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Enhanced oral bioavailability of breviscapine after encapsulation in a liposomal formulation

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This report firstly describes the pharmacokinetic study of liposomal breviscapine (LB) after oral administration in rats. The mean C_{max} and $AUC_{(0\rightarrow t)}$ of LB were 3.3 and 3.1-fold higher than those of breviscapine solution (BS). The oral absorption of breviscapine was significantly increased after encapsulation in the liposomal formulation.

Breviscapine, which is mainly composed of scutellarin (a flavone glucuronide), is a well-known bioactive constituent extracted from the traditional Chinese medicine *Erigeron breviscapus* (Vant.) Hand.-Mazz. Preparations of breviscapine such as injections, tablets etc., are extensively used to treat ischemic cerebrovascular and cardiovascular diseases in China (Chen and Jin 1997; Wang 1999). However, the oral bioavailability of breviscapine was found to be quite poor in rats (Zhong et al. 2003). To increase its oral bioavailability, a liposomal formulation of breviscapine was developed.

The use of liposomes in oral drug delivery has been widely studied and the oral bioavailabilities of some drugs which are difficult to be absorbed are improved by encapsulation into liposomes (Guo et al. 2001; Hassan et al. 1998; Kisel et al. 2001; Lian and Ho 2001; Rogers and Anderson 1998; Woodley 1985). Liposomes may be used as a solubilizing or suspension agents for insoluble drugs, and may potentially enhance the ability of a drug to be absorbed across biological membrane, thus resulting in the improvement of oral bioavailability (Lian and Ho 2001).

In order to assess the viability of the liposomal formulation as a means of increasing the oral bioavailability of breviscapine, pharmacokinetics of liposomal breviscapine (LB) compared with breviscapine solution (BS) in rats was investigated using an improved HPLC method. As the content of scutellarin in breviscapine was more than 90% (Chen and Jin 1997), scutellarin in rat plasma was determined. Scoparone (6,7-dimethoxycoumarin), a commercially available product, was used as the internal standard. Because the retention time of scoparone was only 11.2 min (8.9 min for scutellarin), the analytic time was shortened in comparison with Zhong's method (Zhong et al. 2003). The method was validated for linearity, precision, accuracy, stability etc.

Plasma concentration-time profiles of scutellarin of LB and BS are shown in the Figure, and the relevant pharmacokinetic parameters of LB and BS are listed in the Table.

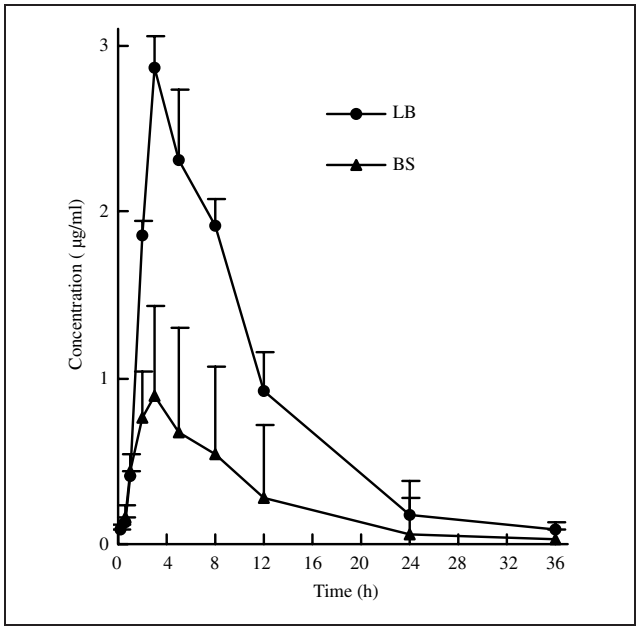

Fig.: Mean scutellarin plasma concentration-time profile of liposomal breviscapine (LB) and breviscapine solution (BS) following oral administration at a dose of 100 mg/kg. Values were expressed as mean + SD (n = 8)

Table: Pharmacokinetic parameters of liposomal breviscapine (LB) and breviscapine solution (BS) following oral administration at a dose of 100 mg/kg (n = 8)

Parameters	LB	BS
C_{max} (μg/ml)	$2.931 \pm 0.320^*$	0.896 ± 0.628
T_{max} (h)	3.50 ± 0.93	3.25 ± 0.71
k_e (1/h)	0.113 ± 0.007	0.103 ± 0.019
$t_{1/2}$ (h)	6.18 ± 0.37	6.97 ± 1.56
$AUC_{(0\rightarrow t)}$ (h · μg/ml)	$28.82 \pm 3.84^*$	9.19 ± 6.79
$AUC_{(0\rightarrow \infty)}$ (h · μg/ml)	$29.58 \pm 3.98^*$	9.59 ± 6.87
$MRT_{(0\rightarrow t)}$ (h)	8.83 ± 0.37	8.50 ± 0.78
$MRT_{(0\rightarrow \infty)}$ (h)	9.73 ± 0.49	10.3 ± 1.6

Each Value was expressed as mean ± SD (n = 8). *: $P < 0.001$

The statistical analysis of data using Student's t-test revealed that the pharmacokinetic parameters, t_{max}, k_{el}, $t_{1/2}$, MRT, showed no significant differences ($p > 0.05$) between LB and BS. However, the C_{max} and $AUC_{(0\rightarrow t)}$ of LB showed a highly significant difference ($p < 0.001$) when compared with those of BS. The C_{max} and $AUC_{(0\rightarrow t)}$ of LB were 2.931 ± 0.320 μg/ml and 28.82 ± 3.84 h · μg/ml, while those of BS were 0.896 ± 0.628 μg/ml and 9.19 ± 6.79 h · μg/ml, respectively. The mean C_{max} and $AUC_{(0\rightarrow t)}$ of LB were 3.3 and 3.1 times as great as those of BS, respectively. The results indicated that the oral bioavailability of breviscapine was enhanced by the liposomal formulation.

The reasons for the improved oral bioavailability of breviscapine after encapsulation into liposomes may be that liposomes may potentially enhance the drug's biological membrane permeability (Guo et al. 2001; Lian and Ho 2001) and that liposomes may increase the solubility of scutellarin in the gastrointestinal tract. Our previous study on the physico-chemical characteristics of scutellarin showed that scutellarin is a weak acid, and its solubility is markedly affected by the pH of the environment. Under the acid condition in the stomach (pH 1–3.5) and the weak acid condition of the small intestine (pH 5–7)

(Gruber et al. 1987), the aqueous solubility of scutellarin is low. Because liposomes may be used as a solubilizing or suspension agent for insoluble drugs (Lian and Ho 2001), the solubility of scutellarin in the gastrointestinal tract was increased after oral administration of liposomal breviscapine.

In conclusion, the encapsulation of breviscapine in a liposomal formulation can lead to improved oral absorption. Considering that phospholipids, as components of both the liposomes and cell membranes, are biocompatible, and that the ethanol injection method used in the preparation of liposomes is easy to scale-up (Naeff 1996; Wagner et al. 2002), the encapsulation of breviscapine into liposomes may be a promising means for the improvement of the oral bioavailability of breviscapine.

Experimental

1. Materials

Breviscapine (scutellarin content, 91.7%) was obtained from Yunnan Wanfang Pharmaceutical Co., Ltd. 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) was a gift kindly provided by Degussa BioActives Deutschland GmbH Co. KG (Germany). Cholesterol was of analytical grade from Tianjin Chemical Reagent Co., Inc. Tetrahydrofuran and acetonitrile were of chromatographic grade from Tianjian Concord Sci. Tech Co., Ltd. All other chemicals were of analytical grade except specified.

2. Preparation of breviscapine solution (BS) and liposomal breviscapine (LB)

Breviscapine was dissolved in distilled water by pH adjustment with 0.1 M NaOH. Sucrose (10%, w/v) was added to isosmotic concentration. Then BS was obtained. LB was prepared by the ethanol injection method with some modification (Batzri and Korn 1973; Pons et al. 1993), which is easy to scale-up (Naeff 1996; Wagner et al. 2002). Briefly, PC (400 mg) and cholesterol (100 mg) were dissolved in 2 ml of warm absolute ethanol. 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 $50 \pm 1\,^{\circ}C$ to get a liposome suspension. Then this suspension was extruded three times through 0.45 μm pores using a 10 ml thermobarrel extruder (Northern Lipids Inc, Vancouver, Canada). The final volume was adjusted to 10 ml by addition of distilled water, to yield LB with an approximate breviscapine concentration of 10 mg/ml. The mean particle size of liposomes was approximately 0.34 μm, and the entrapment efficiency was determined to be $87.7 \pm 2.6\%$.

3. Chromatographic conditions

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.

4. Sample preparation

To a 200 μl aliquot of rat plasma, 50 μl of scoparone solution (20 μg/ml) and 100 μl of phosphoric acid (1 M) were added. 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.

5. Pharmacokinetic study

Wistar rats (female and male, 230–270 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University. The carotid artery of each rat was cannulated 2 days before the experiment. The rats were fasted overnight prior to the study, but had access to water *ad libitum*. Two groups of eight rats each were treated with LB and BS at a dose of 100 mg/kg by oral gavage, respectively. Blood samples (0.5 ml) were collected from the catheter placed in the carotid artery at 0.17, 0.5, 1, 2, 3, 5, 8, 12, 24 and 36 h after oral 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. Plasma concentrations versus time data were analyzed by a non-compartment model using the Topfit 2.0 computer program (Thomae GmbH, Germany). $AUC_{0\rightarrow t}$ was calculated by the trapezoidal rule.

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