acid concentration 200, 2000 and 4000 ng/ml. Then procedures were taken according to the method described in Section 2.5.1. Also, with measured concentration as index of precision, the intra-day and inter-day (3 consecutive days) precision was calculated.

2.6. The pharmacokinetic study of liposomes in rats

2.6.1. Experiment design

Eighteen SD rats (nine male and nine female, provided by the Department of Animals, China Pharmaceutical University), weighing 254 ± 18 g, were randomly assigned into three groups of six rats. All the animals were kept fasting for 12 h prior to the experiments. Glycyrrhetinic acid conventional injection, conventional liposomes and long-circulating liposomes were injected via tail vein at a single dose of 1 mg kg^{-1}, respectively. Blood (0.25 ml) was collected via orbit at 0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 10 h after injection. Blood samples were placed into heparinized tubes. After centrifugation, the obtained plasma was stored at −20 °C until determination.

2.6.2. Data analysis

The pharmacokinetic parameters associated to each animal were estimated by compartmental and noncompartmental methods using DAS Version 2.0 computing program (Drug and Statistic for Windows, produced by the Clinical Research Center of the Shanghai University of TCM, China). The program showed a two compartment open model fitted to the drug concentration–time profiles with the lowest Akaike's number.

2.6.3. Statistical evaluation

Glycyrrhetinic acid pharmacokinetic parameters were compared using analysis of variance (one-way ANOVA) at the 0.05 significance level (Originpro 7.0 software package). The comparison between compartmental and noncompartmental parameters was carried out by paired t-tests.

3. Results

3.1. Characterization of mPEG-PLA

In the Fig. 2, ^{1}H NMR spectra were measured on a Bruker Unity-400 NMR spectrometer at room temperature, with $CDCl_3$ as solvent and TMS as internal reference. Yield: 6.5 g (65%). ^{1}H NMR($CDCl_3$): δ a-5:17 (dd. CH in PLA). b-3.65 (s. methylene-

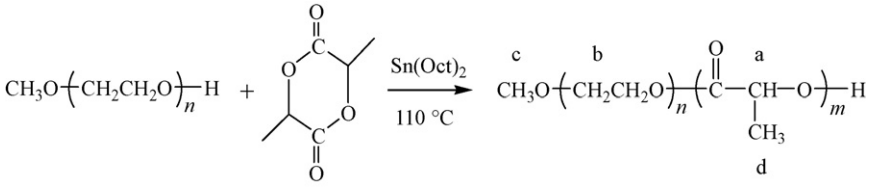

Fig. 1. Synthesis of mPEG-PLA.

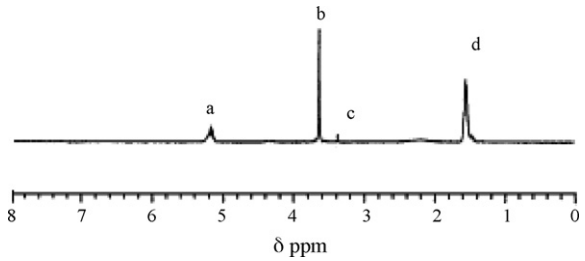

Fig. 2. ^{1}H NMR spectrum of mPEG-PLA.

group of PEG). c-3.38 (s. end group (CH_3O) of PEG).d-1.73 (d. methyl group of PLA). The segment length of the PLA in the blockcopolymer was estimated from the ^{1}H NMR spectrum (Fig. 2) based on the peak intensity ratio of the methylene protons of mPEG (OCH_2CH_2: $\delta = 3.65$ ppm) and the methine proton of the LA unit ($COCH(CH_3)$: $\delta = 5.17$ ppm), assuming the Mn of mPEG to be 2000. The Mn of the PLA segment thus determined was 450, which agreed well with the calculated value based on the initial molar ratio of initiator to lactide.

From IR spectra of mPEG-PLA, characteristic absorption band of mPEG, υC-H(2900 cm^{-1}) υC-O(1200 cm^{-1}) and the characteristic absorption band of LA, υC = H(1700 cm^{-1}) were obvious (Fig. 3).

The DSC were measured on a NETZSCH DSC 204 range from 20–300 °C (10 °C/min) using a crucible of Pan AL, open. The T_m of mPEG-PLA showed no peak of lactide and the T_m of mPEG was degraded, which means mPEG-PLA was polymerized successfully (Fig. 4).

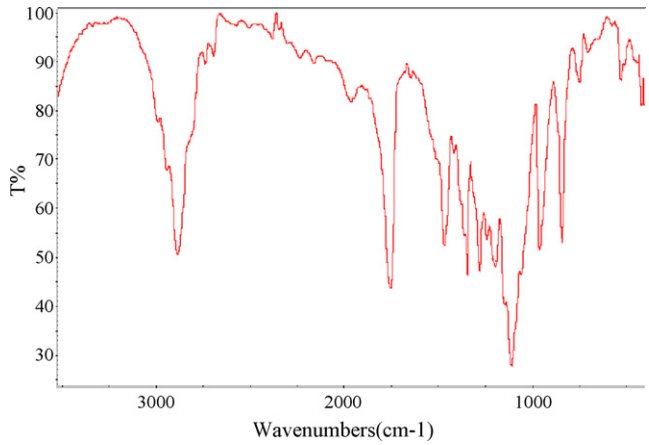

Fig. 3. IR spectrum of mPEG-PLA.

3.2. The particle size, distribution, the content and in vitro release of glycyrrhetinic acid in proliposomes

3.2.1. Mean particle size and size distribution

The C-LP and LC-LP prepared through film dispersion have suitable particle size and ideal entrapmency efficient, as liposomes prepared by supporter deposit-liposome method or alcohol injection method are not satisfactory as shown in Table 1.

Therefore, 2.3% (w/w) mPEG-PLA (80/20, w/w) was found to be an optimum concentration for the formulation of the LC-LP by film-dispersion method. The results showed that the mean particle size was about 240 nm and the entrapment efficiency was over 90%. In addition, they could be easily redispersed in

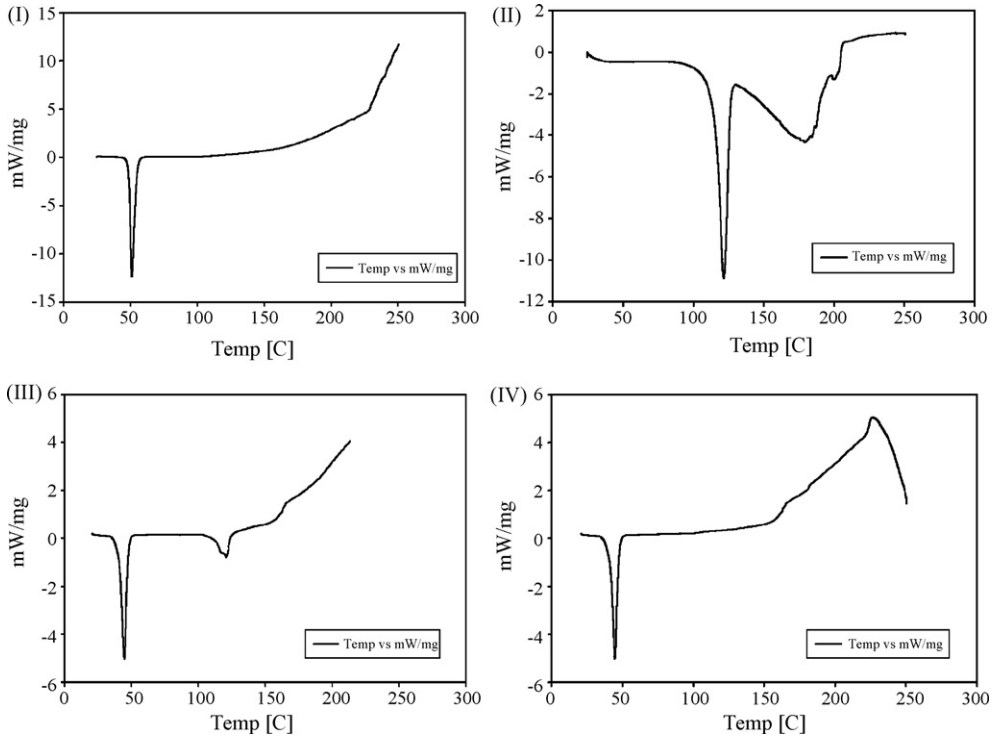

Fig. 4. DSC of mPEG2000 (I), Lactide (II), blend of Lactide and mPEG (III), mPEG-PLA (IV).

Table 1
Particle size, particle distribution index and entrapment efficient of C-LP and LC-LP following different preparation methods ($n = 3$, mean ± S.D.)

	Type[a]	Particle size (nm)	Particle distribution index	Entrapment efficient (%)
C-LP	II	221.2 ± 23.8	0.311 ± 0.114	92.9 ± 1.2
	I	315.6 ± 43.7	0.469 ± 0.214	84.9 ± 4.9
LC-LP	II	243.3 ± 17.1	0.355 ± 0.136	91.4 ± 0.8
	III	217.6 ± 25.9	0.298 ± 0.227	80.3 ± 3.3

[a] Type I by pre-liposome method; Type II by film-dispersion method; Type III by alcohol injection method.

aqueous media. On this basis, 5% lactose (w/v) was selected as the optimal concentration for the lyophilization process.

3.2.2. Transmission electron microscopy

Both the C-LP and LC-LP are integrated ellipse-spherite as shown in Fig. 5 by transmission electron microscopy. Both the C-LP and LC-LP were comparatively integrated ellipsoid, and LC-LP was more uniform. From transmission electron photographs we found an interesting phenomenon that phosphotungstic acid was more obviously absorbed on superficies of LC-LP than C-LP. This could be explained for mPEG-PLA on superficies of LC-LP, showing mPEG-PLA had a idio-adsorption with phosphotungstic acid.

3.2.3. In vitro drug release study

The results of the in vitro release of drug solution and liposomes from our previous study have been used for comparison. As shown in Fig. 6, there is a certain degree of difference between the CL-LP and C-LP although their release rates significantly decreased compared to drug solution. Within 48 h at 37 °C, the LC-LP released about 60% of the drug while the C-LP released 67%. Considering that the release of glycyrrhetinic acid was rapid and almost completed within 12 h from the drug solution based formulation of conventional injection, the incorporation of mPEG-PLA into the liposome probably did not destroy the structure of liposome and may have certain structural stability to liposomes. Most of the glycyrrhetinic acid must have remained in liposomes in the formulations under the condition studied.

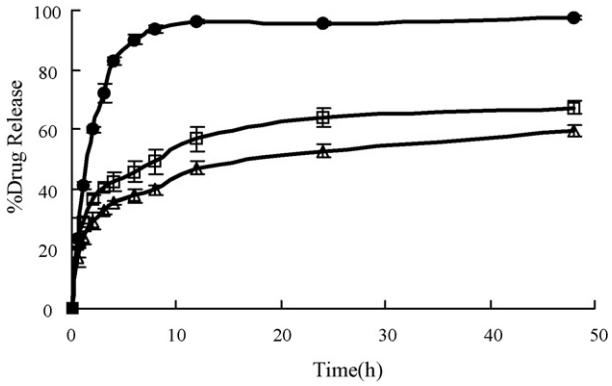

Fig. 6. In vitro release of glycyrrhetinic acid solution (●), conventional liposomes (□) and long-circulating liposomes (□) ($n = 6$).

We also noticed that drug released rapidly within 2 h, the prompt release of the drug adsorbed on the surface of the liposome might be the reason for this phenomenon.

3.3. HPLC method

The impurities in plasma did not interfere with the determination of the drug in the samples. The t_R of glycyrrhetinic acid was about 13.35 min (Fig. 7). The detection limit was 20 ng/ml. There was a good linearity between A and C ($A = 15.832C - 258.7$, $r = 0.9998$). The recovery ranged from 83.45% to 95.97%. The R.S.D. of intra-day and inter-day was 1.23–2.81% and 2.61–3.63%, respectively.

3.4. The pharmacokinetic study

The concentration–time curve after a single dose intravenous infection of glycyrrhetinic acid in C-LP, LC-LP and conventional injection in rats is shown in Fig. 8. All data were carefully conducted and the results showed that the pharmacokinetic behaviors of glycyrrhetinic acid in C-LP, LC-LP and conventional injection fitted two-compartment models. The main pharmacokinetic parameters by compartmental model are listed in Table 2. The data fitness was evidenced by the high correlation ($R^2 > 0.999$) between computer conducted and experimental gly-

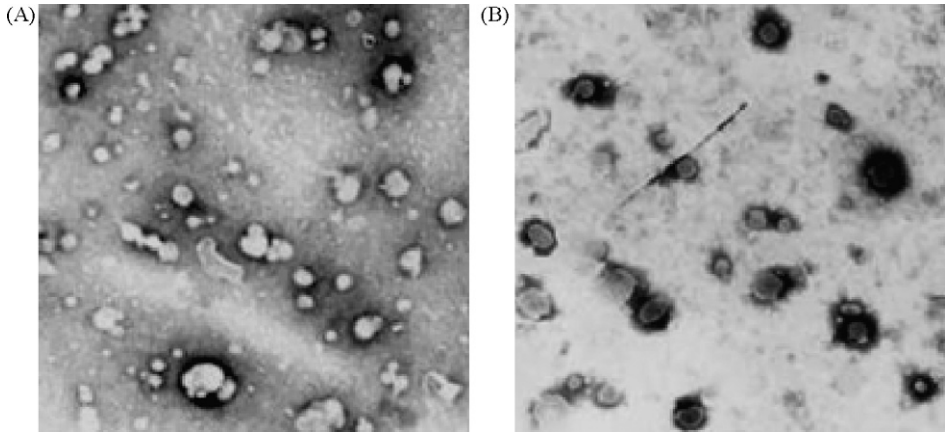

Fig. 5. Transmission scanning electron micrographs of C-LP (A) (×12,000) and LC-LP (B) (×15,000).

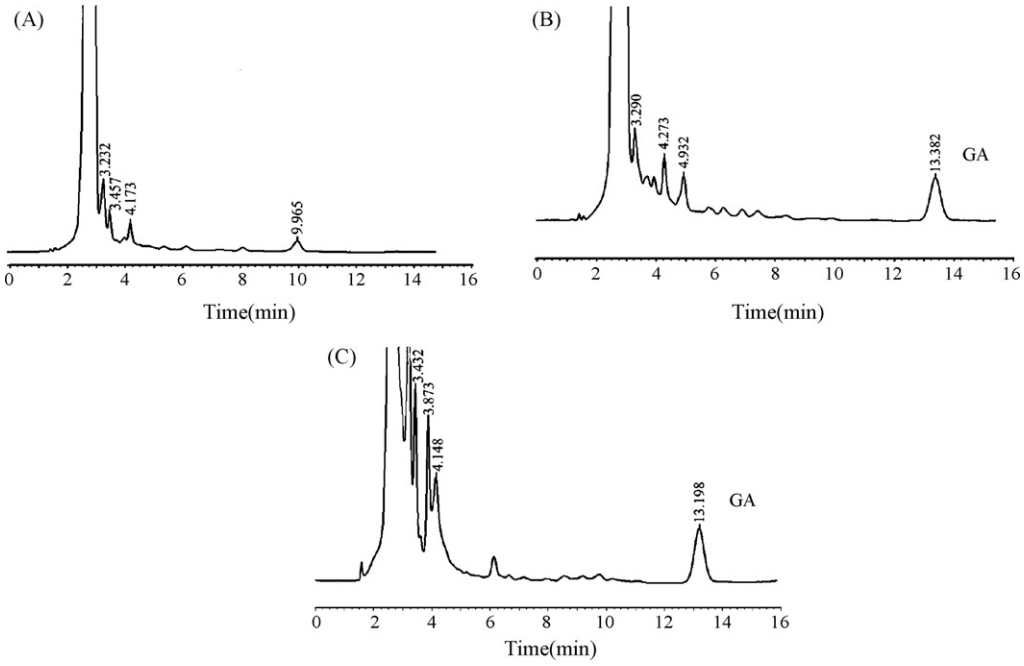

Fig. 7. Chromatograms of glycyrrhetinic acid in rat plasma. (A) blank rat plasma; (B) plasma spiked with glycyrrhetinic acid; (C) plasma sample after iv administration (1mg•kg^{-1})of glycyrrhetinic acid long-circulating liposomes in rats.

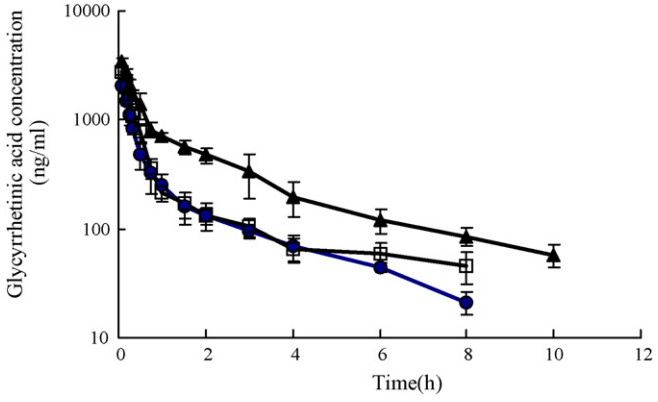

Fig. 8. Drug concentration in plasma ($n = 6$, mean ± S.D.) following a single i.v. administration (1 mg kg^{-1}) of glycyrrhetinic acid conventional injections (●), conventional liposomes (□) and long-circulating liposomes (▲) in rats.

cyrrhetinic acid blood concentrations (Molpeceres et al., 1998). The concentration–time curves of conventional injections and C-LP were comparative coincidence while the LC-LP curve was much different. The inter-compartmental drug distribution rates (k_{10}, k_{12}, k_{21}) showed statistically significant differences as shown in Table 2. In a global sense, k_{10} of conventional injection and C-LP was statistically the same, but different for conventional injections and LC-LP. k_{12} and k_{21} of three preparations showed statistically significant differences. The half-life of Alpha ($T_{1/2\alpha}$) and half-life of Beta ($T_{1/2\beta}$) showed no statistically significant differences ($P > 0.05$) except $T_{1/2\alpha}$ of conventional injections compared with C-LP. The apparent volume of distribution (V) showed significant differences, for LC-LP to other two preparations. The drug $AUC_{0\rightarrow 8(10)}$ as well as systemic clearance (CL) showed interclass statistical significant differences.

Table 2
Compartmental pharmacokinetics parameters of glycyrrhetinic acid conventional injections, C-LP and LC-LP following a single bolus i.v. injection of 1 mg kg^{-1} ($n = 6$, mean ± S.D.)

Parameter	Conventional injections	C-LP	LC-LP
k_{10} (1/h)	4.216 ± 0.840	3.647 ± 0.430	3.160 ± 0.595***
k_{12} (1/h)	0.383 ± 0.182*	0.176 ± 0.045**	0.608 ± 0.147***
k_{21}(1/h)	0.366 ± 0.144*	0.215 ± 0.074**	0.407 ± 0.080
V (l/kg)	2.427 ± 0.872	2.890 ± 1.069**	0.867 ± 0.110***
$T_{1/2\alpha}$ (h)	0.153 ± 0.023*	0.183 ± 0.019	0.186 ± 0.035
$T_{1/2\beta}$ (h)	2.365 ± 0.866	3.939 ± 2.021	2.140 ± 0.408
CL (l/h/kg)	0.715 ± 0.082*	0.531 ± 0.064**	0.286 ± 0.044***
$AUC_{0\rightarrow 10}$ (ng h/ml)	1278.1 ± 154.4*	1658.6 ± 95.1**	3449.2 ± 487.2***

* $P < 0.05$ vs. C-LP.
** $P < 0.05$ vs. LC-LP.
*** $P < 0.05$ vs. conventional injections.

Table 3
Noncompartmental pharmacokinetics parameters of glycyrrhetinic acid conventional injections, C-LP and LC-LP following a single bolus i.v. injection of 1 mg kg^{-1} ($n = 6$, mean ± S.D.)

Parameter	Conventional injections	C-LP	LC-LP
C_{max} (ng/ml)	2074.0 ± 100.4*	2788.1 ± 220.2**	3441.2 ± 252.2***
$AUC_{0\rightarrow 10}$ (ng h/ml)	1302.8 ± 151.3*	1697.1 ± 87.1**	3588.6 ± 483.6***
$AUMC_{0\rightarrow 10}$	1580.8 ± 243.1*	2474.6 ± 431.4**	7426.7 ± 1543.4***
$MRT_{0\rightarrow 10}$ (h)	1.209 ± 0.050*	1.453 ± 0.194**	2.052 ± 0.165***

* $P < 0.05$ vs. C-LP.
** $P < 0.05$ vs. LC-LP.
*** $P < 0.05$ vs. conventional injections.

The mean pharmacokinetic parameters derived from a noncompartmental analysis are presented in Table 3 (Sasaki et al., 2003). The $AUC_{0\rightarrow 8(10)}$ of LC-LP was nearly 2.75-fold to conventional injection while $MRT_{0\rightarrow 8(10)}$ was 1.7-fold. These results indicated that LC-LP circulated longer in the blood circulating system than conventional injection. The contrast between C-LP and conventional injections was smaller, as 1.3-fold and 1.2-fold.

The coincidence between the two pharmacokinetic analysis was assessed by comparing the AUC estimates from the compartmental model with those derived from the noncompartmental analysis. The lines of identity and regression showed a slope −1.03 and $r^2 = 0.997$. Paired t-tests did not show any statistically significant difference between the model derived pharmacokinetic parameters and those calculated by noncompartmental analysis ($P > 0.05$).

4. Discussion

In our study, a new kind of adjuvant, the block copolymers with longer hydrophilic chain of mPEG2000-PLA (80/20) was synthesized successfully. We designed to find if the glycyrrhetinic acid contained in mPEG-PLA modified liposomes was more stable in vivo. The particle size distribution was controlled by the film method and unencapsulated drugs could be removed simultaneously for the low dissolubility of glycyrrhetinic acid. Sodium deoxycholate was used to decrease the particle size and increase the encapsulation efficiency. The kinetics of glycyrrhetinic acid in plasma, as shown in the above tables, AUC and MRT of LC-LP are much bigger than C-LP and conventional injection groups. It is obviously that glycyrrhetinic acid in plasma decreased rapidly in conventional injection group, the concentration is only moiety compare to LC-LP group 5 min after i.v. injection. One reason of this phenomenon could be the low solubility of glycyrrhetinic acid. Glycyrrhetinic acid educe rapidly into small particles in plasma and concentrated in liver or micrangium. The C-LP did not reveal significant ascendancy than conventional injections. This can well be explained by the theory that conventional liposomes (0.1–1.0 μm) are taken up preferentially by cells of the reticuloendothelial system (RES), located principally in the liver and spleen. The LC-LP was degraded fast in the first 1 h like C-LP and conventional injections group. The big particle size of liposomes may elucidate this phenomenon. One hour after injection, the LC-LP group showed an obviously different elimination rate. We thought mPEG-PLA and lipid phase of liposomes in vivo were composed of farrago-micelles. These farrago-micelles could reduce interaction with cells of the mononuclearphagocytic system (MPS) and remain in the systemic circulation. All in all, glycyrrhetinic acid circulated for a longer time in the blood following i.v. administration of LC-LP, which could make more glycyrrhetinic acid accumulate in the target tissue and increase the pharmacodynamic actions of glycyrrhetinic acid. Next, we hope to improve the technology to get liposomes smaller than 100 nm with higher in vivo quality. Also we plan to investigate the distribution of glycyrrhetinic acid in different organs after giving LC-LP and set up pharmacodynamic-pharmacokinetic models.

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