Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 1147–1153

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Enhanced distribution and extended elimination of glycyrrhetinic acid in mice liver by mPEG-PLA modified (mPEGylated) liposome

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ARTICLE INFO

Article history:
Received 24 July 2009
Received in revised form 31 October 2009
Accepted 8 November 2009
Available online 13 November 2009

Keywords:
Glycyrrhetinic acid
Chronic hepatitis
PEGylation
Liposome
Pharmacokinetics
Distribution

ABSTRACT

A rapid and simple method of high-performance liquid chromatography with UV detector for the quantification of glycyrrhetinic acid (GA) in mice plasma and tissues has been developed and validated. With the established assay method, the pharmacokinetic profiles and tissue distribution of GA in different formulations are compared in mice after intravenous administration of the drug (25 mg/kg). The results showed that mPEG-PLA modified (mPEGylated) GA liposome (PL-GA) significantly prolonged the mean residence time (*MRT*) of GA in mice plasma and liver (*MRT*: 0.43 ± 0.13 and 1.72 ± 0.11 h, respectively) than the normal GA liposome (L-GA) (*MRT*: 0.23 ± 0.01 and 1.07 ± 0.31 h, respectively) and GA sodium injection (S-GA) (*MRT*: 0.13 ± 0.01 and 0.95 ± 0.08 h, respectively). Moreover, PL-GA specifically increased GA uptake in liver ($AUC_{0-\infty,\ liver}$ value of 1.6-fold and 1.3-fold higher than that for S-GA and L-GA, respectively) and reduced its distribution into other tissues after dosing. Due to these pharmacokinetic properties, it may be promising to develop PL-GA further as a new pharmaceutical preparation for GA on the treatment of various chronic hepatic diseases.

1. Introduction

The dried root of licorice (*Glycyrrhiza glabra*), also called "*Gancao*" in China, is a traditional Chinese Medicine which has been used for treatment of various chronic diseases in China and worldwide [1,2]. Previous research has identified triterpene saponins to be the main chemical constituents from licorice extract. Among them, Glycyrrhizin (GL) and its aglycone Glycyrrhetinic acid (GA, Fig. 1) have been identified as potent anti-hepatotoxic agents [3,4] and widely used for the treatment of various hepatic diseases in clinical practice [5–7]. One of the commercialized glycyrrhizin formulations, named stronger neominophagen C (SNMC), has been used for treating chronic liver diseases [8], subcute hepatic failure, chronic hepatitis and cirrhosis [9] in Japan and Europe for many years. In several pharmacokinetic studies, oral administration (p.o.) of GL has been reported to be completely metabolized to GA by the intestinal bacteria prior to absorption [10–12]. GL is barely detectable in human plasma at the therapeutic dosage (1.4–1.8 mg/kg) while GA is highly expressed [13]. Moreover, GA has also been demonstrated to exhibit higher anti-viral and antibacterial activities than GL [14] and processes several pharmacological benefits including as anti-hepatotoxicity [15,16], anti-neoplastic activity [17,18] and some anti-viral activities [14,19–21] both *in vitro* and *in vivo*. These findings suggest that GA is more potent than GL, which may become a more suitable candidate for being a new drug for clinical application.

However, some *in vivo* investigations have indicated that GA declines biexponentially from plasma and distributes minimally into body tissues after a bolus intravenous (i.v.) administration to rats [22,23]. The constant plasma-to-blood concentration ratio of 1.79 (2–12 mg/kg, i.v.) suggests a poor uptake of GA into the erythrocytes. The maximum tissue-to-blood concentration ratio is found in kidney (approximately 0.121) which suggests its higher distribution in kidney. When radioactive GA is orally administered to rats (60 mg/kg), an average of 86% of the radioactivity is recovered in 1–3 days with 83% in the feces, 1% in the urine and 4% remained in liver [24]. When the radioactive drug is given subcutaneously (60 mg/kg), 74% of the radioactivity is recovered with 73% in feces and 1% in the urine. Of the radioactivity recovered in the feces, about 7.4% is unchanged GA. The results have indicated that most of the administered GA is metabolized *in vivo* and its metabolites are rapidly excreted into the bile. In addition, pseudo-aldosteronism, such as sodium retention, hypokalaemia and hypertension, is a well-known side-effect caused by GA [25]. The high concentration of GA in kidney can inhibit the renal 11-β-hydroxy-steroid-dehydrogenase and increases cortisol level, which can ultimately lead to the hypermineralocorticoid effect. There-

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0731-7085/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jpba.2009.11.005

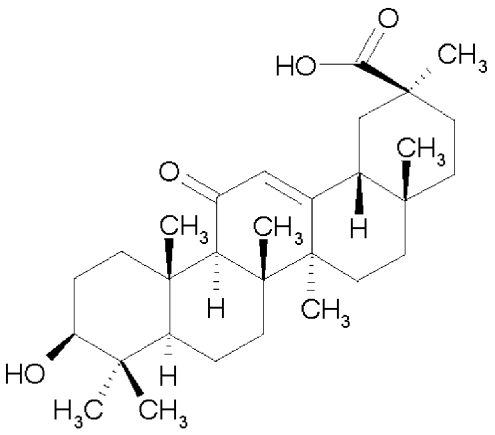

Fig. 1. Chemical structure of glycyrrhetinic acid ($C_{30}H_{46}O_4$).

fore, the development of a new hepatocyte-specific drug/formula instead of using the conventional ones is very important in increasing its therapeutic effects in the liver.

Previously, a methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA) modified GA liposome (PL-GA) has been developed in our research group and demonstrated to present a long-circulating property in rats after a bolus i.v. administration [26]. Comparing to the conventional GA injection and GA lipsome (L-GA), PL-GA prolongs the mean residence time (*MRT*) of GA in rat blood (1.7-fold and 1.4-fold, respectively), exhibits a larger area under blood concentration–time curve (*AUC*) (2.7-fold and 2.1-fold, respectively) and reduces blood clearance (CL_b) (0.4-fold and 0.5-fold, respectively). The observed long-circulating effect caused by PL-GA has significantly extended the retention of GA in rat blood and slowed its elimination from rat vascular system.

In this study, the efficiency of delivering GA to liver by various formulations was evaluated. A high-performance liquid chromatographic method was developed and validated for the quantitative determination of GA in mice plasma and various tissues. With this established bio-analytical method, the tissue distribution of GA in mice following intravenous administration of GA sodium (S-GA), L-GA and PL-GA injections (25 mg/kg, in respect of GA) was investigated.

2. Experimental

2.1. Chemical and regents

Glycyrrhetinic acid (purity >99%) was purchased from Xi'an Fujie Pharmaceutical Co. Ltd. (Xi'an, China). Soybean phospholipid (SP) was purchased from Shanghai Taiwei Co. Ltd., (Shanghai, China). Cholesterol (CH) and sodium deoxycholate (SD) were purchased from Sigma Chemical Co. (Shanghai, China) as analytical grade. Fenofibrate (purity >99.5%) was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, 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 (MW of 2000 Da) as described previously [27]. Acetonitrile (ACN) was purchased from Merck Company (Darmstadt, Germany) as HPLC grade. Methanol and acetic acid were of HPLC grade (Qualigens, China). Water was prepared using a Millipore (Bedford, MA, USA) Milli-Q purification system and was filtered through a 0.22 μm filter prior to liquid chromatography.

2.2. Preparation and characterization of S-GA, L-GA and PL-GA

2.2.1. Preparation of S-GA, L-GA and PL-GA

S-GA was synthesized by the reaction of GA (13 g) and sodium hydroxide (1.5 g) in ethanol (500 mL). The mixture was reflux-heated under stirring until the color of the solution became yellow. The reacted solution was decolorized with activated carbon and filtered by vacuum filtration. The filtrate was concentrated and then refrigerated overnight. The recrystallized S-GA was collected and dried for animal experiments.

The L-GA and PL-GA liposomes were prepared by using an ethanol injection method [28]. For PL-GA preparation, GA, SP, CH, mPEG-PLA and SD at a mass ratio of 1:25:4:1.8:1.5 were dissolved in 40 mL absolute ethanol and then regularly injected into 100 mL dispersant solution with rapid stirring at room temperature. After thoroughly stirring, the ethanol was evaporated and the final volume was adjusted to 100 mL with the dispersant solution. To get the homogenous suspension, the crude liposome was extruded in turn through 0.45 and 0.22 μm micropore filter (cellulose nitrate membrane) by Extruder (Northern Lipid INC., CA) for five times. The powdered PL-GA was obtained by freeze-drying after addition of 15% (w/v) lactose.

The powdered L-GA was prepared with the same protocol as PL-GA but without supplement of mPEG-PLA.

2.2.2. Characterization of liposomes

Droplet size distribution of liposomes was determined using photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., Malvern, UK) based on laser light scattering. An aliquot of lyophilized glycyrrhetinic acid liposomes (L-GA and PL-GA) was dispersed in deionized water at concentration of 1 mg/mL (equal to GA). Measurements were performed at a fixed angle of 90° to the incident light and data were collected over a period of 3 min.

To evaluate the loading efficacy of GA into liposomes, the prepared liposomes were dispersed in water and filtered by micropore film (0.22 μm, cellulose nitrate membrane). A fixed amount of filtered liposomes was dissolved into suitable concentration with methanol and measured by HPLC analysis.

2.3. Liquid chromatography

The sample determination was performed by using an HPLC system (SHIMADZU LC-10AD pump liquid chromatograph). Separation of GA from fenofibrate (FB), the internal standard (IS), was achieved by a Diamonsil C-18 analytical column (250 mm × 4.6 mm I.D., 5 μm, Dikma Technology Company, China) maintained at 30 °C. The isocratic elution with a mobile phase of methanol–1% acetic acid (82:18, v/v) was used at a flow rate of 1.0 mL/min for separation of analytes. The analytes were monitored at the UV wavelength of 250 nm.

2.4. Standard and working solutions

Individual standard stock solutions of GA (400 μg/mL) and fenofibrate (IS, 10 μg/mL) were prepared by accurately weighing the required amounts into volumetric flasks and dissolved in methanol. Further dilutions were made from this stock solution with methanol to yield the working concentrations from 0.4 to 10 μg/mL for plasma samples and from 0.2 to 10 μg/mL for tissue samples. All prepared solutions were stored at 4 °C in amber glass tubes and brought to room temperature before use.

The calibration curves for GA were prepared by spiking 100 μL of blank plasma or tissue homogenate (0.25 g/mL, w/v) with 50 μL of the working solutions (dried before the addition of

plasma or homogenate) to achieve concentrations in the range of 0.4–10 μg/mL and 0.2–10 μg/mL, respectively. Quality control (QC) samples at three different concentrations of 0.4, 4 and 10 μg/mL for plasma, and 0.2, 4, 10 μg/mL for tissue homogenates were prepared separately. The QC samples were used to access the accuracy and precision of the assay methods. All the calibration and QC samples were extracted by the method described in the subsequent section and then analyzed. The QC samples were stored along with the test samples at −20 °C until analysis.

2.5. Sample preparation

The mice plasma (100 μL, containing GA 0.4–10 μg/mL) or tissue homogenate (100 μL, containing GA 0.2–10 μg/mL) was added into a dried Eppendorf tube (pre-treated with 50 μL IS solution, 10 μg/mL) and then followed by 400 μL acetonitrile. The mixture was vortex-mixed for 1 min and centrifuged at 12,000 × *g* for 10 min. 20 μL of the supernate was injected into the HPLC for analysis. The calibration curves were conducted by plotting the concentrations of GA in plasma or tissues as a function of peak area ratio of GA to IS.

2.6. Method validation

The specificity of the method was investigated by comparing the chromatograms of blank plasma or tissue homogenate with those samples collected after i.v. administration of PL-GA liposomes; or those obtained from the blank plasma or tissue homogenate spiked with authentic standard of GA.

2.6.1. Precision and accuracy

The intra-day precision was determined within 1 day by analyzing six replicates of QC samples at concentrations of 0.4, 4 and 10 μg/mL (plasma) or 0.2, 4 and 10 μg/mL (tissue homogenate). The inter-day precision was determined on five separate days for the QC samples. The intra-day and inter-day precisions were defined as the relative standard deviation (R.S.D.) and the accuracy was defined by calculating the relative error (R.E.).

2.6.2. Sensitivity

The limit of determination (LOD) was defined by the lowest concentration of the drug resulting in a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) was defined by the lowest concentration of spiked plasma or tissue samples that can be determined with sufficient precision and accuracy, i.e. R.S.D. less than 20% and R.E. of −20 to 20% [29].

2.6.3. Stability

Freeze-thaw stability of each sample was evaluated by exposing QC samples to three freeze-thaw cycles before sample preparation. The stability of the prepared samples in auto-sampler was evaluated by analyzing extracted QC samples after being placed in the auto-sampler at room temperature for 24 h.

2.6.4. Recovery

The extraction recovery was estimated by comparing the peak area of analytes of pre-spiking samples with those of post-spiking samples [30]. Pre-spiking sample means spiking GA and IS to yield the same concentrations as QC samples before sample preparation, while post-spiking sample means spiking neat solutions of GA and IS at the same concentrations as QC samples into the extracted blank plasma or homogenate samples. The matrix effect was estimated by comparing the peak area of analytes of post-spiked samples with neat solutions of GA and IS.

2.7. Tissue distribution

Male and female ICR mice (body weight of 20 ± 2 g) supplied by the Experimental Animal Center of China Pharmaceutical University were fed on a standard laboratory diet with free access to water under the controlled temperature at 20–22 °C and relative humidity of 50% with 12 h light/dark cycle. Before drug administration, mice were fasted but allowed to have free access to water overnight. All protocols and procedures were approved by the Institutional Animal Care and Use Committee in the China Pharmaceutical University.

S-GA solution and L-GA or PL-GA liposome dispersions were prepared in water at the concentration of 1 mg/mL (equal to GA) and filtered through a 0.22 μm filter before injection. For tissue distribution studies, 90 mice were randomly assigned into three groups and intravenously (i.v.) administered with S-GA, L-GA or PL-GA via the tail vein at the dose of 25 mg/kg (equal to GA). Mice were sacrificed at 5, 15, 30, 60 and 120 min after dosing ($n = 6$ for each group). Plasma was collected by centrifuging the heparinized blood at 3000 × *g* for 10 min. Various organs (heart, liver, spleen, lung and kidneys) were isolated on ice and homogenized in 4-fold volume of saline (w/v). All the bio-samples were store at −20 °C until analysis.

2.8. Data analysis

Pharmacokinetic parameters for tissue distribution, including area under concentration–time curve (*AUC*), mean residence time (*MRT*), apparent tissue clearance (*CL*) and elimination half-life ($t_{1/2}$) were calculated by the commercially available software WinNonlin™ Professional Version 5.0.1 (Phar-sight, Mountain View, CA, USA) with a non-compartmental approach. Results were presented as mean ± S.D. Differences between the three formulations in parameters were statistically evaluated by a one-way analysis of variance test using SPSS Version 11.5. A value of $P < 0.05$ was considered significant for all tests.

3. Results and discussion

3.1. Characterization of S-GA, L-GA and PL-GA

S-GA appeared as white crystal and its structure was identified by MS and NMR determination. The purity of S-GA was higher than 99% as determined by HPLC analysis.

L-GA and PL-GA were white and porous powder. Both of them were easily dispersed in aqueous media and exhibited as mildly blue translucent liposome dispersion. The mean particle size and particle size distribution of L-GA and PL-GA were determined as 83.6 ± 5.3 nm and 72.9 ± 4.7 nm, respectively. The drug entrapment efficiency was 97.49 ± 2.45% and 98.10 ± 1.87%; and the drug loading was 2.76 ± 0.91% and 2.43 ± 0.62%, respectively.

3.2. Analytical method

In the current study, an HPLC-UV method was optimized and validated for the quantitative determination of GA in mice plasma and tissues. Fenofibrate was served as the internal standard (IS) based on its similar chromatographic behavior to GA. Comparing to the chromatograms of blank plasma or tissue samples, blank plasma or tissue samples spiked with GA, and plasma or tissue samples obtained from the PL-GA dosed mice (i.v., 25 mg/kg) (Fig. 2), GA could be chromatographically separated from the IS with no endogenous interferences observed at the retention times of the analytes and the IS.

The calibration curves for GA in plasma and various tissues exhibited a good linear response ($r^2 = 0.9995–0.9999$) over the

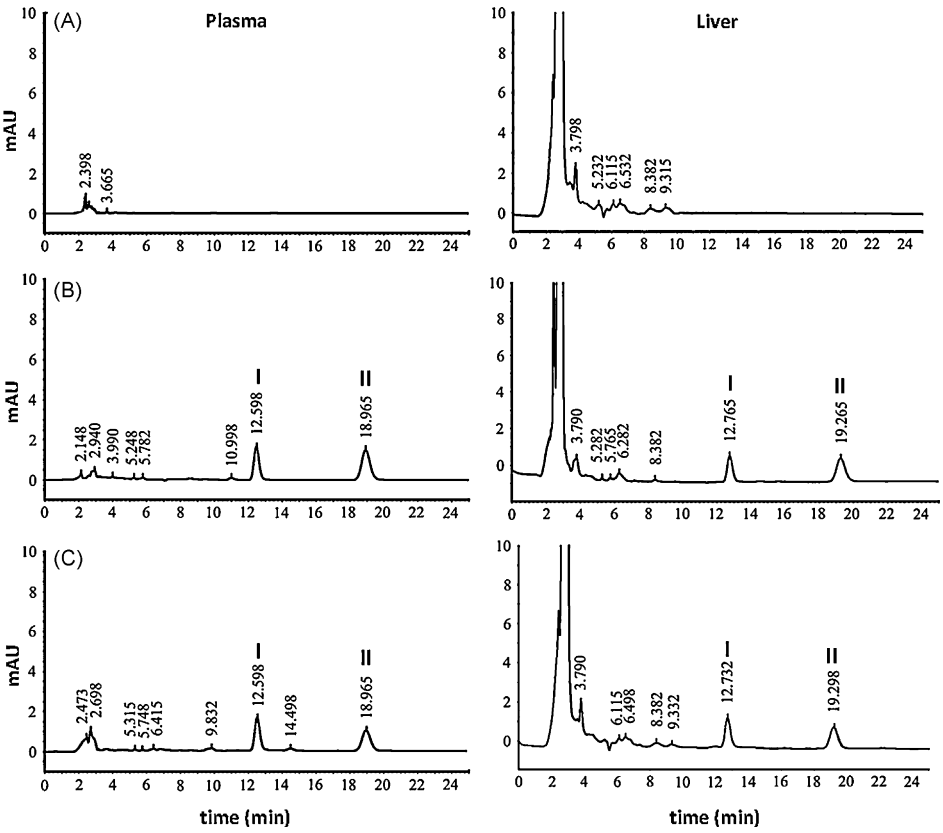

Fig. 2. HPLC chromatograms of (A) blank plasma (left) or liver homogenate (right), (B) plasma (left) or liver homogenate (right) spiked with GA (2 μg/mL) and IS (5 μg/mL); (C) plasma sample (left) or liver homogenate sample (right) at 5 min after intravenous administration of PL-GA injection (25 mg/kg, equal to GA). Peak I: Fenofibrate (IS); Peak II: GA.

ranges 0.4–10 μg/mL in plasma and 0.2–10 μg/mL in tissue. The LOQ were 0.4 μg/mL in plasma and 0.2 μg/mL in tissue samples, and the LOD was 0.05 μg/mL for all bio-samples. The intra-day and inter-day precisions for GA were below 4.99% and 4.94% (%R.S.D.) in the tested concentration ranges, respectively (Table 1). The absolute recoveries for GA in plasma or tissue samples were ranged from 90.4% to 105.0% at three concentrations; while of 95.6% for the IS.

The above experimental results indicated that GA was very stable for at least three freeze-thaw cycles (with 93.4–96.4% remained for concentrations ranged from 0.4 to 10 μg/mL for

Table 1
Intra- and inter-day precision and accuracy (mean ± S.D.) for the quantification of glycyrrhetinic acid in mice plasma and tissues.

Tissues	Intra-day (n = 6)				Inter -day (n = 5)		
	Spiked concentration (μg/mL or μg/g)[a]	Determined concentration (mean ± SD, μg/mL or μg/g)[a]	R.S.D.[b] (%)	R.E.[c] (%)	Determined concentration (mean ± SD, μg/mL or μg/g)[a]	R.S.D.[b] (%)	R.E.[c] (%)
Plasma	0.40	0.36 ± 0.01	3.59	−9.5	0.38 ± 0.02	4.19	−4.5
	4.0	3.92 ± 0.16	3.98	−2.0	3.96 ± 0.16	4.04	−1.0
	10.0	9.60 ± 0.37	3.81	−4.0	10.23 ± 0.37	3.58	2.3
Liver	0.20	0.18 ± 0.01	3.28	−8.5	0.19 ± 0.01	4.15	−3.5
	4.0	3.77 ± 0.12	3.10	−5.8	3.75 ± 0.18	4.80	−6.3
	10.0	9.99 ± 0.28	2.80	−0.1	9.71 ± 0.26	2.68	−2.9
Spleen	0.20	0.20 ± 0.01	3.55	−1.5	0.20 ± 0.01	4.93	1.5
	4.0	4.20 ± 0.10	2.45	5.0	3.85 ± 0.11	2.86	−3.8
	10.0	9.75 ± 0.13	1.36	−2.5	9.63 ± 0.40	4.15	−3.7
Heart	0.20	0.19 ± 0.01	2.12	−5.5	0.19 ± 0.01	3.61	−3.0
	4.0	3.74 ± 0.10	2.65	−6.5	3.94 ± 0.05	1.27	−1.5
	10.0	9.43 ± 0.36	3.85	−5.7	9.64 ± 0.44	4.56	−3.6
Lung	0.20	0.19 ± 0.01	4.30	−7.0	0.21 ± 0.01	4.78	4.5
	4.0	3.75 ± 0.13	3.57	−6.3	3.72 ± 0.13	3.49	−7.0
	10.0	9.22 ± 0.15	1.63	−7.8	9.34 ± 0.38	4.07	−6.6
Kidney	0.20	0.21 ± 0.01	3.90	2.5	0.20 ± 0.01	3.94	1.5
	4.0	3.73 ± 0.17	4.53	−6.8	3.85 ± 0.19	4.94	−3.8
	10.0	9.49 ± 0.47	4.99	−5.1	10.05 ± 0.46	4.58	0.5

[a] The unit of drug concentration in plasma: μg/mL. The unit of drug concentration in tissue: μg/g.
[b] R.S.D. (%) (relative standard deviation) = (S.D./mean concentration) × 100.
[c] R.E. (%) = [(mean concentration determined/concentration spiked) − 1] × 100.

plasma and 0.2 to 10 μg/mL for tissues). In addition, GA was found to be very stable (96.8–100.1% remained) in the prepared samples after being placed in the auto-sampler at room temperature for 24 h.

3.3. Tissue distribution of GA after intravenous administration of S-GA, L-GA and PL-GA injections

The plasma concentrations and tissue contents of GA were determined at certain time schedule between 5 and 120 min after i.v. administration by the developed HPLC-UV method. The pharmacokinetic profiles and parameters of different formulations of GA in mice plasma and liver were illustrated in Fig. 3 and summarized in Table 2.

As shown in Fig. 3A, the pharmacokinetic profile of GA after i.v. bolus injection of S-GA, L-GA or PL-GA (25 mg/kg, equal to GA) showed that its elimination in mice plasma was a first-order kinetic process with biexponential decline manner. Comparing to S-GA, liposomal encapsulization significantly increased the plasma levels of GA which led to relative higher $AUC_{0-\infty,\,plasma}$ values ($AUC_{0-\infty,\,plasma}$: 16.81 ± 0.81 h μg/mL for S-GA, 23.31 ± 1.65 h μg/mL for L-GA and 31.53 ± 1.62 h μg/mL for PL-GA, respectively). Although the normal liposomal formulation (L-GA) did not change the elimination rate of GA from mice plasma ($t_{1/2}$: 0.32 ± 0.05 h for S-GA and 0.36 ± 0.05 h for L-GA), mPEG-PLA modification enhanced the stability of GA liposome in plasma and also slowed the release of GA from the liposomal vesicle, which therefore decreased the elimination of GA from mice plasma ($t_{1/2}$: 0.81 ± 0.44 h). In addition, the mean plasma clearances (*CL*) for PL-GA (0.79 ± 0.04 L/(h kg)) and L-GA (1.08 ± 0.08 L/(h kg)) were found to be less than that of S-GA (1.49 ± 0.07 L/(h kg)). Both PL-GA and L-GA extended the mean resident time (*MRT*) of GA in mice plasma (*MRT*: 0.43 ± 0.13 h and 0.23 ± 0.01 h) when compared with that of S-GA (*MRT*: 0.13 ± 0.01 h) and also prolonged GA retention in mice vascular system.

The tissue distributions of GA after i.v. injection of three different formulations to mice were illustrated in Fig. 4. S-GA was found to be highly concentrated in various organs (heart, lung and kidney) 5 min after dosing. However, L-GA and PL-GA significantly decreased GA uptake by at least two times in those organs. GA is famous for causing side-effects such as sodium retention, hypokalaeia and hypotension by inhibiting the 11-β-hydoxy-steroid-dehydrogenase. These two particular formulations may therefore reduce the side-effects of GA since they would reduce the concentrations of GA in the kidney. Although the liposomal formulations did not change the C_{max} (0.21 ~ 0.23 μg/g), V_d (1.83 ~ 2.19 (mg/kg)/(μg/g)) and *CL* (1.16 ~ 1.86 (mg/kg)/h (μg/g)) of GA in mice liver (Fig. 3B and Table 2), the liver $AUC_{0-\infty,\,liver}$ values of GA for PL-GA (21.73 ± 2.56 h μg/g) and L-GA (16.41 ± 3.23 h μg/g) were increased at least 1.6-fold and 1.2-fold higher than that of S-GA (13.68 ± 2.20 h μg/g), respectively. Whereas L-GA exhibited similar t_{max} (0.19 ± 0.10 h) and *MRT* (1.07 ± 0.31 h) to those of S-GA (t_{max}: 0.19 ± 0.10 h and *MRT*: 0.95 ± 0.08 h), PL-GA significantly delayed its lag-time until reaching a peak concentration (t_{max}: 0.33 ± 0.14 h) and slowed down the elimination ($t_{1/2}$: 1.18 h) of GA thus extended its residence (*MRT*: 1.72 ± 0.11 h) in mice liver. These results suggest that the mPEGylated formulation may contribute

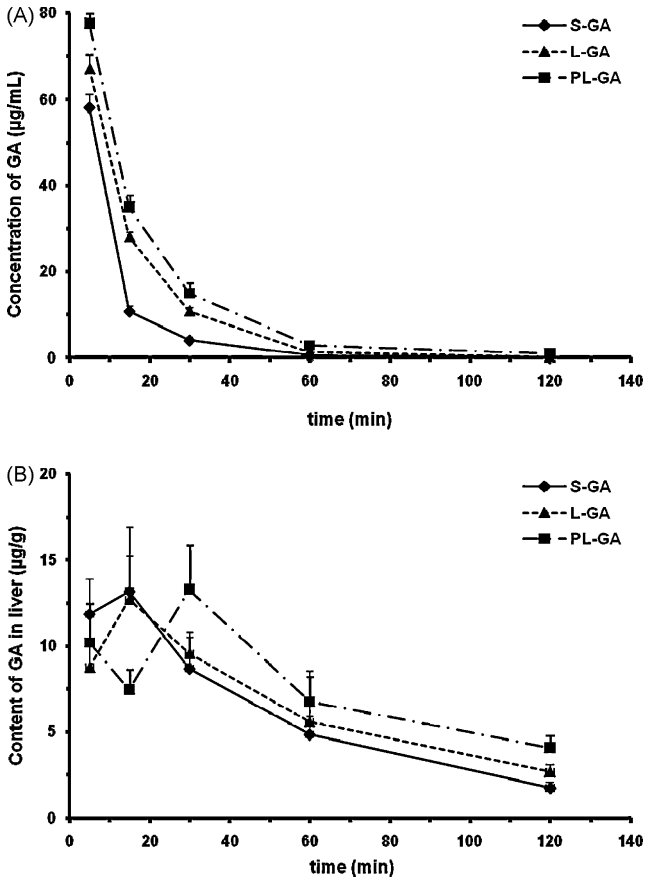

Fig. 3. Concentration–time profiles of GA in mice plasma (A) and liver (B) after i.v. injection of S-GA (♦) L-GA (▲) and PL-GA (■) in mice at a dose of 25 mg/kg. Data were expressed as mean ± SD (n = 6).

Table 2
Pharmacokinetic parameters of glycyrrhetinic acid in mice plasma and liver after the intravenous administration of liposomal and injectable formulations (25 mg/kg, equal to GA, n = 6).

Parameters	Plasma			Liver		
	S-GA	L-GA	PL-GA	S-GA	L-GA	PL-GA
AUC_{0-t} (h μg/mL) or (h μg/g)	16.79 ± 0.82	23.21 ± 1.64*	30.04 ± 2.06*△	11.96 ± 2.06	12.80 ± 3.54	14.88 ± 1.87
$AUC_{0-\infty}$ (h μg/mL) or (h μg/g)	16.81 ± 0.81	23.31 ± 1.65*	31.53 ± 1.62*△	13.68 ± 2.20	16.41 ± 3.23	21.73 ± 2.56**
$t_{1/2}$ (h)	0.32 ± 0.05	0.36 ± 0.05	0.81 ± 0.44*	0.68 ± 0.09	0.95 ± 0.19*	1.18 ± 0.12**
t_{max} (h)	–	–	–	0.19 ± 0.10	0.19 ± 0.10	0.33 ± 0.14*
C_{max} (μg/mL) or (μg/g)	–	–	–	0.23 ± 0.03	0.21 ± 0.07	0.23 ± 0.04
V_d (L/kg) or (mg/kg)/(μg/g)	0.69 ± 0.14	0.56 ± 0.07	0.93 ± 0.52*	1.83 ± 0.43	2.19 ± 0.76	1.98 ± 0.35
CL (L/(h kg)) or (mg/kg)/h (μg/g)	1.49 ± 0.07	1.08 ± 0.08*	0.79 ± 0.04*△	1.86 ± 0.33	1.56 ± 0.28	1.16 ± 0.14
MRT (h)	0.13 ± 0.01	0.23 ± 0.01*	0.43 ± 0.13*△	0.95 ± 0.08	1.07 ± 0.31	1.72 ± 0.11*

Data were calculated by using non-compartmental analysis. AUC_{0-t}: area under the concentration-time curve from time 0 to 2 h; $AUC_{0-\infty}$: area under the concentration-time curve from time 0 to infinity; $t_{1/2}$: elimination half-life; t_{max}: time to peak concentration; C_{max}: maximum concentration; V_d: the volume of distribution; *CL*: total clearance; *MRT*: mean residence time.
S-GA: glycyrrhetinic acid sodium; L-GA: glycyrrhetinic acid liposome; PL-GA: mPEG-PLA modified glycyrrhetinic acid liposome.
*P < 0.05 **P < 0.01 *vs.* S-GA; △P < 0.05 *vs.* L-GA.

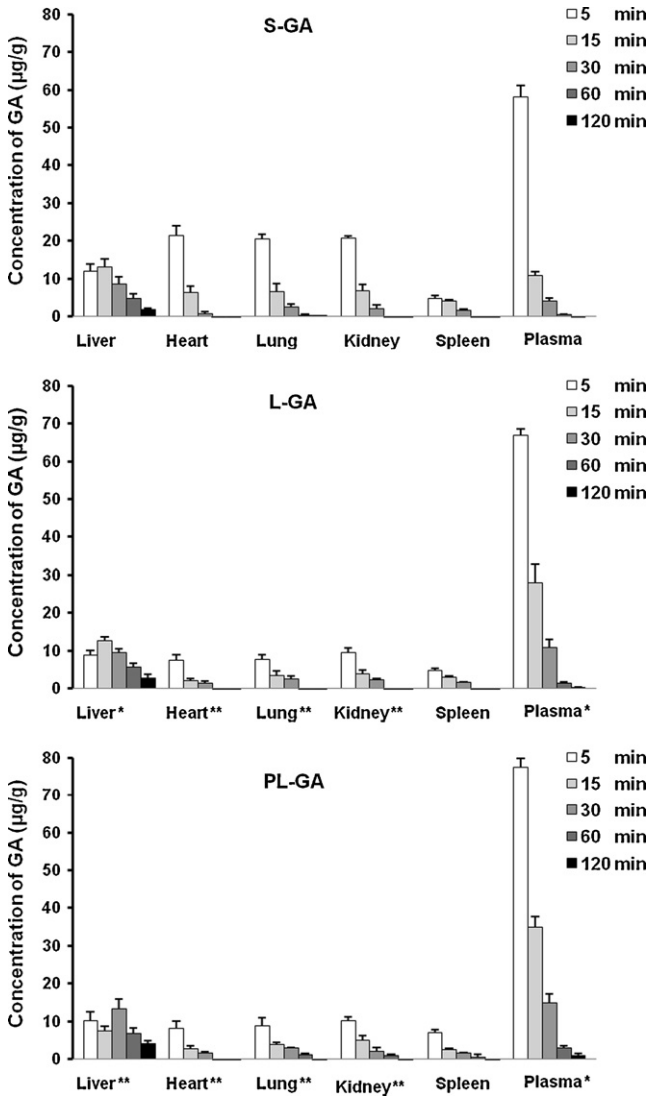

Fig. 4. Distributions of GA in different organs and plasma after i.v. injection of aqueous solutions and liposomal formulations at a dose of 25 mg/kg. Data were expressed as mean ± SD ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* S-GA.

to the higher concentrations of GA in liver, which may therefore enhance its therapeutic values.

Many previous investigations have demonstrated that liposomes are promising drug carrier systems and can be applied to clinical use because of their ability to alter pharmacokinetic profiles of various drugs and also to reduce their associated toxicity *in vivo* [31]. Long-circulating liposomes such as PEGylated liposomes may be used in targeted delivery to tumor or inflammatory regions and to improve the therapeutic index of the encapsulated drugs [32]. In this study, both L-GA and PL-GA formulations were found to reduce the distribution of GA in kidney, which might help to lower its renal toxicity and eliminate its potential pseudo-aldosteronism (i.e. sodium retention, hypokalaemia and hypertension). Moreover, PL-GA significantly delayed the elimination of GA from mice plasma than L-GA and S-GA. The long-circulating effect and delayed release of GA from the mPEGylated liposomal vesicle in vascular system could increase the liver uptake and prolong its pharmacological duration in liver. These findings suggested that mPEGylated GA liposome might have a potential to be developed as a promising pharmaceutical preparation for the treatment of chronic hepatitis.

4. Conclusion

In summary, a novel HPLC-UV method for quantitative determination of GA in mice plasma and tissues is established and successfully applied to measure the bio-samples obtained from mice dosed with S-GA, L-GA and PL-GA with no interference from endogenous substance. The developed assay is simple, accurate, specific and with good reproducibility. By using this method, the pharmacokinetics and tissue distribution of GA in various formulations (all administered at 25 mg/kg in respect to GA, i.v.) in mice were first studied and reported. The liposomal formulations, especially the mPEG-PLA modified liposome, have long-circulating effects in mice plasma with relatively higher liver uptake and lower kidney distribution of GA. Therefore, the mPEG-PLA modified liposome has more favorable pharmacokinetic properties and may have a potential to be developed as a new formulation for GA in the treatment of chronic hepatic diseases.

Acknowledgements

This work was kindly supported by the National High Technology Foundation of China (863 Projects, Ref.: 2003AA2Z347A) and the National 863 Projects (Ref.: 2004AA2Z3020).

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