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Multivesicular liposome formulation for the sustained delivery of breviscapine

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Abstract

Breviscapine, a well-known bioactive flavonoid ingredient extracted from the traditional Chinese medicine, has been extensively used in clinic to treat ischemic cerebrovascular and cardiovascular diseases in China. In order to prolong the duration of the drug in the circulation, reduce the frequency of injection administration and subsequently afford patient compliance, multivesicular liposome (MVL, namely DepoFoam) was utilized as a sustained-delivery system for breviscapine. In vitro release and in vivo pharmacokinetics of MVLs containing breviscapine (bre-MVLs) following intramuscular injection to rats were investigated compared with those of traditional liposomes containing breviscapine (bre-TLs). The drug durations both in vitro and in vivo were significantly prolonged for the bre-MVL, and that the drug release in vitro and the absorption in vivo showed a good linear correlation ($R = 0.9834$), which provided an evidence for the suitability to select human plasma as the medium of drug release from MVLs in vitro. Drug release from bre-MVLs (triolein/tricaprylin, 10/0) in vitro extended a long period of 5–6 days, while the bre-TLs released 80% within only 4 h. The mean residence time (MRT) obtained from the pharmacokinetics study of bre-MVL was about 16.6- and 5.04-fold longer than those of breviscapine solution (BS) and bre-TL, respectively. A duration in vivo for a period of 4–5 days was fulfilled for bre-MVL. In conclusion, MVL can be successfully used as a sustained delivery system of breviscapine.
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Keywords: Breviscapine; Multivesicular liposomes; Sustained delivery; In vitro–in vivo correlation

1. Introduction

Nowadays, the importance of traditional Chinese medicine (TCM) together with its bioactive ingredients (BI) has been more and more recognized due to their effectiveness and safety. However, many of TCM and BI are manufactured in a primitive rough way in China and do not keep up with the times. To improve their

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performance, it is necessary to renovate the dosage forms for TCM and BI with the modern advanced technology.

Breviscapine, a well-known bioactive flavonoid ingredient extracted from the TCM *Erigeron breviscapus* (Vant.) Hand.-Mazz., is mainly composed of scutellarin. Recent studies have shown that scutellarin possesses potent pharmacological effects (Chen and Jin, 1997; Wang, 1999). The injection preparation of breviscapine is extensively used in clinic to treat ischemic cerebrovascular and cardiovascular diseases in China, such as cerebral infarction, apoplexy, coronary heart disease and angina pectoris, etc. (Chen and Jin, 1997; Wang, 1999). Due to the prominent efficacy of breviscapine in the clinical treatment of these diseases, the research of breviscapine has become a hot topic in China in recent years. More than 40 Chinese patents on breviscapine have been published since 2001. Over half of the patents focus on the renovation of the dosage form of breviscapine. However, to our knowledge, there is no research report on the sustained-delivery injectable formulation for breviscapine up to now.

The ischemic cerebrovascular and cardiovascular diseases dealt with breviscapine are chronic and always need a long period of treatment time. However, the residence time of breviscapine in the circulation is short (Ge et al., 2003; Jiang et al., 2003; Liu et al., 2003). In order to prolong the duration of the drug in the circulation, effectively maintain the therapeutic drug levels in the blood for a long time, reduce the frequency of injection administration and therefore afford patient compliance, multivesicular liposomes (MVLs, namely DepoFoam) were utilized as drug delivery vehicles for sustained release of breviscapine in this study.

Multivesicular liposome (MVL), a unique lipid-based depot-delivery system, has been demonstrated as an effective sustained-delivery system with a release duration from days to several weeks (Ye et al., 2000; Mantripragada, 2002). The MVL formulations of many drugs have been studied for sustained delivery, including small molecular drugs, such as amikacin (Roehrborn et al., 1995; Huh et al., 1998), methotrexate (Chatelut et al., 1994), gentamicin (Grayson et al., 1993), cisplatin (Xiao et al., 2004) and morphine (Kim et al., 1993, 1996), and peptide/protein drugs, such as leridistim (Langston et al., 2003), progenipoietin (Ramprasad et al., 2003), apolipoprotein E (Ramprasad et al., 2002) and human interferon α-2b (Bonetti and Kim, 1993). Two products based on MVL technology, Depocyt® and DepoDur™, have been approved for clinical use. MVLs are distinct from traditional liposomes (TLs) in structure, composition and size. The MVLs consist of numerous non-concentric closely-packed internal aqueous chambers separated by a network of lipid membranes (Mantripragada, 2002). Because of the unique structure characteristics, these MVL particles have higher mechanical strength and are more stable than TLs. Furthermore, the structure of MVLs confers a longer duration of drug release since a single or several breaches of lipid membranes of MVL particle will not result in a total release of the internal aqueous contents, which is not the same as unilamellar and multilamellar liposomes. The uniqueness of the structure is attributed to the presence of triglycerides in the MVL particles. The triglyceride acts as hydrophobic space filler at the junctions between the internal lipid chambers to stabilize the junctions and can be used as a tool to adjust drug release rate (Ellena et al., 1999; Mantripragada, 2002; Ramprasad et al., 2003). The particle size of MVLs is about 10 times larger than that of TLs. Typically, the particle size of MVLs is about 5–50 μm, while that of TLs is about 0.1–5 μm. Due to the large size, these MVLs are not rapidly cleared by tissue macrophages and can act as a drug-depot (Ramprasad et al., 2003). MVL can be injected via many routes, including intramuscular, intrathecal, epidral, subcutaneous, and intraocular, which has been reviewed in reference (Howell, 2001).

In this study, MVL formulations of breviscapine (bre-MVL) for its sustained delivery were prepared. In vitro release behavior and in vivo pharmacokinetics characteristics of bre-MVL following intramuscular injection to rats were investigated and compared with those of bre-TL. The in vitro–in vivo correlation (IVIVC) for bre-MVL formulation was also studied.

2. Materials and methods

2.1. Materials

Breviscapine (scutellarin content, 91.7%) was obtained from Yunnan Wanfang Pharmaceutical Co. Ltd. (China). Scutellarin was from Kunming Institute of Botany (98.0%, China). Scoparone (98.5%) was purchased from the Center Delta Natural Organic

Compound (China). Phosphatidylcholine (PC, 92% Epikuron 200) and phosphatidylglycerol (PG, >90%) were gifts kindly provided by Degussa BioActives Deutschland GmbH & Co. KG (Germany). Triolein (TO) was obtained from Zhejiang Huangma Chemical (Group) Co. Ltd., Tricaprylin (TC) was purchased from Sigma. Cholesterol was of analytical grade from Tianjin Chemical Reagent Co. Inc.

2.2. Preparation of bre-MVLs and bre-TLs

Bre-MVLs were prepared by a double emulsification process, as described previously (Kim et al., 1983; Katre et al., 1998). Briefly, a lipid solution containing 40 mg of PC, 8 mg of PG, 40 mg of cholesterol, designed amounts of triolein or tricaprylin (the molar ratio of PC to the total triglyceride is 5.75:1) in 1 ml of chloroform–diethyl ether (1:1 v/v) was emulsified with 1 ml of an aqueous solution (the first aqueous solution) to prepare a water-in-oil emulsion. The first aqueous solution was 40 mg/ml of breviscapine and sucrose (4%, w/v) in 50 mM arginine-containing buffers (pH 7). The emulsification condition was 10,000 rpm for 8 min with a mixer (T 18 basic ULTRA-TURRAX®, IKA Works, Guangzhou). A subsequent emulsification (6000 rpm for 40 s) of the water-in-oil emulsion with the second aqueous solution containing 3.4% glucose and 40 mM L-Lysine, yielded a water-in-oil-in-water double emulsion (the second emulsion). The second emulsion was transferred to a 250 ml Erlenmeyer flask (bottom diameter, 8 cm). Chloroform and diethyl ether were removed by flushing nitrogen over the surface of the mixture at approximately 29–30 °C. Before the experiments of in vitro release and in vivo pharmacokinetics, the free drug was removed from the resulting MVLs by centrifugation at $600 \times g$ for 5 min and then resuspended in appropriate volume of buffered saline solution. The final drug content in MVL was approximately 10 mg/ml.

The lyophilization-reconstitution bre-TLs were prepared in the experiment. The liposomes before lyophilization were prepared by the ethanol injection method with some modification (Batzri and Korn, 1973; Pons et al., 1993). Briefly, PC (400 mg) and cholesterol (100 mg) were dissolved in 2 ml of absolute ethanol by stirring at a water bath at about 50 °C. The ethanol solution of lipids was rapidly injected into 10 ml of magnetically stirred breviscapine solution (10 mg/ml). Ethanol was removed under reduced pressure at 50 ± 1 °C. The final volume was adjusted to 10 ml by addition of distilled water, to yield a liposome suspension with an approximate breviscapine concentration of 10 mg/ml. Then this suspension was extruded three times through 0.8 μm pores using a 10 ml thermobarrel extruder (Northern Lipids Inc., Vancouver, Canada). A 2 ml of the extruded liposomes was mixed with 0.5 ml of (40%) sucrose solution, frozen and lyophilized on a laboratory freeze drier (FD-1, Beijing Bioking Technology Company, Beijing, China) for 36–48 h. The lyophilized preparation was rehydrated with 2 ml of bidistilled water before the next experiment.

2.3. Particle characterization of MVLs and TLs

Particle size was measured by a Laser Diffraction Particle Size Analyzer (LS 230, Beckman Coulter Inc.). The morphology was estimated by a Digital Biological Microscope, equipped with a computer-controlled image analysis system (DMBA 450, Motic China Group Co. Ltd.).

2.4. HPLC analysis of scutellarin

As the content of scutellarin in breviscapine was more than 90% (Chen and Jin, 1997), scutellarin in the breviscapine preparations or rat plasma samples was determined by a HPLC method. The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT pump, an SPD-10A UV detector set at 335 nm. The analyte was determined at room temperature on a 200 mm × 4.6 mm, 5 μm Kromasil ODS column (Dalian Institute of Chemical Physics, China). The mobile phase consisted of acetonitrile–tetrahydrofuran–sodium phosphate buffer (20 mM) (18:7:75 v/v/v). The sodium phosphate buffer was adjusted to pH 2.5 with 1 M phosphoric acid before mixing with acetonitrile and tetrahydrofuran. The mobile phase was pumped through the system at a rate of 0.8 ml/min.

2.5. Determination of encapsulation efficiency

Bre-MVL preparations (0.5 ml), to which 4.5 ml of normal saline was added, were centrifuged at $600 \times g$ for 5 min to separate the free scutellarin (in the supernatant) from the MVLs encapsulated

scutellarin (in the pellet). The amount of drug encapsulated in the MVLs (D_{en}) was determined by HPLC analysis after the pellet was dissolved in appropriate methanol containing 1% (v/v) 1 M HCl (acidified methanol). The total content of drug (D_{tot}) in the breviscapine preparations was also determined after the liposomes were dissolved in appropriate acidified methanol. Encapsulation efficiency of MVLs (En%) was calculated from the percentage ratio of D_{en}:$D_{tot.}$

To bre-TLs, the free drug (D_{free}) was separated by ultrafiltration and quantified using HPLC method. The total content of drug (D_{tot}) in the breviscapine preparations was also determined after the liposomes were dissolved in appropriate acidified methanol. Encapsulation efficiency (En%) of TLs was estimated by the following equation:

$$E_{\mathrm{n}}\,(\%) = (D_{\mathrm{tot}} - D_{\mathrm{free}})/D_{\mathrm{tot}} \times 100$$

2.6. In vitro release

An amount of 2 ml of each MVL suspension was diluted and incubated with four-fold of human plasma containing 0.01% NaN_3 under stirring condition at 37 °C. An amount of 0.5 ml of samples was taken from the diluted suspension according to the planned schedule and 4.5 ml normal saline was added to each sample. Particle pellets were then obtained by centrifugation at 10 000 × *g* for 10 min. The retained drug in particles (D_{ret}) was analyzed by HPLC after the pellets were dissolved in appropriate acidified methanol. The release percent at each time point was calculated by the following equation:

$$\mathrm{Release}\,(\%) = (D_0 - D_{\mathrm{ret}})/D_0 \times 100$$

where D_0 is the drug amount encapsulated in MVLs before mixing with the plasma.

In vitro drug release from TL dispersions was determined by using dialysis bags (MW cutoff 12–14K, Ustars-Bio Corportion, China). A 2 ml of the liposomal suspension was transferred to the dialysis bags, which was tied to the paddle of the dissolution apparatus, ZRS-8G Dissolution Tester (Tianjin University, Precision Instrument Factory, China). The dissolution apparatus was lowered into 250 ml beakers containing 250 ml PBS (pH 7.4) as dissolution medium. The contents of the beaker were stirred at 50 rpm at 37 °C throughout the experiment. Samples (5 ml) were withdrawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 12 h. Each withdrawal was followed by replacement with fresh medium and the samples were analyzed by HPLC method.

2.7. Pharmacokinetics and in vitro–in vivo correlation (IVIVC) study

2.7.1. Animals

Wistar rats (female and male, 230–270 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University. All the animals use procedures were in accordance with the Regulation of Experimental Animal Administration issued by State Committee of Science and Technology of PR China on 14 November, 1988. The carotid artery of each rat was cannulated 2 days before the experiment. The rats were fasted overnight prior to the study, but were allowed water ad libitum.

2.7.2. Drug administration and blood sampling

Three groups of six rats each were treated with bre-MVL, bre-TL and breviscapine solution at a single dose of 40 mg/kg via intramuscular injection at the central of the right thigh muscle (musculi rectus), respectively. Blood samples (0.3 ml) were collected from the catheter placed in the carotid artery at predetermined time intervals after administration. An equal volume of normal saline (0.9% NaCl) was injected after each withdrawal. The heparinized blood was immediately centrifuged at 3000 × *g* for 10 min. Rat plasma was obtained and stored at −20 °C until analysis.

2.7.3. Sample preparation

An amount of 20 μl of scoparone solution (internal standard solution, 20 μg/ml) and 50 μl of phosphoric acid (1 M) were added to a 100 μl aliquot of rat plasma. This mixture was extracted with 3 ml of ethyl acetate by shaking for 15 min. The organic phases were separated by centrifugation at 3000 × *g* for 10 min, transferred to a 5 ml tube and evaporated to dryness under nitrogen stream in a 40 °C water bath. The residue was dissolved in 100 μl mobile phase. A 50 μl aliquot of the solution was injected into the HPLC system for analysis.

2.7.4. Date analysis

Plasma concentrations versus time data were analyzed by a non-compartmental model using the Topfit

2.0 computer program (Thomae GmbH, Germany). The maximum plasma concentration (C_{max}) and the time to reach this maximum (T_{max}) were obtained directly from the concentration–time profile. The terminal elimination rate constant (k_e) was determined by linear least squares regression of the terminal portion of the plasma concentration–time curve. The area under the plasma concentration–time curve up to the last time (t_{last}) showing a measurable concentration (C_{last}) of the analyte ($AUC_{0\to t}$) was calculated by the linear trapezoidal rule, with extrapolation to infinity ($AUC_{0\to\infty}$) by the equation $AUC_{0\to t} + C_{last}/k_e$. The mean residence time (MRT) was calculated from the area under the moment curve divided by the area under the curve. The percentage of absorbed drug in vivo for the IVIVC study was calculated by Loo–Riegelman method (Gibaldi and Perrier, 1982). Our previous study showed that pharmacokinetics behavior of breviscapine following intravenous injection administration could be described by a two-compartment model. The transfer rate constant from central compartment to peripheral compartment (k_{12}) and the one from peripheral compartment to central compartment (k_{21}) were 0.516 and 0.349, respectively. And the elimination rate constant (k_{10}) was 1.929.

Statistical analysis was performed using Student's t-test with $p < 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Particle characterization of MVLs and TLs

The formulation of bre-MVL was optimized by single factor experiment and orthogonal experimental design in our previous study. The morphology of MVLs at 400× magnification with an optical microscope is shown in Fig. 1. The bre-MVLs were spherical with internal appearance looked like aggregates of small particles, which were like that described previously (Kim et al., 1983). Fig. 2 shows the volume weighted size distribution profile of the optimized bre-MVL formulation. The profile showed a single narrow peak. The mean diameter of bre-MVLs was 17.9 μm and above 90% of the particles were in the size range of 5–40 μm. The encapsulation efficiencies of bre-MVLs were determined as 81.2 ± 3.1%.

The mean particle size measured from the lyophilization-reconstitution bre-TLs was 0.54 μm and more than 90% of the bre-TL particles were in the size range of 0.30–0.80 μm. The encapsulation efficiencies obtained from the preparation were 87.9 ± 3.1%. The high encapsulation efficiency was due to the slight molecular interaction between the drug and phospholipids, which will be discussed in detail later.

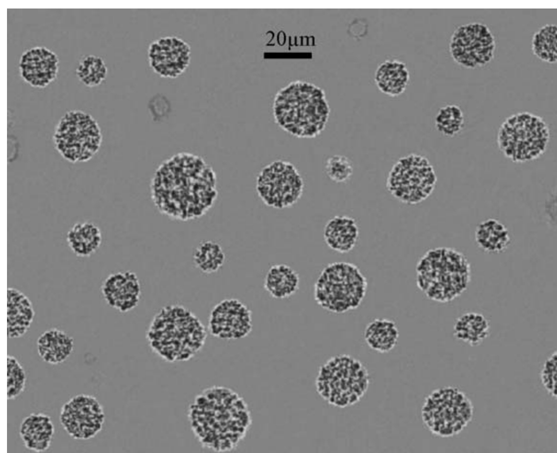

Fig. 1. A morphological picture of bre-MVLs at 400× magnification taken by a light microscope, which is equipped with a computer-controlled image analysis system.

3.2. In vitro release

The in vitro release profiles for bre-MVL (triolein/tricaprylin, 10/0) and bre-TL are shown in Fig. 3.

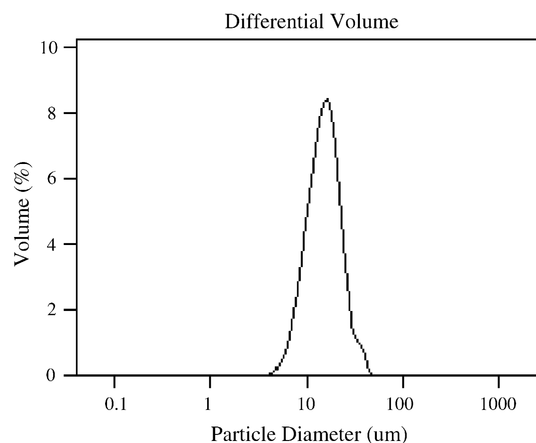

Fig. 2. Particle size distribution of bre-MVLs (triolein/tricaprylin, 10/0).

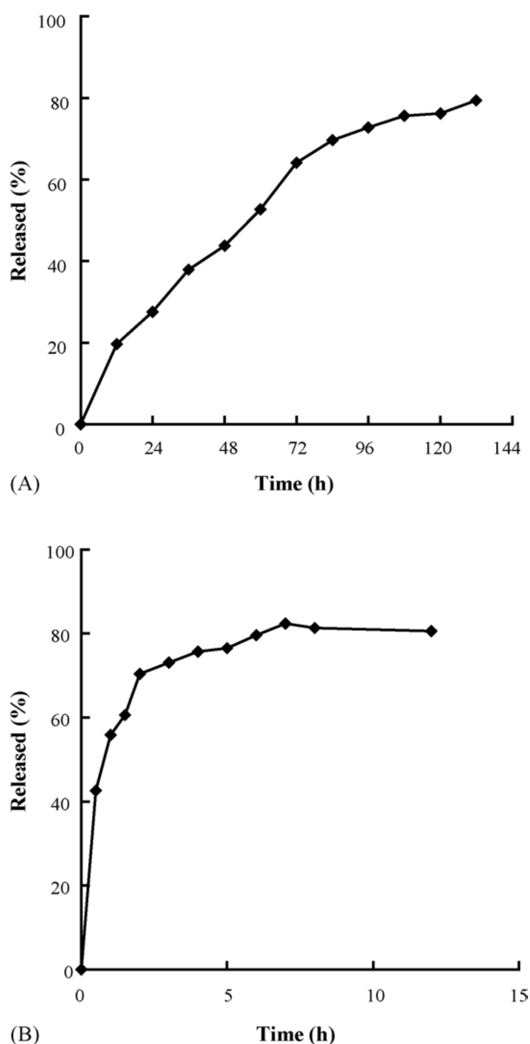

Fig. 3. In vitro release profile of bre-MVLs (triolein/tricaprylin, 10/0) and bre-TLs: (A) bre-MVLs incubated with human plasma at 37 °C; and (B) bre-TLs dialysed against PBS at 37 °C. The points were expressed with the mean values on the basis of three experiments. The S.D. were calculated as less than 3% of the mean values.

Although the release condition of bre-MVL, incubating with human plasma, was more rigorous than that of bre-TL, the drug release rate from bre-MVLs (triolein/tricaprylin, 10/0) was much slower than that from bre-TLs. The release duration from the bre-MVLs extended as long as 5–6 days, while the bre-TLs released 80% of drug within 4 h and subsequently the release system reached equilibrium.

Why was the drug release from bre-TLs so rapid? This phenomenon may be related to the location site of scutellarin molecule binding at the liposomes. Scutellarin is a flavonoid compound. Perhaps there is some molecular interaction between the scutellarin and the polar headgroups of phospholipids. This is the case for many other flavonoid compounds, such as quercetin, rutin, morin (Ratty et al., 1988; Saija et al., 1995). Thus a relative portion of the scutellarin is located at the polar headgroups of phospholipids, which leads to the high apparent drug encapsulation efficiency of bre-TLs. However, the molecular interaction is so weak that the scutellarin located at the polar headgroups is released rapidly when bre-TLs are exposed in dialysis medium. Additionally, perhaps the other portion of scutellarin encapsulated in the TLs is easy to penetrate through the lipid membrane and diffuse into the external medium.

Unlike TLs, MVLs have a much higher aqueous volume-to-lipid ratio. The percent of encapsulated aqueous volume is typically 95% for the MVL particles (Ye et al., 2000; Langston et al., 2003). Therefore, breviscapine is mainly encapsulated in the internal aqueous chambers. So the release of drug from MVLs becomes slower. Moreover, the slow release of encapsulated scutellarin from MVLs may also be attributed to the unique structure of MVL particles. The MVL particles are composed of numerous nonconcentric internal aqueous chambers containing encapsulated drug. Each chamber is separated from adjacent chambers by lipid membranes. Thus, a single or several breaches of lipid membranes will not lead to a total drug release from the internal aqueous contents, which is not like TLs. Additionally, the different release mechanism of MVLs from that of TLs may be another reason for the slow release rate of MVLs. The mechanism of release from MVL particles is not exclusively classical diffusion. It also includes surface erosion of exterior vesicles causing release of encapsulated drug to the external medium and lipid reorganization, together with coalescence of interior chambers leading to extrusion of the excess lipid at the surface (Mantripragada, 2002).

Adjusting the molar ratio of long and short chain triglycerides can modify the release rate of encapsulated drug in MVLs. Long chain triglycerides result in slower drug release from MVLs than short chain triglycerides (Mantripragada, 2002; Ramprasad et al., 2003). It has been suggested that long and short chain

triglycerides stabilize the structure of MVLs to a different extent, and that the hydrocarbon chain length of the triglyceride and the concentration of the longer versus shorter chain triglycerides determine the rate at which the internal membrane reorganization and coalescence take place during drug release from MVLs (Mantripragada, 2002).

In all kinds of long-chain triglycerides, triolein is always selected, and tricaprylin is always used in short chain triglycerides. To study the effect of the ratio of triolein to tricaprylin on the scutellarin release from MVLs, bre-MVL formulations containing different molar ratio of triolein and tricaprylin with the total molar amount of triglycerides keeping constant were prepared. The varying triglyceride ratios had little effect on the drug encapsulation efficiency of MVL formulations (data not shown).

Fig. 4 shows in vitro release profiles in human plasma at 37 °C for various bre-MVL formulations with different molar ratios of triolein to tricaprylin. Generally, the release rate increased with the increase of the tricaprylin/triolein ratio. Especially, the trends became significant when the tricaprylin/triolein ratio increased from 5/5 to 8/2.

Although in vitro release characteristics for the drug delivery system may not accurately predict and describe the in vivo behavior, it can provide a guide in evaluating as well as screening for various formulations and provide a basis for in vivo studies. As the formulation in which the ratio of triolein/tricaprylin was 10/0

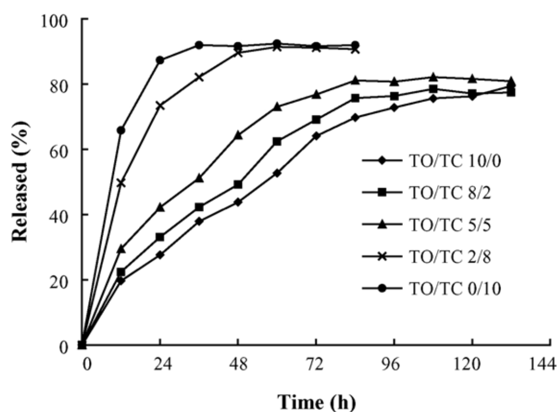

Fig. 4. In vitro release profile of bre-MVLs formulated with different molar ratios of triolein (TO) to tricaprylin (TC). The experiments were repeated three times. The S.D. was found to be less than 3% of the mean values.

showed the best release behavior in vitro, this formulation was selected for the pharmacokinetics study in vivo.

3.3. Pharmacokinetics study

Pharmacokinetics study on the bre-MVL formulation (triolein/tricaprylin, 10/0) was performed in rats via a single intramuscular injection administration at a dose of 40 mg/kg in comparison with those of bre-TL and simple breviscapine solution (BS). Fig. 5 shows the profiles of the scutellarin blood concentration versus time for the three formulations of breviscapine. The pharmacokinetics parameters calculated by Topfit computer program are also shown in Table 1.

The $\text{AUCs}_{0\rightarrow t}$ of bre-MVL and bre-TL were not significantly different when compared with that of BS, although the former was a little larger and the latter was smaller than that of BS. The plasma scutellarin concentration increased rapidly to peak level at 5–15 min after im administration of BS, which indicated the rapid absorption of the free drug in rats via im administration. Both TL and MVL lagged behind BS with regard to the $T_{\max}$.

As for the $C_{\max}$, both bre-MVL and bre-TL were significantly lower than BS, especially, for bre-MVL. The terminal elimination rate constants (k_e) obtained from the two liposomal formulations were both smaller than that obtained from BS. In contrast with TL, the pharmacokinetics parameters of bre-MVL, such as k_e and AUC showed no significant difference, while $T_{\max}$ of bre-MVL was a little delayed.

The most valuable parameter among the results obtained from the pharmacokinetics study was the mean residence time (MRT). The mean $\text{MRT}_{0\rightarrow t}$ of BS was only 1.99 h, which was in agreement with that reported in the literatures (Ge et al., 2003; Jiang et al., 2003; Liu et al., 2003). The short MRT of BS and the chronic property of the diseases treated with breviscapine suggested that it be necessary to develop the sustained-delivery formulation for breviscapine.

A means for drug sustained-delivery is intramuscular injection administration of TL formulation (Arrowsmith et al., 1984; Schreier et al., 1987; Cabanes et al., 1998; Storm et al., 2000). It has been reported that when the particle size of TLs is above 0.1 μm, the liposomes remain at the injection site and act as a sustained drug release system for maintaining prolonged

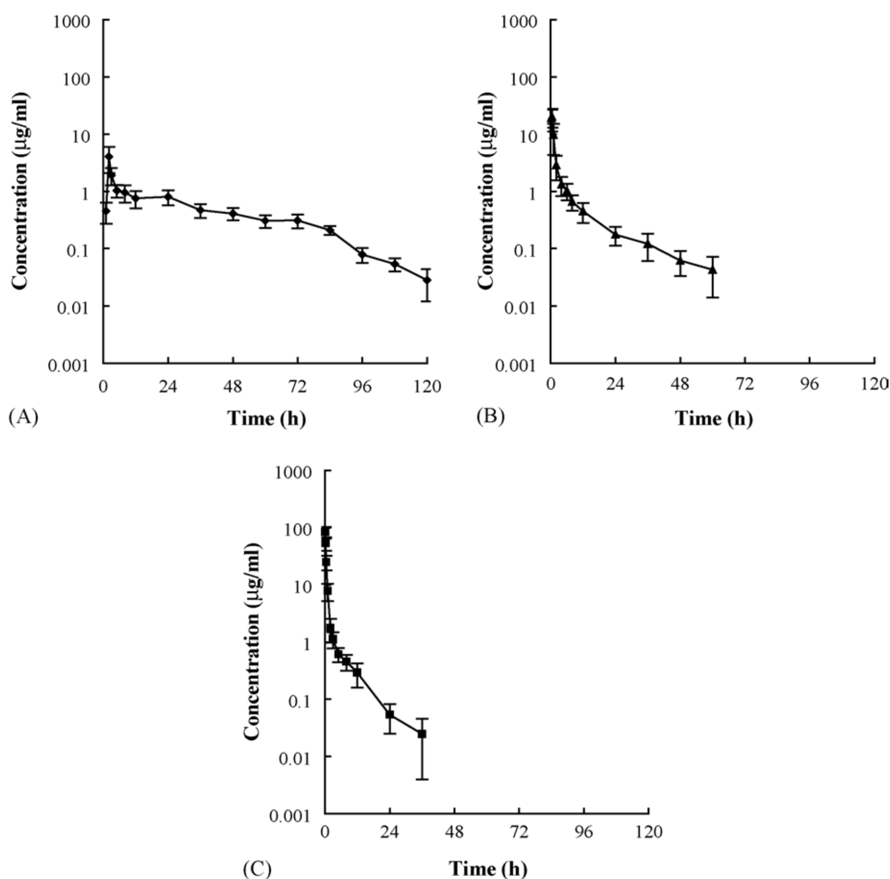

Fig. 5. Mean scutellarin plasma concentration–time profile of (A) bre-MVL (triolein/tricaprylin, 10/0), (B) bre-TL and (C) breviscapine solution (BS) following im administration at a dose of 40 mg/kg. Values were expressed as mean ± S.D. ($n = 6$).

drug concentration in the blood compartment (Storm et al., 2000).

From the results, MRTs of the two lipsomal formulations of breviscapine were significantly prolonged when compared with BS. It seems that both two liposomal formulations can be used to the sustained delivery for breviscapine. However, although the mean $MRT_{0\to t}$ of TL had a 3.29-fold increase compared with that of BS, it was only 6.55 h. Therefore, there is no significant merit for bre-TL in clinic compared

Table 1
Pharmacokinetic parameters of bre-MVL (triolein/tricaprylin, 10/0), bre-TL and breviscapine solution (BS) following im administration at a dose of 40 mg/kg ($n = 6$)

	BS	Bre-TL	Bre-MVL
C_{max} (μg/ml)	84.85 ± 17.36	25.01 ± 5.33	4.27 ± 1.64
T_{max} (h)	0.11 ± 0.07	0.50 ± 0.27	2.33 ± 0.52
k_e (h^{-1})	0.114 ± 0.024	0.042 ± 0.008	0.052 ± 0.008
$AUC_{0\to t}$ (μg h/ml)	46.55 ± 10.65	38.79 ± 9.09	51.27 ± 12.79
$AUC_{0\to\infty}$ (μg h/ml)	46.77 ± 10.73	39.94 ± 9.43	51.86 ± 12.78
$MRT_{(0\to t)}$ (h)	1.99 ± 0.54	6.55 ± 1.26	32.98 ± 1.31
$MRT_{0\to\infty}$ (h)	2.19 ± 0.67	8.78 ± 2.57	34.25 ± 1.9

with the simple injection preparation of breviscapine in market. This motivated us to design a better formulation for breviscapine in order to provide a longer sustained-delivery duration. In contrast with the other injectable sustained–release systems, such as synthetic polymer based microsphere and microcapsule, MVL possesses many advantages, for example, the efficiency of drug encapsulation is high due to its large percent of encapsulated aqueous volume, furthermore, the formulation components are biocompatible. The lipids, used to formulate MVLs, namely phospholipids, cholesterol and triglycerides, are naturally occurring. So the lipids used in the manufacture of MVLs are both biodegradable and biocompatible. It has been reported that the MVLs particle itself does not show any local or systemic toxicity in humans or animals, and there is no foreign body response at the injection site after subcutaneous injection (Katre et al., 1998; Ye et al., 2000). Therefore, MVL technology was utilized to supply the better sustained-delivery for breviscapine. As shown in Table 1, the mean MRT obtained from the pharmacokinetics study of bre-MVL was 33.0 h, which was markedly increased by about 16.6- and 5.04-fold when compared with BS and bre-TL, respectively. The duration of drug delivery in vivo from the MVL formulation can last 4–5 days. MVL provides a depot for the delivery of encapsulated breviscapine over a period of relatively long time in a sustained manner.

It should be noted that there was a 19.9-fold decrease of C_{max} for bre-MVL in comparison with BS and that the general plasma drug level of bre-MVL were markedly lower than that of BS due to the sustained delivery of MVLs. So a larger single dose will be required to maintain the effective blood drug concentration if bre-MVL is applied to the clinic. Because a daily dose of the simple breviscapine injection preparation in market is only 10 mg, a larger single dose for bre-MVL is easy to realize in clinic.

3.4. IVIVC study

The extents of the prolonged duration in the circulation for the two liposomal formulation of breviscapine were consistent with the results obtained from the release in vitro. The drug release in vitro from bre-MVLs was much slower than that from bre-TLs and the much longer duration in vivo for bre-MVLs can also be observed.

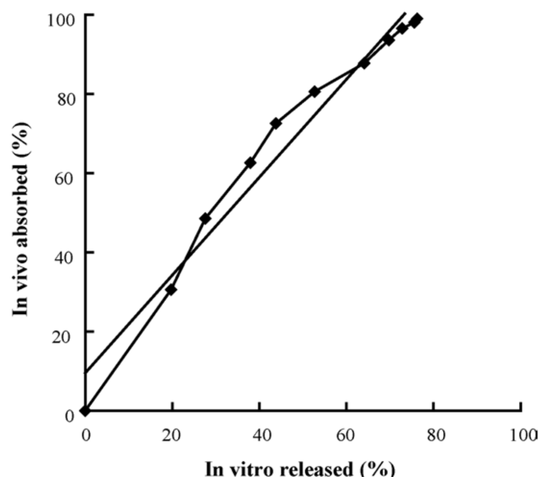

Fig. 6. Correlation of percentage of drug absorbed in vivo and percent drug release in vitro for bre-MVL (triolein/tricaprylin, 10/0).

The in vitro–in vivo correlation (IVIVC) for bre-MVL formulation was explored by comparing the drug absorption in vivo with the release in vitro. The results in Fig. 6 indicated an approximate linear correlation between the percent of absorbed drug in vivo and that of accumulated drug release in vitro. A slope of 1.23 with an intercept of 9.48 ($R = 0.9834$) was obtained. An attempt to correlate in vitro release profile from bre-TLs with in vivo absorption profile was not performed because drug release from the formulation was too fast. Additionally, although there is only one formulation for bre-MVL, an IVIVC is still meaningful since the medium of the release in vitro, human plasma, is used in almost all of the studies on MVLs. However, to our knowledge, there was no report on the IVIVC study of multivesicular liposomes. Our study provides an evidence for the suitability to select human plasma as the medium of drug release from MVLs in vitro.

4. Conclusions

The sustained-delivery durations of bre-MVL both in vitro and in vivo prolonged significantly compared with those of bre-TL. A lipid depot–delivery system, MVLs may be successfully utilized to the sustained delivery for breviscapine. This study provides an attempt and a reference for the research on the dosage form renovation of traditional Chinese medicine

together with its bioactive ingredients. Furthermore, the results from the study of IVIVC preliminarily indicated that it might be feasible to use human plasma as the medium of the release in vitro for MVLs.

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