Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education West China School of Pharmacy, Sichuan University, P.R. China

Enhanced oral bioavailability of salvianolic acid B by phospholipid complex loaded nanoparticles

QIANG PENG, TAO GONG, JIAO ZUO, JIE LIU, DONG ZHAO, ZHIRONG ZHANG

Received February 25, 2008, accepted April 24, 2008

Prof. Zhirong Zhang; Dr. Tao Gong, Key Laboratory of Drug Targeting and drug delivery systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Southern Renmin Road, No. 17, Section 3, Chengdu, 610041, P.R. China
zrzzl@vip.sina.com; gongtaoy@126.com

Pharmazie 63: 661–666 (2008) *doi: 10.1691/ph.2008.8053*

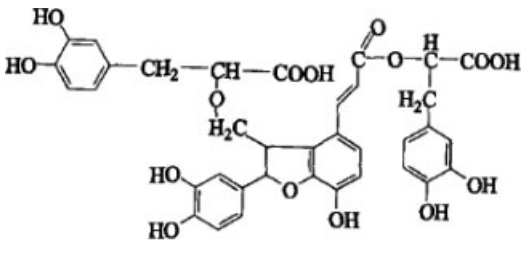
With a simple and feasible method, a salvianolic acid B-phospholipid complex was prepared to increase the absorption of salvianolic acid B in the gastrointestinal (GI) tract. Because the solubility of the complex in water was very poor, the complex was encapsulated into nanoparticles to facilitate its administration. The physicochemical properties of the complex were investigated by differential scanning calorimetry (DSC), infrared scanning, ultraviolet scanning and X-ray diffraction (XRD), and the solubility of salvianolic acid B and the complex in water or n-octanol was measured. The pharmacokinetic characteristics and bioavailability were compared after oral administration of salvianolic acid B (500 mg/kg) and the complex nanoparticles (450 mg/kg equivalent to salvianolic acid B). The results proved the drastic decrease in the solubility of salvianolic acid B in water after successful formation of the drug-phospholipid complex. After oral administration the peak plasma concentration (C_{max}) of salvianolic acid B given by the complex nanoparticles was 3.4 μg/ml which was much higher than that of salvianolic acid B sample ($C_{max} = 0.9$ μg/ml), with T_{max} of 75 min, 45 min and AUC of 664, 257 μg/ml · min, respectively. The relative bioavailability (F) reached 286%.

1. Introduction

Danshen, a well-known traditional Chinese medicine, is the dried roots of *Salvia miltiorrhiza* Bunge, and has been extensively used for the treatment of many kinds of disease, such as ischemic heart disease, heart-stroke, coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, menorrhalgia and insomnia (Tang and Eisenbrand 1992; Lu and Foo 2002; Lin and Chang 2000; Xu et al. 2007). The effective chemical ingredients of *Danshen root* can be classified as lipid-soluble and water-soluble components. Previous research studies were mainly focused on the lipid-soluble components, mainly tanshinones (diterpenoid quinones). In recent years, however, much attention has been paid to the biologically active water-soluble, mainly phenolic compounds (Li et al. 2002), among which salvianolic acid B is one of the primary ingredients. Various pharmacological activities have been reported, such as removal of oxygen free radicals, protection of heart and brain against injuries induced by ischemia-reperfusion, reduction of low density lipoprotein, inhibition of platelet aggregation and protection of liver from fibrosis (Lin et al. 2006; Chen et al. 2001; Tang et al. 2002; Zhang et al. 2004).

In spite of the attractive biological activities, salvianolic acid B presents a poor oral bioavailability in animal bodies (Wu et al. 2006), as far as we are concerned, which may be due to its extremely high water solubility. This disadvantage must be the capital reason limiting its practical use as a potentially active agent. Therefore, the purpose of this study was to reduce the water solubility of salvianolic acid B so that it can be easily absorbed after oral administration with a proper lipid/water distribution coefficient.

Phospholipid, one of the important components of biological cell membrane, plays a very important role in the activities of cells. Considering phospholipid is an endogenetic agent, numerous novel drug carriers based on phospholipids are investigated extensively, such as nanoemulsions, liposomes, micelles, solid lipid nanoparticles, niosomes, lipid microbubbles, etc. (Rabinovich-Guilat et al. 2005; Duzgunes 2001). Salvianolic acid B combining with phospholipid would be a rational method to regulate the lipid/water distribution coefficient due to the am-



Salvianolic acid B

phiphilic character of phospholipids. With this method, the therapeutic efficacy of some molecules, such as silybin and curcumin which have poor oral bioavailability, could be enhanced due to increasing oral absorption (Xiao et al. 2006; Kuntal et al. 2007).

2. Investigations, results and discussion

2.1. Characterization of salvianolic acid B-phospholipid complex

2.1.1. Differential scanning calorimetry (DSC)

For the characterization of the complex, DSC was employed. The samples of salvianolic acid B, phospholipids, the salvianolic acid B-phospholipid complex or the physical mixture of salvianolic acid B and phospholipids were sealed in the aluminum crimp cell, and then heated at a speed of 10 °C/min from 0 °C to 300 °C under nitrogen atmosphere. Peak transition top temperatures of the four samples were determined and compared.

In DSC, a possible interaction is indicated by elimination of endothermic peaks, appearance of new peaks, changes in peak shape and its onset or peak temperature and relative peak area or enthalpy (Kuntal et al. 2007). Fig. 1 shows the DSC curves of salvianolic acid B sample (A), phospholipids (B), the salvianolic acid B-phospholipid complex (C) and the physical mixture of salvianolic acid B and phospholipids (D). Salvianolic acid B sample shows three endothermic peaks with maximum points at 52.1 °C, 121.8 °C and 169.4 °C respectively. The melting event recorded at 169.4 °C was generated by free salvianolic acid B because its melting point is around this temperature, and the others might be due to the minority of the impurities contained in salvianolic acid B sample. Phospholipids show two different endothermic peaks, the first one (139.2 °C) is mild due to the hot movement of phospholipids polar head groups. However, the second peak (238.2 °C) appears very sharp, which is owing to the phase transition from gel state to liquid crystal state, and the non-polar carbon-hydrogen tail in phospholipids perhaps happened to be melt, isomerous or the crystal changes during this period. The complex exhibits a broad peak at 72.2 °C, which is different from the peaks of salvianolic acid B and phospholipids. The peaks of free salvianolic acid B and the phospholipids disappear in the thermogram of the complex and the phase transition temperature decreases compared to phospholipids. The physical mixture of salvianolic acid B and phospholipids shows a peak at 86.2 °C and a fluctuation at 169.4 °C. The former is similar to the peak of the complex (72.2 °C), and the latter has the same temperature with the peak temperature of free salvianolic acid B. This might be attributed to the fact that when the temperature increases phospholipids melt and salvianolic acid B is then dissolved in the phospholipids and partly forming the complex. According to Venema and Weringa (1988) and Lasonder and Weringa (1990), there might be some interaction between salvianolic acid B and phospholipids, such as the formation of hydrogen bonds or van der Waals forces. After the interaction between the phenolic hydroxyl group of salvianolic acid B and the polar parts of the phospholipids molecule, the carbon-hydrogen tail of phospholipids could turn freely and their polar head resulting in a decrease of the sequence of the phospholipids hydrocarbon chains and the disappearance of the second endothermal peak of phospholipids and lowering the phase transition temperature.

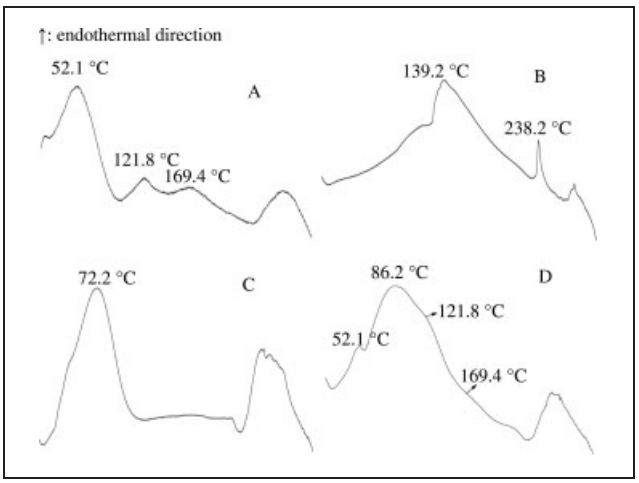


Fig. 1: DSC thermograms of salvianolic acid B sample (A), phospholipids (B), the salvianolic acid B-phospholipid complex (C) and the physical mixture of salvianolic acid B and phospholipids (D)

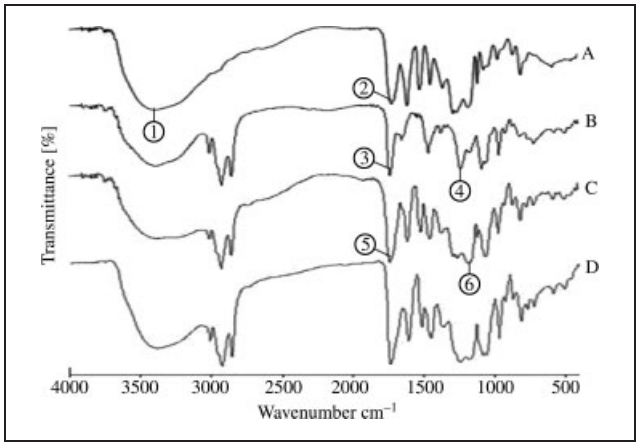


Fig. 2: FTIR spectras of salvianolic acid B sample (A), phospholipids (B), the salvianolic acid B-phospholipid complex (C) and the physical mixture of salvianolic acid B and phospholipids (D). The wavenumber of the peaks of 1–6 are 3385.93, 1718.74, 1734.34, 1236.18, 1734.80 and 1180.90 cm^{-1} respectively

2.1.2. Infrared scanning

Fourier transform infrared spectroscopy (FTIR) samples were obtained by grinding the 4 kinds of samples into KBr and compressing under vacuum. The pellets were used to record the FTIR spectra in a Nicolet FTIR 20SXB. The wavenumber range was from 400 to 4000 cm^{-1}.

FTIR analysis of salvianolic acid B, phospholipids, salvianolic acid B-phospholipid complex and the physical mixture of salvianolic acid B and phospholipids is reported in Fig. 2. It is shown that the peak of phenolic hydroxyl group of salvianolic acid B at 3386 cm^{-1} (A-1) is broad. After physically mixed with phospholipids the shape or wavenumber of this peak is almost unchanged (D). After the formation of the complex, however, the shape of this peak is much broader (C). It is considered that there must be some interactions between phenolic hydroxyl group of salvianolic acid B and phospholipids. The peak of P=O of phospholipids is at 1236 cm^{-1} (B-4). After physically mixed with salvianolic acid B this peak is not shifted. In the complex, however, this peak is at 1181 cm^{-1} (C-6) which obviously moves to lower wavenumbers. It is as-

sumed that the phenolic hydroxyl group of salvianolic acid B and the P=O in phospholipids are involved in the interaction, such as the formation of hydrogen bonds or van der Waals forces. The peak of C=O of phospholipids at 1734 cm^{-1} (B-3) is similar to that of the physical mixture (D) and the complex (C-5), which indicates that the group of C=O of phospholipids might be not involved in the formation of the complex.

2.1.3. X-ray diffraction (XRD)

The X-ray diffraction was recorded on a D/max-ra X-ray diffractometer (Rigaku, Japan). The CuKα radiation was employed to be X-ray source. Spectra of graphs were plotted from 2θ of 0.00 to 60.00° with a step width of 0.02° and step time of 0.4 s at room temperature. The dried samples of certain weight were added into the slide for packing prior to X-ray scanning.

Fig. 3 presents the XRD graphs of salvianolic acid B, phospholipids, salvianolic acid B-phospholipid complex and the physical mixture of salvianolic acid B and phospholipids. No crystalline peak appears on the XDR graph of salvianolic acid B, which indicates that salvianolic acid B exists in a non-crystalline state. Phospholipids show obvious diffraction peaks at about 4°, 6°and 7.5° indicating its crystalline characteristics. There is still an apparent diffraction peak at 7.5° after physically mixing salvianolic acid B and phospholipids. However the diffraction peak disappears in the graph of the complex. It is presumed that during the period of forming the complex, a directional combination happened between salvianolic acid B and the polar parts of phospholipids. Therefore salvianolic acid B and phospholipids actually stay in a highly dispersed state, resulting in the masking of their own crystalline characteristics.

2.1.4. Ultraviolet scanning

The 4 samples dissolved in ethanol were used for UV spectra analysis which was recorded on a CARY 100 Conc UV-Visible spectrophotometer (VARIAN, U.S.A.). The wavelength range was from 200 nm to 400 nm.

Fig. 4 shows the ultraviolet scanning spectra of salvianolic acid B, phospholipids, salvianolic acid B-phospholipid complex and the physical mixture of salvianolic acid B and phospholipids. There is no absorbance for phospholipids in the range of 250–400 nm. The spectra of salvianolic acid B, the complex and the physical mixture are similar, in which absorbance peaks appear at about 254 nm and 286 nm. It is concluded that the structure of chromophore of salvianolic acid B is not changed after forming the complex with phospholipids.

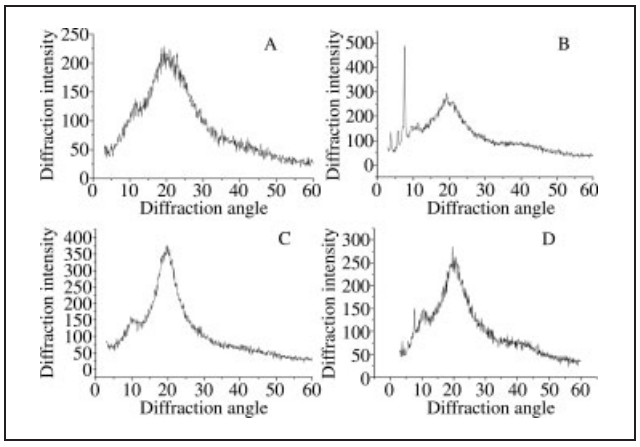


Fig. 3: XDR graphs of salvianolic acid B sample (A), phospholipids (B), the salvianolic acid B-phospholipid complex (C) and the physical mixture of salvianolic acid B and phospholipids (D)

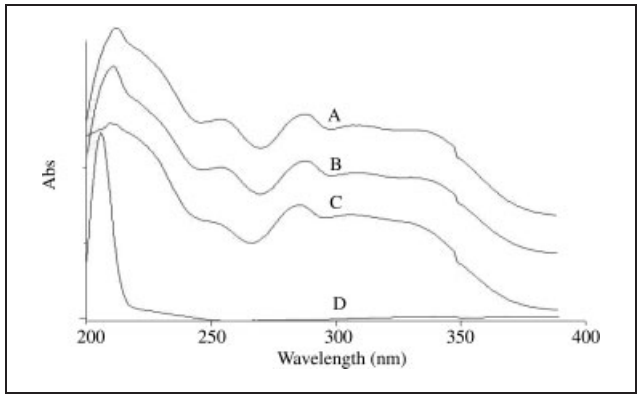


Fig. 4: Ultraviolet scanning spectras of the salvianolic acid B-phospholipid complex (A), salvianolic acid B sample (B), the physical mixture of salvianolic acid B and phospholipids (C) and phospholipids (D)

2.1.5. Solubility and content determination

Table 1 presents the solubility of salvianolic acid B, the phospholipid complex and the physical mixture of salvianolic acid B and phospholipids in water and n-octanol. It is shown that the solubility of salvianolic acid B in water is a little stronger than that in n-octanol. But the solubility of the phospholipid complex in n-octanol is about 175 times higher than that in water. The content of salvianolic acid B in the phospholipid complex was 23.36 ± 0.18% (w/w) (values are mean ± S.D., n = 3).

2.2. Characterization of the complex nanoparticles

Once the solubility of the complex in water was very poor, complex nanoparticles were prepared (see section 3.6.) to facilitate its administration.

An aliquot of the prepared lyophilized nanoparticles powder was re-dispersed in deionized water. The mean particle size analysis was recorded with Malvern zetasizer Nano ZS90 (Malvern instruments Ltd., UK) at 25 °C.

The morphology of nanoparticles was observed with a scanning electron microscope (SEM) (JSM–5900 LV, JEOL, Japan) at an accelerating voltage of 20 kV. One drop of the nanoparticles suspension was placed on a gra-

Table 1: Solubility of salvianolic acid B, the complex and the physical mixture in water or n-octanol at room temperature

Samples	Solubility in water[a]	Solubility in n-octanol (mg/ml)[a]
Salvianolic acid B	447.98 ± 0.99 mg/ml	263.02 ± 1.80
Salvianolic acid B-phospholipids complex	516.58 ± 4.18 μg/ml	90.32 ± 1.83
Physical mixture of salvianolic acid B and phospholipids	1.79 ± 0.01 mg/ml	138.83 ± 0.80

[a] Values are mean ± S.E.M., n = 3

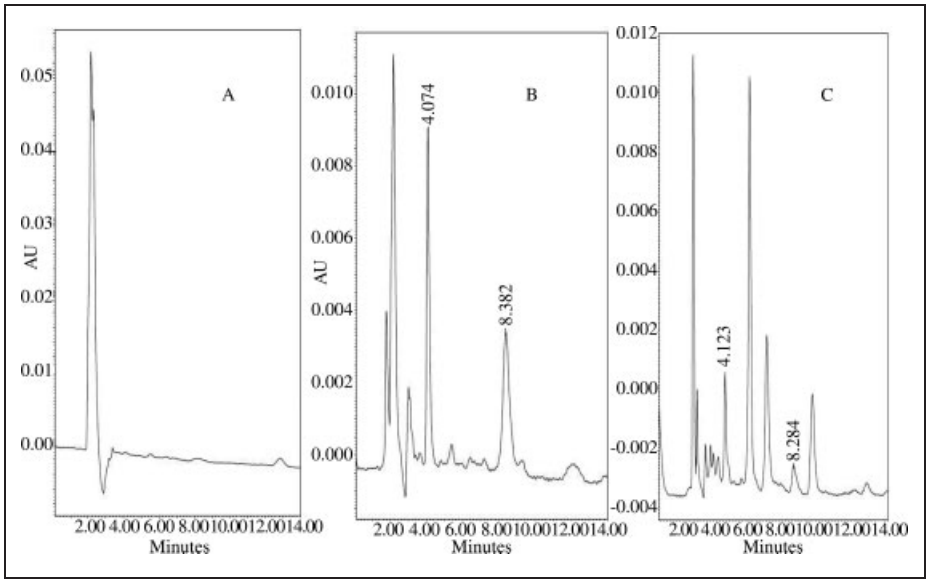


Fig. 5:
Typical chromatograms of salvianolic acid B: Blank plasma (A); blank plasma spiked with salvianolic acid B and internal standard (B); a sample after oral administration of the complex nanoparticles (C). (tR about 4.1 min: internal standard; tR about 8.3 min: salvianolic acid B)

phite surface. After dryness, the sample was coated with gold using an Ion Sputter.

The value of the mean particle size of nanoparticles was 206 ± 3 nm[a] with the polydispersity index of 0.144 ± 0.015[a]. The morphology image observed by SEM shows that the nanoparticles were sphere-like in shape. The value of the encapsulation efficiency (EE) (calculated as the following equation) was $86.07 \pm 0.13\%$[a]. The content of salvianolic acid B in nanoparticles was $6.36 \pm 0.20\%$[a] (w/w). The prepared nanoparticles were fit for oral administration. ([a] values are mean $\pm$ S.D., $n = 3$)

EE = (real amount of drug in nanoparticles/theoretical amount of drug in nanoparticles) $\times$ 100

2.3. Bioavailability studies in rats

The concentration of salvianolic acid B in rat plasma was determined using HPLC with ultraviolet detection. The typical chromatograms of salvianolic acid B are shown in Fig. 5 from which we can see that salvianolic acid B and internal standard p-hydroxybenzoic acid could be well separated from other ingredients by this HPLC system. Calibration curves ranging from 0.503–5.03 μg/ml were linear ($r = 0.9940$). The recoveries of salvianolic acid B of high, middle and low concentrations were 81.14, 86.07 and 83.56%, respectively. The inter-days R.S.D. were 2.8, 7.9 and 5.9% respectively, and the intra-days R.S.D. were 3.8, 3.1 and 8.2%, respectively. The lowest detection limit was 0.2 μg/ml.

Fig. 6 shows the plasma concentration-time curve of salvianolic acid B in rats after oral administration of salvianolic acid B (500 mg/kg) and the complex nanoparticles (450 mg/kg equivalent to salvianolic acid B). The main pharmacokinetic parameters, listed in Table 2, were calculated by DAS pharmacology software package (Drug And Statistics, Anhui, China). The peak plasma concentration (C_{max}) of 0.90 ± 0.14 μg/ml was obtained after oral administration of salvianolic acid B with T_{max} of about 45 min. As for the complex nanoparticles, however, at the T_{max} of about 75 min the C_{max} is 3.41 ± 0.28 μg/ml which is much higher than that of salvianolic acid B even though the dose was lower. It is also shown that the complex nanoparticles stayed for a longer time in rats than salvianolic acid B with a higher plasma concentration. The pharmacokinetic data show that two compartment

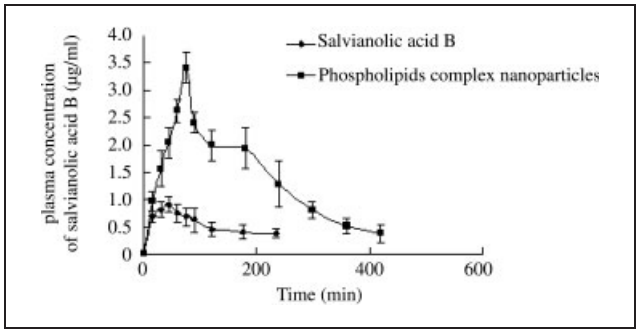


Fig. 6: Mean plasma concentration-time curve of salvianolic acid B in rats after oral administration of salvianolic acid B (500 mg/kg) and the complex nanoparticles (450 mg/kg equivalent to salvianolic acid B) ($n = 5$)

model and one-order absorption were fitted for both salvianolic acid B and the complex nanoparticles plasma concentration-time course in rats. The relative bioavailability (F) (calculated as the following equation) of the complex versus salvianolic acid B was about 286%.

$$F = (AUC_{complex}/dose)/(AUC_{salvianolic\ acid\ B}/dose)$$

This enhanced bioavailability might be attributed to drastically decreasing the water-solubility of salvianolic acid B by forming the phospholipids complex, as a result, the lipophilicity was increased. In addition, as an important component of biomembrane, phospholipid improved the biocompatibility of the drug from which the absorption of salvianolic acid B in GI tract was beneficial. In further

Table 2: Main pharmacokinetic parameters after administration of salvianolic acid B and nanoparticles of the complexx ($n = 5$)

Parameters	Salvianolic acid B	Nanoparticles of the complex
C_{max} (μg/ml)[a]	0.9022 ± 0.1391	3.4113 ± 0.2779
T_{max} (min)	45	75
AUC (μg/ml · min)	257.421	664.382
CL/F (L/min/kg)	1.942	0.677

[a] Values are mean $\pm$ S.E.M., $n = 5$

studies, we will investigate the absorption machanism of salvianolic acid B through GI tract.

3. Experimental

3.1. Materials

The phospholipid (soybean lecithin) was purchased from Shanghai Tai-wei Pharmaceutical Co. Ltd. (China). Salvianolic acid B was extracted by ourselves, with a purity higher than 85% by HPLC and by which salvianolic acid B could be well separated from impurities. Salvianolic acid B standard (purity higher than 98% by HPLC) was provided by Zhongxin Innova Laboratories (Tianjin, China). Poloxamer188 was provided by Nanjing Well Chemical Co., Ltd. (China). All the other chemical reagents were of analytical grade or better.

3.2. Animals

Healthy male Sprague-Dawley rats (210–280 g) were purchased from the Laboratory Animal Center of Sichuan University, (Chengdu, P.R. China). Prior to use, the rats were breeded in a temperature and humidity controlled animal observation room (25 °C, 55% air humidity) with free access to water and standard rat chow. All experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University.

3.3. Preparation of the salvianolic acid B-phospholipid complex

At a weight ratio of 1 : 2, about 1.5 g of salvianolic acid B and 3.0 g of phospholipids were taken in a 250 ml round bottom flask and dissolved in 100 ml acetic ether. The mixture was stirred at room temperature for 2 h resulting in a clear solution. After acetic ether was evaporated off under vacuum at 45 °C, 30 ml of dichloromethane was added under continuous stirring for 20 min in order to precipitate the free salvianolic acid B. Another clear solution was gained with a little precipitate, after filtration the filtrate was evaporated under vacuum at 30 °C for more than 30 min to remove solvent off. The resultant salvianolic acid B-phospholipid complex was sealedly kept in a desiccator at room temperature. The same as the complex, the weight ratio of salvianolic acid B to phospholipids in physical mixture was 1 : 2.

3.4. Determination of salvianolic acid B content in the complex

The content determination of salvianolic acid B in the complex was carried out as follows. Approximately 5 mg of the complex were dissolved in 10 ml ethanol, and 10 μl aliquot of the resultant solution was injected into an HPLC system.

3.5. Solubility studies

Determination of solubility characteristics of salvianolic acid B, the phospholipid complex and the physical mixture of salvianolic acid B and phospholipids was carried out by adding excess of above-mentioned samples to a certain volume of water or n-octanol in sealed glass containers. The liquids were pendulated for 24 h at room temperature and centrifuged at 4500 rpm for 10 min. The obtained supernatant of 100 μl aliquot was diluted with water or methanol at different times according to the estimated concentration of each sample so that the resultant concentration of each solution was fit for the linear range. A 10 μl aliquot of the resultant solution was injected into the HPLC system.

3.6. Preparation of nanoparticles of the salvianolic acid B-phospholipid complex

About 3.01 g of the salvianolic acid B-phospholipid complex was dissolved in 25 ml of ethanol, and the solution was quickly injected into the 110 ml stirring aqueous solution containing appropriate amount of poloxamer 188 (1.0%, w/v), and then the ethanol was completely removed under vacuum at 45 °C, resulting in a homogeneous suspension. After high pressure homogenization, 5.5 g of sucrose was dissolved in the suspension. The mixture was frozen at −50 °C for more than 5 h and then lyophilized for 24 h. The lyophilized powder was obtained and stored in the desiccator until use.

3.7. Encapsulation efficiency of nanoparticles

A 4 ml aliquot of the nanoparticles suspension was ultracentrifuged for 3 h at 4 °C at 50,000 rpm by a Himac CP60E (Hitachi Koki Co., Ltd. Tokyo, Japan). Then the supernatant was removed, and the nanoparticle precipitates were dissolved and diluted with ethanol by 100 times. A 10 μl aliquot of the diluted solution was injected into the HPLC system.

3.8. Determination of salvianolic acid B content in the nanoparticles

Approximately 10 mg of the nanoparticles were dissolved in 10 ml ethanol, and a 10 μl aliquot of the resultant solution was injected into the HPLC system.

3.9. Bioavailability studies in rats

3.9.1. Calibration curves and method validation

Different volume of salvianolic acid B standard stocking solution and 30 μl of internal standard (p-hydroxybenzoic acid) stocking solution were added to the 1 ml centrifuge tube. Methanol was used as the solvent of both stocking solution. After methanol was removed under air flow at 30 °C, 100 μl of blank plasma and one drop of 1 M HCL were added. The resultant concentrations of salvianolic acid B were 0.503, 1.006, 2.012, 3.018, 4.024 and 5.03 $\mu g \cdot ml^{-1}$. These calibrations were subjected to the whole analytical procedure so that the linearity, precision and accuracy of the method could be tested.

3.9.2. Sample preparation

The rats were anaesthetized by ether, and 250 μl blood was collected from the caudal veins and put into the 1 ml centrifuge tube coated with heparin. The plasma separated by centrifuging (10 min, 3500 rpm) was stored at −20 °C until use.

30 μl of internal standard stocking solution was added to the 1 ml centrifuge tube and methanol was removed under air flow at 30 °C. Then 100 μl of blank plasma and one drop of 1 M HCL were added, the mixture was agitated for 30 s. After addition of 350 μl acetic ether the mixture was shaken for 1 min and centrifuged at 1×10^4 rpm for 1 min and the supernatant was separated. After repetition of the extracting process one more time, the supernatant was combined and evaporated to dryness under air flow at 30 °C. The residue was dissolved in 60 μl double-distilled water by agitating for 3 min and centrifuged at 1×10^4 rpm for 1 min. Aliquots (20 μl) of the supernatant were injected for HPLC analysis.

3.9.3. Pharmacokinetic study of salvianolic acid B and the phospholipid complex in rats

Ten Sprague-Dawley male rats (body weight 210–280 g) were divided into two groups and fasted for 12 h, but had free access to water. One group was for oral adminstration of salvianolic acid B at a dose of 500 mg/kg and the other group was for adminstration of the complex nanoparticles at a dose equivalent to 450 mg/kg of salvianolic acid B. For the adminstration of the complex nanoparticles, a lower dose was adopted because under the same diluting folds a higher concentration made it difficult to form the steady suspension after re-dispersing nanoparticles powder in water.

3.10. HPLC analysis

The HPLC system used in this study consisted of a Waters 2690 separation module and a 996 Photodiode Array (PDA) detector; and data were collected and processed using Millennium software version 3.2 (all equipment from Waters, Milford, MA, USA). The stationary phase, Diamonsil C_{18} column (200 mm × 4.6 mm, 5 μm) plus a Shimadzu Shim-pack G guard column (C_{18}, 10 mm × 4 mm, 5 μm) (Chiyoda-Ku, Tokyo, Japan), was kept at 35 °C. The mobile phase consisted of acetonitrile, methanol, and 0.1% phosphoric acid at a ratio of 25 : 10 : 65, and was newly prepared and filtered through a 0.22 μm membrane filter before use and degassed via an online degasser. The flow rate was 1.0 ml/min, and a wavelength of 286 nm was used for detection.

References

Chen YH, Lin SJ, Ku HH, Shiao MS, Lin FY, Chen JW, Chen YL (2001) Salvianolic acid B attenuates VCAM-1 and ICAM-1 expression in TNF-treated human aortic endothelial cells. J Cell Biochem 82: 512–521.

Duzgunes N (2001) Lipid assemblies for drug delivery. Adv Drug Deliv Rev 47: 137–138.

Kuntal M, Kakali M, Arunava G, Bishnu PS, Pulok KM (2007) Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm 330: 155–163.

Lasonder E, Weringa WD (1990) An NMR and DSC study of the interaction of phospholipid vesicles with some anti-inflammatory agents. J Colloid Interface Sci 139: 469–478.

Li HB, Lai JP, Jiang Y, Chen F (2002) Preparative isolation and purification of salvianolic acid B from the Chinese medicinal plant *Salvia miltiorrhiza* by high-speed counter-current chromatography. J Chromatogr A 943: 235–239.

Lin HC, Chang WL (2000). Diterpenoids from Salvia miltiorrhiza. Phytochemistry 53: 951.

Lin YH, Liu AH, Wu HL, Westernbroek C, Song QL, Yu HM, Horst GJT, Li XJ (2006) Salvianolic acid B, an antioxidant from Salvia miltiorrhiza, prevents $A\beta_{25-35}$-induced reduction in BPRP in PC12 cells. Biochem Biophys Res Commun 348: 593.

Lu YR, Foo LY (2002) Polyphenolics of Salvia - a review. Phytochemistry 59: 117–140.

Rabinovich-Guilatt L, Dubernet C, Gaudin K, Lambert G, Couvreur P, Chaminade P (2005) Phospholipid hydrolysis in a pharmaceutical emulsion assessed by physicochemical parameters and a new analytical method Eur J Pharm Biopharm 61: 69–76.

Tang MK, Ren DC, Zhang JT, Du GH (2002) Effect of salvianolic acids from Radix Salviae miltiorrhizae on regional cerebral blood flow and platelet aggregation in rats. Phytomedicine 9: 405–409.

Tang W, Eisenbrand G (1992) Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer, Berlin, pp. 891–902.

Venema FR, Weringa WD (1988) The interactions of phospholipid vesicles with some anti-inflammatory agents. J Colloid Interface Sci 125: 484–500.

Wu YT, Chen YF, Hsieh YJ, Jaw I, Shiao MS, Tsai TH (2006) Bioavailability of salvianolic acid B in conscious and freely moving rats. Int J Pharm 326: 25–31.

Xiao YY, Song YM, Chen ZP, Ping QN (2006) The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm 307: 77–82.

Xu J, Tan TW, Janson IC (2007) One-step simultaneous purification of three water-soluble constituents in crude extracts from Danshen by adsorption chromatography on oligo-β-cyclodextrin substituted agarose gel media, Process Biochem 42: 480–485.

Zhang Y, Akao T, Nakamura N, Duan CL, Hattori M, Yang XY, Liu JX (2004) Extremely low bioavailability of magnesium lithospermate B, an active component from Salvia miltiorrhiza, in rat. Plant Med 70: 138–142.