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Improved solubility and pharmacokinetics of PEGylated liposomal honokiol and human plasma protein binding ability of honokiol

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ABSTRACT

PEGylated liposomal honokiol had been developed with the purpose of improving the solubility and pharmacokinetics compared with free honokiol. Human plasma protein binding ability of honokiol was also investigated. PEGylated liposomal honokiol was prepared by thin film evaporation-sonication method. Its mean particle size was 98.68 nm, mean zeta potential was −20.6 mV and encapsulation efficiency were 87.68 ± 1.56%. The pharmacokinetics of PEGylated liposomal honokiol was studied after intravenous administration in Balb/c mice. There were significant differences of parameters $T_{1/2\beta}$ and $AUC_{0\to\infty}$ between them and liposome lengthened $T_{1/2\beta}$ and $AUC_{0\to\infty}$ values. The mean $T_{1/2\beta}$ value of PEGylated liposomal honokiol and free honokiol were 26.09 min and 13.46 min, respectively. The $AUC_{0\to\infty}$ ratio of PEGylated liposomal honokiol to free honokiol was about 1.85-fold (219.24 μg/mL min/118.68 μg/mL min) ($P = 0.000$). Examination of protein binding ability showed that honokiol with 0.5, 8.0 and 20 μg/mL concentrations in human plasma achieved the percent of bound between 60% and 65%. The results suggested that PEGylated liposomal honokiol improved the solubility, increased the drug concentration in plasma, and withstanded the clearance. Besides, the percent of protein bound of honokiol in human plasma was between 60% and 65%.

1. Introduction

Honokiol has been isolated and identified from the stem bark of *Magnolia officinalis Rehd. et Wils.* It has been reported to have strong pharmacological effects including against microbial (Syu et al., 2004), platelet aggregation (Pyo et al., 2002), oxidation (Haraguchi et al., 1997), anxiety (Kuribara et al., 1998, 1999, 2000), depression (Watanabe et al., 1983) and antitumor (Bai et al., 2003; Hu et al., 2008). However, its poor solubility makes it difficult to be well dispersed in the hydrous solution and restricts greatly its application. Liposomes have been regarded as a drug delivery system to overcome drug poor solubility in aqueous solution, increase the therapeutic efficiency, reduce the side-effects of drug (Papahadjopoulos et al., 1991; Drummond et al., 1999), and enhance oral bioavailability and absorption (Zou et al., 2008). However, one of the major shortcomings of liposomal formulation is its rapid clearance from blood due to the adsorption of plasma protein to the phospholipid membrane of liposomes, thereby triggering the recognition and uptake of the liposomes by mononuclear phagocytic system (Schnyer and Huwyler, 2005). Fortunately, the uptake of mononuclear phagocytic system can be sluggish, when the surface of liposomes is modified with a flexible hydrophilic polymer such as PEG. Therefore, studied on PEGylated liposomal honokiol as a long active liposome is necessary to increase the aqueous solubility and improve the pharmacokinetics of honokiol.

Up to date, studies on pharmacokinetics of honokiol have not yet been paid much attention. Some reports only focused on the pharmacokinetics of free honokiol *via* rectum (Wu et al., 2003), vein (Tsai et al., 1994) and *per os* (Su et al., 2008) administration. In present study, we developed PEGylated liposomes as a delivery system of honokiol and compared the pharmacokinetic difference between PEGylated liposomal honokiol and free honokiol by intravenous administration in mice.

Besides, the ability to bind to plasma protein is an important parameter to influence the pharmacokinetic and pharmacodynamic properties of drugs. The amount of unbound drug fraction may influence its volume of distribution, drug clearance and final concentration at site of action (Wilkinson, 1983; Pacifici and Viani,

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1992). The ability to bind to human plasma protein of honokiol would be also reported for the first time.

2. Materials and methods

2.1. Materials

Honokiol (≥98% in purity) was separated and purified in our laboratory, as reported previously (Chen et al., 2007). Honokiol standard substance (≥98% in purity) and diphenyl (>99% in purity, internal standard, IS) were purchased from the National Institute for the Control of Pharmaceuticals and Biological Products (Chengdu, China). Phosphatidylcholine, cholesterol and $MPEG_{2000}$-DSPE were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Human plasma was obtained from one healthy volunteer. Pooled human plasma from healthy individuals in this study was obtained from the blood and transfusion center at the West China Hospital and was stored frozen at −20 °C until use. The SpectraPor dialysis membranes (molecular weight cutoff 10 kDa) were purchased from Spectrum Laboratories Inc. (Los Angeles, CA, USA). Isotonic buffer was prepared: 4.00 g $Na_2HPO_4{\cdot}2H_2O$, 0.77 g $NaH_2PO_4{\cdot}H_2O$ and 5.78 g NaCl were dissolved in 1 L of water, then the pH of this solution was adjusted with NaH_2PO_4 to 7.35, and the resulting isotonic buffer had an ionic strength of 0.168, which was close to that of plasma.

Chloroform and methanol (analytical grade) were purchased from Kelong Chemicals (Chengdu, China). Acetonitrile (HPLC grade) was purchased from Fisher Chemical (Loughborough, UK). All chemicals and solvents were ACS analytical grade or HPLC grade. And water was purified using a Millipore (AK, USA) laboratory ultra pure water system (0.2 μm filter).

2.2. Chromatographic equipment and conditions

Chromatographic analyses were performed with an Alliance 2965 HPLC system (Waters, Milford, MA, USA) consisting of a column heater and an autosampler, and detection was carried out by using UV detector at 209 nm. Chromatographic separation of honokiol was carried out on a reversed-phase column (Atlantis C18 column, 150 × 4.6 mm, 5 μm, Waters). The column temperature was maintained at 28 °C. Samples were eluted using acetonitrile and water (60:40, V/V) at a flow rate of 1.0 mL/min. Empower software was applied to collect and analyze the data from chromatogram.

2.3. Preparation and physicochemical properties of PEGylated liposomal honokiol

PEGylated liposomal honokiol was prepared by thin film evaporation-sonication method (Hou et al., 2008) and described briefly as follows: phosphatidylcholine, cholesterol, $MPEG_{2000}$-DSPE and honokiol in weight ratios of 1.00:0.15:0.24:0.22 were mixed and dissolved in 15 mL chloroform/methanol (1:4, v/v) in a round-bottomed flask. The mixture was dried to form a thin film in the rotary evaporation apparatus under vacuum in water bath at 40 °C. The resulting film was left overnight and then hydrated with 5% glucose solution while sonication for 10 min. The solution was put into cillin bottles and mixed with cryoprotectant of mannitol. The mixture was freeze-dried in the freeze dryer. Before use, the lyophilized PEGylated liposomal honokiol was directly dissolved in 5% glucose solution for study, with a resulting concentration of honokiol at 4 mg/mL.

The morphology of PEGylated liposomal honokiol was observed by the transmission electron microscope (TEM) (H-6009IV, Hitachi, Japan): Liposomes were diluted with distilled water and placed on a copper grid covered with nitrocellulose, then the sample was negatively stained with phosphotungstic acid and dried at room temperature before observation.

The particle size and zeta potential of prepared liposomes were measured by Malvern Nano-ZS90 laser particle size analyzer (Nano-ZS, Malvern Instruments Ltd., UK) at room temperature after diluting with 5% glucose solution. Each sample was analyzed in quadruple. To investigate the stability of PEGylated liposomal honokiol, the liposome solution was kept at 4 °C for 1 day, 3 days, 5 days and 7 days, and then particle size and zeta potential were measured.

The encapsulation efficiency of PEGylated liposomal honokiol was determined by protamine aggregation method. A 0.1 mL of liposome suspension and 0.1 mL of protamine solution (10 mg/mL) were respectively added into a clean 2 mL test tube. After vortex mixing for 60 s, the mixed solution was kept for 3 min at room temperature. Then 1.0 mL of normal sodium was also added into this tube, mixed and centrifuged at 3000 rpm for 30 min. The supernatant was removed and determined by HPLC to obtain the amount of free honokiol in the liposome. Encapsulation efficiency of PEGylated liposomal honokiol was calculated according to the following equation:

$$\mathrm{EE\%} = \left[1 - \left(\frac{M_{\mathrm{free}}}{M_{total}}\right)\right] \times 100$$

where M_{total} is the total amount of honokiol in PEGylated liposomal honokiol and M_{free} is the amount of free honokiol not encapsulated in the liposome.

Liposomal leakage ratio after storage at 4 °C was determined as followings: PEGylated liposomal honokiol suspension was stored at 4 °C for 0 day, 1 day, 3 days, 5 days and 7 days. At the five time points, 0.1 mL of liposome suspension was removed for determine the liposomal leakage ratio. Meanwhile, free honokiol of 0.1 mL of liposome suspension was separated using protamine aggregation method and the amount of free honokiol was determined by HPLC. Liposomal leakage ratio was calculated according to the following equation:

$$\text{Liposomal leakage ratio}\,(\%) = \left(\frac{M_{\mathrm{free}(t)} - M_{\mathrm{free}(0)}}{M_{\mathrm{total}} - M_{\mathrm{free}(0)}}\right) \times 100$$

where M_{total} is the total amount of honokiol in PEGylated liposomal honokiol, $M_{\mathrm{free}(0)}$ is the amount of free honokiol not encapsulated in the liposome which was stored at 4 °C for 0 day and $M_{\mathrm{free}(t)}$ is the amount of free honokiol not encapsulated in the liposome which was stored at 4 °C for 1 day or 3 days or 5 days or 7 days.

2.4. Plasma samples pretreatment

An aliquot (0.1 mL) of plasma was accurately transferred to a clean 1.5 mL test tube and 10 μL of diphenyl solution (25 μg/mL in acetonitrile) as internal standard was added. Following briefly vortex-mixing, 0.2 mL of acetonitrile was added into the mixture to precipitate the plasma protein. After incubating at room temperature for 10 min, the mixture was centrifuged at 10,000 rpm for 5 min. The resulting supernatant solution was transferred into a clean 1.5 mL test tube containing 40–50 mg sodium chloride. After vortex mixing for 5 s, the suspension was kept for 10 min at room temperature and divided into the organic layer and aqueous layer. 10 μL of upper organic layer was directly injected into HPLC system for analysis.

2.5. Drug administration and plasma samples collection

The study protocol of the animal experiments complied with the Institute Guidelines on Animal Experimentation of Sichuan University in China. Male and female Balb/c mice (6–8 weeks old, 18–22 g) were obtained from the Laboratory Animal Center of Sichuan Uni-

versity and were randomly divided into two groups to be injected with free honokiol solution and PEGylated liposomal honokiol, respectively. The animals were allowed to recover overnight with free access to water. Then the free honokiol solution and PEGylated liposomal honokiol were respectively administered to the mice at a single dose of 20 mg/kg body weight *via* caudal vein. Free honokiol solution was prepared by dissolving 160 mg honokiol in 1 mL mixture of polyethoxylated castor oil (Cremophor EL) and ethanol (1:1, v/v) followed by dilution with 5% glucose solution to 40 mL to generate final concentration of 4 mg/mL. PEGylated liposomal honokiol was directly dissolved in 5% glucose solution to generate the same concentration compared to free honokiol.

During the pharmacokinetic studies, at designated time points (five mice per point, $n = 5$) for 0, 5 min, 10 min, 15 min, 30 min, 45 min, 60 min and 120 min following injection, the mice were sacrificed, respectively. About 0.20 mL of blood samples were collected into tubes containing heparin sodium. Plasma samples were obtained after immediate centrifugation of blood at 3000 rpm for 10 min at 4 °C and were stored at −20 °C until analysis.

2.6. Human plasma protein binding studies in vitro

Equilibrium dialysis was performed to determine the human plasma protein binding of honokiol. Before the equilibrium dialysis experiment, pooled human plasma was incubated for 20 min in an incubator with 9% CO_2 at 37 °C. Prior to use, the dialysis membrane was soaked for 1 h in isotonic buffer, and rinsed twice with water, and then placed into the equilibrium dialysis unit between the two compartments of dialysis chamber to separate free and total honokiol. Human plasma was mixed with honokiol to obtain final concentrations of 0.5, 8.0 and 20 μg/mL. Eight hundred microlitres of the samples were introduced into one side of the cell, while 800 μL of isotonic buffer were added on the other side of the cell. The cell was then sealed and placed on a dialysis cell shaker at 37 °C until equilibrium was reached. The time required to reach equilibrium was 4 h. At the end of the experiment, 0.1 mL samples were taken from each side of the cell for analyzing. The total honokiol concentration in the human plasma compartment (B) and the free honokiol concentration in the isotonic buffer compartment (A) were measured by HPLC. The percent of bound (Fb (%)) was calculated according to the equation described previously (Barre et al., 1985): $\text{Fb}(\%) = [(B - A)/B] \times 100$.

2.7. Drug stability in human plasma

The stability study of honokiol containing 0.5, 8.0 and 20 μg/mL in human plasma was performed to ensure that plasma protein binding data was not affected by drug instability in plasma. The experiment was evaluated as follows: honokiol containing 0.5, 8.0 and 20 μg/mL in human plasma were incubated at 37 °C for 4 h on a shaker, and 0.1 mL of the samples were respectively transferred and analyzed at intervals of 0, 1, 2, 3 and 4 h. The test data of every time were applied to evaluate the stability study of honokiol in human plasma. The same experiment was performed in triple.

2.8. Statistical analysis

T_{max} and C_{max} values of the plasma and tissues were recorded directly from the measured data. Then $T_{1/2}$, CL, Vd and $AUC_{0\to\infty}$ values were calculated. The Drug and Statistics (DAS) software, version 2.1.1, edited and published by the Mathematical Pharmacology Professional Committee of China, was applied to pharmacokinetic analyses. The determination of human plasma protein binding was performed in triplicate and the data were expressed as mean ± SD.

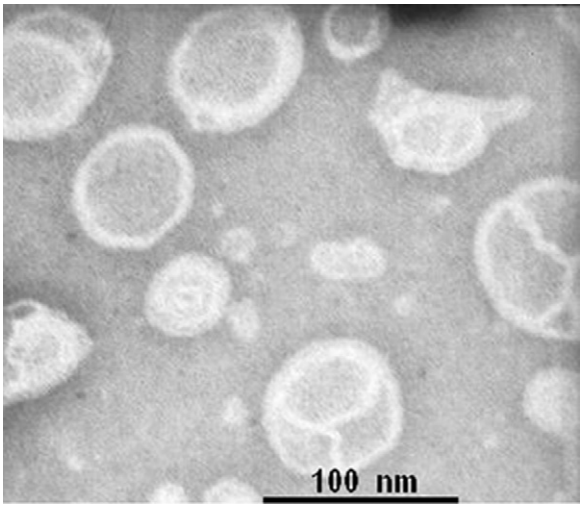

Fig. 1. Transmission electron microscope (TEM) photograph of PEGylated liposomal honokiol.

3. Results

3.1. Physicochemical properties of PEGylated liposomal honokiol

The morphology of PEGylated liposomal honokiol was sphere or oval observed by the transmission electron microscope (TEM) as shown in Fig. 1. According to the TEM image, the mean particle size of PEGylated liposomal honokiol was about 75 nm.

The mean particle size of PEGylated liposomal honokiol was 98.68 nm (Fig. 2A) measured by Malvern Nano-ZS90 laser particle size analyzer. Its mean zeta potential was −20.6 mV and its zeta potential distribution could be seen in Fig. 2B. The data of stability investigation of PEGylated liposomal honokiol were shown in Table 1, and the results revealed that PEGylated liposomal honokiol kept stable at least 7 days at 4 °C with stable particle size (99.87 ± 1.66 nm) and zeta potential (20.81 ± 1.09 mV).

PEGylated liposomal honokiol with various weight ratios of phosphatidylcholine, cholesterol, PEG_{2000}-DSPE and drug were prepared and their encapsulation efficiencies were determined. The results showed that the encapsulation efficiency was the highest (87.68 ± 1.56%) when the weight ratios of phosphatidylcholine, cholesterol, PEG_{2000}-DSPE and honokiol were 1.00:0.15:0.24:0.22. Liposomal leakage ratio was also measured and the results were

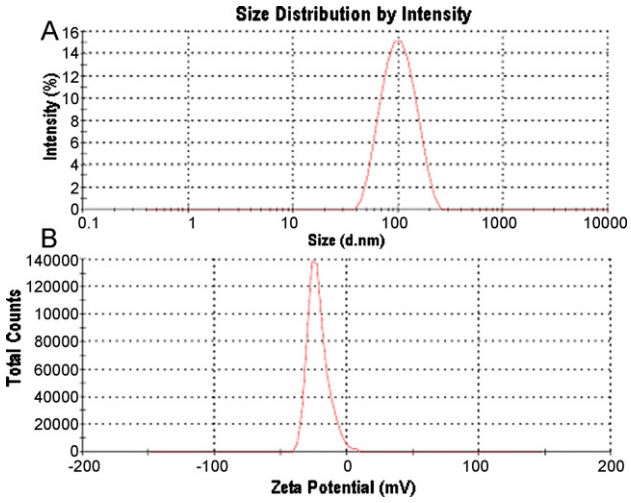

Fig. 2. Particle size and zeta potential distribution of PEGylated liposomal honokiol.

Table 1
Stability of PEGylated liposomal honokiol at 4 °C for 0 day, 1 day, 3 days, 5 days and 7 days.

Days	Particle size (nm)	Mean particle size ± SD (nm)	Zeta potential (mV)	Mean zeta potential ± SD (mV)
0	98.68		−20.60	
1	98.13		−20.51	
3	99.28	99.87 ± 1.66	−19.25	20.81 ± 1.09
5	101.85		−21.96	
7	101.39		−21.96	

respectively 0.00%, 0.21%, 0.49%, 0.63% and 0.81% at 4 °C for 0 day, 1 day, 3 days, 5 days and 7 days. The liposomal leakage ratio was less than 1% at 4 °C environment during 7 days.

Due to its poor solubility, free honokiol was hard to dissolve in the hydrous solution except some suspending agent or auxiliary solvent such as Cremophor EL and ethanol were added, which might result in some toxic effects including hypersensitivity and neurotoxicity. However, PEGylated liposomal honokiol could directly dissolve in 5% glucose solution to generate the same concentration compared to free honokiol. Therefore, the solubility of honokiol could be improved and the toxic effects of it were also greatly decreased when it was prepared into liposomes.

3.2. Validation of analytical method

Peak identification of honokiol was performed by comparison of retention time and UV spectra of the eluting peak with honokiol standard substance. The retention times of honokiol and IS were about 6.5 min and 9.5 min, respectively. No interfering peaks with similar retention times of the two substances peaks were observed in the blank plasma samples. The endogenous substances in plasma did not interfere in the analysis.

The calibration curves of honokiol in plasma had good linearity by determining the standard concentrations of 0.5–20 μg/mL. The regression equation was $y = 0.9568X + 0.6286$ ($r = 0.9991$, $n = 5$). The lowest limit of detection (LLOD) was 10 ng/mL, defined as the peak–area ratios of honokiol versus noise was 3:1.

Recovery and precision were calculated in plasma, spiked with a honokiol standard at concentrations of 0.5, 5.0 and 20 μg/mL. For the low, medium and high concentration, the recoveries were 91.26 ± 4.82%, 101.16 ± 3.97%and 103.52 ± 1.68%. The intra-day R.S.D was less than 9.65%, and the inter-day R.S.D was less than 13.97%.

3.3. Pharmacokinetics

The mean plasma concentration–time curves of honokiol in mice after intravenous administration of free honokiol and PEGylated liposomal honokiol as a long active liposome at a single dose of 20 mg honokiol/kg body weight were shown in Fig. 3. Both curves fit the open two-compartment model. The mean pharmacokinetic parameters were listed in Table 2. The results showed that there were significant differences for most of these parameters between them, and the $T_{1/2\beta}$ ratio of PEGylated liposomal honokiol to free honokiol was about 2-fold (26.09 min/13.46 min) ($P = 0.000$) and the $AUC_{0\to\infty}$ ratio of PEGylated liposomal honokiol to free honokiol was about 1.85-fold (219.24 μg/mL min/118.68 μg/mL min) ($P = 0.000$). The CL value of PEGylated liposomal honokiol was less

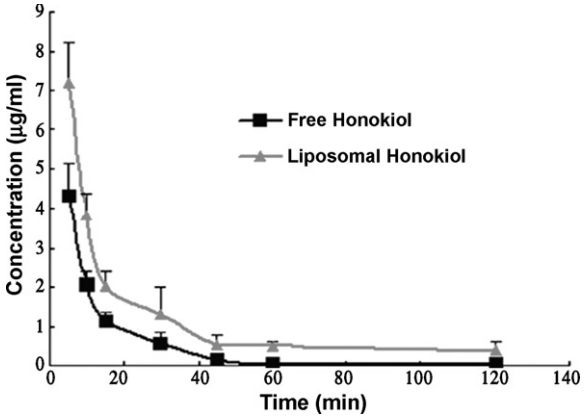

Fig. 3. Mean plasma concentration–time curves of honokiol in mice after intravenous administration of free honokiol and PEGylated liposomal honokiol at a single dose of 20 mg/kg body weight. Each point with bar represented the mean + SD ($n = 5$).

Table 3
The percent of bound data of honokiol in pooled human plasma in vitro ($n = 4$).

Concentration (μg/mL)	The percent of bound data (%) mean	SD
0.5	64.69	0.07
8.0	62.78	0.03
20	64.46	0.03

compared with that of free honokiol ($P = 0.017$). Therefore, the results revealed that free honokiol rapidly cleared in bloodstream, and PEGylated liposomal honokiol did increase the drug concentration in plasma and withstand the clearance.

3.4. Human plasma protein binding studies in vitro

The time taken to reach equilibrium for honokiol between pooled human plasma and isotonic buffer was investigated. Pooled human plasma spiked with honokiol (8.0 μg/mL) was dialyzed against isotonic buffer at 1, 2, 3, 4, 5 and 6 h. Honokiol crossed the dialysis membrane rapidly and reached a steady state in human plasma within 4 h at 37 °C on a shaker. Therefore, equilibrium dialysis time was set at 4 h to investigate all subsequent equilibrium dialysis experiments. The incubated temperature was selected at 37 °C similar to human body temperature. The percent of bound data of honokiol in vitro in pooled human plasma were shown in Table 3. The results indicated that the percent of bound between 60% and 65% was achieved within the range of honokiol concentrations from 0.5 to 20 μg/mL in pooled human plasma. The data of

Table 2
The mean plasma pharmacokinetic parameters of honokiol after intravenous administration of free honokiol and PEGylated liposomal honokiol in mice ($n = 5$).

Parameters	$T_{1/2a}$ (min)	$T_{1/2\beta}$ (min)	$AUC_{0\to\infty}$ (μg/mL min)	CL (mL/min/g)	K10 (1/min)	K12 (1/min)	K21 (1/min)
Free honokiol	2.75	13.46	118.68	0.17	0.097	0.117	0.089
Liposomal honokiol	3.47	26.09	219.24	0.09	0.075	0.101	0.051
P-Value	0.000	0.000	0.000	0.017	>0.05	>0.05	0.003

Table 4
Stability of honokiol in human plasma at 37 °C over a 4 h time-course ($n = 3$).

Sample concentration (μg/mL)	n	Mean concentration (μg/mL)	Precision (R.S.D%)	Accuracy (%)
0.5	1	0.48	6.25	96.0
	2	0.49	5.82	98.0
	3	0.48	6.93	96.0
8.0	1	7.96	3.56	99.5
	2	8.03	4.17	100.4
	3	7.98	4.61	99.8
20	1	20.23	2.81	101.2
	2	21.05	3.96	105.3
	3	21.41	3.15	107.1

the percent of bound in human plasma might explain the pharmacokinetic characters and pharmacodynamic properties of honokiol and these results were reported for the first time.

3.5. Drug stability in human plasma

The stability of honokiol (0.5, 8.0 and 20 μg/mL) was studied in human plasma at 37 °C and differences were observed over a 4 h time-course. The results were given in Table 4. These data showed that no significant differences (R.S.D values were between 2.81% and 6.93%) were found in different times, which indicated that honokiol in human plasma could be kept for at least 4 h without resulting in significant losses at 37 °C and honokiol was stable over the time-course of protein binding assay.

4. Discussion

The liposomal formulation is considered to be a low-toxic technology with considerable potential for encapsulating lipophilic drugs among the different drug delivery system (Souto, 2009). In our study, PEGylated liposomal honokiol was developed and could directly dissolve in 5% glucose solution and the final concentration of honokiol was 4 mg/mL. In contrast, free honokiol could only dissolve in some suspending agent or auxiliary solvent including Cremophor EL, ethanol, tween 80 and carboxymethylcellulose sodium, which might result in some toxic effects. Therefore, PEGylated liposomal honokiol improved greatly the solubility of honokiol.

When the weight ratios of phosphatidylcholine, cholesterol, PEG_{2000}-DSPE and honokiol were 1.00:0.15:0.24:0.22, the mean particle size of PEGylated liposomal honokiol measured by Malvern Nano-ZS90 laser particle size analyzer was 98.68 nm, its mean particle size measured by TEM image was 75 nm, its mean zeta potential was −20.6 mV and its encapsulation efficiency were the highest (87.68 ± 1.56%). There was a difference of mean particle size by measuring using the two methods and the mean particle size measured by TEM image was smaller than that measured by Malvern Nano-ZS90 laser particle size analyzer. The reason might be that liposome solution would be dried before observation when particle size of it was measured by TEM, however, the liposome solution would be directly observed when its particle size was measured by Malvern Nano-ZS90 laser particle size analyzer. Due to the swelling phenomenon, the particle size of liposome solution without drying would be bigger compared to liposome solution dried. Therefore, the mean particle size of PEGylated liposomal honokiol measured by TEM image was smaller compared to Malvern Nano-ZS90 laser particle size analyzer. The encapsulation efficiency of liposomes was determined using usually sephadex chromatography, equilibrium dialysis and ultrafiltration (Perkins et al., 1993). Each method had its advantages and disadvantages. With regarding to the determination of encapsulation efficiency of liposomal honokiol, sephadex chromatography, equilibrium dialysis and ultrafiltration methods were not suitable to the PEGylated liposomal honokiol due to the little honokiol adsorption and long experiment time. Protamine contained a number of basic amino acids and its isoelectric point was between 10 and 12, which made protamine having positively charged in solution. Due to its positively charged, protamine could interact with negatively charged PEGylated liposomal honokiol through electrostatic interaction without interacting with free honokiol. Therefore, protamine aggregation method was a better method to separate the PEGylated liposomal honokiol and free honokiol and determine the encapsulation efficiency of PEGylated liposomal honokiol. In our study, protamine aggregation method was chosen as a method to determine the encapsulation efficiency of PEGylated liposomal honokiol.

It was very easy for liposomes to be phagocytized by mononuclear phagocytic system in vivo. However, when the surface of liposomes was modified with PEG, the phagocytosis could be retarded (Torchilin and Trubetskoy, 1995). This resulted in increasing the drug concentration of PEGylated liposomal honokiol in plasma and withstanding the clearance. Therefore, PEGylated liposomal honokiol could improve the pharmacokinetics of drug. Our results showed that significant differences of pharmacokinetic parameters between PEGylated liposomal honokiol and free honokiol did exist. The elimination half-life value of PEGylated liposomal honokiol was about 2-fold increase compared with that of free honokiol ($P = 0.000$). And the $AUC_{0\to\infty}$ value of PEGylated liposomal honokiol was about 1.85-fold increase compared with that of free honokiol ($P = 0.000$). The CL value of PEGylated liposomal honokiol was less compared with that of free honokiol ($P = 0.017$).

It had been well known that the protein binding ability of drug in plasma, especially in human plasma, was a very important factor influencing the pharmacokinetics and pharmacodynamics of drug. However, there had been no investigation on the protein binding ability of honokiol. In the present studies, the results indicated that the percent of binding of honokiol were between 60% and 65%. There was no concentration dependent binding at the concentrations of 0.5, 8.0 and 20 μg/mL in pooled human plasma. These results had provided us some information for understanding the properties of honokiol on pharmacokinetics and pharmacodynamics.

5. Conclusions

One of the most significant findings of this study was that PEGylated liposomal honokiol improved the solubility of free honokiol without Cremophor EL, ethanol, tween 80 and carboxymethylcellulose sodium as suspending agent or auxiliary solvent which would result in severe anaphylaxis and peripheral nerve toxicity and so on. Second, PEGylated liposomal honokiol as a long active liposome improved significantly its pharmacokinetics in Balb/c mice and lengthened its $T_{1/2\beta}$ and $AUC_{0\to\infty}$ values. Finally, the percent of protein bound of honokiol in human plasma was between 60% and 65% which was reported for the first time.

Conflict of interest

No potential conflicts of interest were disclosed.

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