International Journal of Pharmaceutics 359 (2008) 118–122

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

# Comparative pharmacokinetics of breviscapine liposomes in dogs, rabbits and rats

Wenli Lv [a], Jianxin Guo [b], Qineng Ping [a,*], Yunmei Song [a], Jin Li [a]

[a] School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
[b] Technology Department, Talecris Biotherapeutics, Clayton, NC 27520, USA

ARTICLE INFO

*Article history:*
Received 28 October 2007
Received in revised form 10 March 2008
Accepted 27 March 2008
Available online 11 April 2008

*Keywords:*
Breviscapine
Scutellarin
Liposomes
Comparative pharmacokinetics
Allometric equation
RP-HPLC

ABSTRACT

*Aim:* To compare pharmacokinetics of intravenous breviscapine liposomes in Beagle dogs, rabbits and rats.
*Methods:* Six Beagle dogs, 6 rabbits and 12 rats were intravenously administrated with breviscapine liposomes and commercial injection (breviscapine solution) by the crossover design (two periods), respectively. Plasma concentration of scutellarin, the main active component in breviscapine, at different time intervals was determined by reverse phase-HPLC. The pharmacokinetic parameters including area under the curve (AUC), clearance ($CL_{(s)}$) and volume of distribution ($V_{(c)}$) were calculated.
*Results:* The plasma concentration–time profiles were fitted to a two-compartment model. Breviscapine liposomes exhibited significant difference from injection in $CL_{(s)}$ and AUC, examined by a one-way analysis of variance (ANOVA). With regard to both breviscapine liposomes and commercial injection (free breviscapine), the logarithm values of AUC, $CL_{(s)}$ and $V_{(c)}$ were all related to the logarithm of the body weight by the allometric equation: $Y = a \times W^b$.
*Conclusion:* The allometric equation might be applied to extroplate dosage for human from animal data and also for dosage adjustment of breviscapine liposomes in order to achieve same AUC as commercial injection. Compared with the breviscapine solution, breviscapine liposomes delivered more scutellarin into the plasma.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

Breviscapine is a flavonoid extracted from Chinese herb *Erigerin breviscapus* (Vant.) Hand-Mazz. Scutellarin is a flavone glycoside. It is the primary active ingredient of breviscapine (Zhang et al., 2000). In China, breviscapine products are extensively used in clinic for the treatment of cerebrovascular diseases (Zhang et al., 2002). It can protect against cerebral ischemia-reperfusion injury (Zhou et al., 1992; Shi et al., 1998; Shuai and Dong, 1998). In recent years, studies have provided evidences for the neuroprotective properties of scutellarin (Liu Hong et al., 2003; Hong and Liu, 2004). The pharmacokinetics of breviscapine at a single intravenous dose to rabbits and dogs (Li Su-hua et al., 2003; Liu Yi-ming et al., 2003) showed that scutellarin distributed very fast and eliminated rapidly from the blood. In rabbits and dogs, the $T_{1/2\alpha}$ of breviscapine was $(12.0 \pm 6.6)$ min and $(6.99 \pm 2.74)$ min, respectively. In this study, breviscapine liposomes were prepared to extend the residence time of breviscapine in blood and achieve slow release of the drug. This work also provided some information on comparative pharmacokinetics in different animal species such as beagle dogs, rabbits and rats for both intravenous breviscapine liposomes and commercial injection (free breviscapine).

## 2. Experimental

### 2.1. Materials

Breviscapine was purchased from Yunnan Plant Pharmaceutical Industry Ltd. (Yunnan, China). Breviscapine liposomes were prepared by a rotary evaporation-sonication method. The average vesicle diameters were $401 \pm 47$ nm and the encapsulations efficient were $88.64 \pm 0.97\%$. *Injectio Breviscapine*, an injection solution of breviscapine (20 mg/5 mL), was manufactured by Dilong pharmacy Ltd. (Heilongjiang, China). Scutellarin (purity 96.4%) and rutin (internal standard, IS) were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile were of HPLC-grade. $NaH_2PO_4$ and other chemicals used were of analytical grade. Water was double-distilled water.

* Corresponding author. Tel.: +86 25 83271098; fax: +86 25 83301606.
*E-mail address:* pingqn@cpu.edu.cn (Q. Ping).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.03.047

### 2.2. Animals

Beagle dogs ($10.0 \pm 1.2$ kg), New Zealand white rabbits ($1.87 \pm 0.058$ kg) and Wistar rats ($200 \pm 20$ g body weight) were provided by the Animal Center of China Pharmaceutical University).

### 2.3. Chromatographic analysis

The concentration of scutellarin was determined by HPLC. The HPLC system was consisted of two pumps (HP1100, Agilent, USA), a Diamonsil C18 column (4.6 mm × 250 mm, 5 μm, Dikma, Beijing, China) with a Shim-park C18 precolumn (4 mm × 3.0 mm, 5 μm, Simadzu, Tokyo, Japan) maintained at 35 °C, an UV detector (HP1100, Agilent, USA) at 335 nm and an autosampler (HP1100, Agilent, USA). The HP1100 ChemStation software was applied on the HPLC system. The mobile phase consisted of methanol–acetonitrile–0.5% phosphoric acid (35:10:65, v/v/v) and the flow-rate was 1.0 mL $min^{-1}$ (Wenli Lv et al., 2005).

### 2.4. Drug administration and biological sample collection

The animal experiments were approved by the ethic committee of China Pharmaceutical University. Six Beagle dogs, 6 rabbits and 12 rats were administered at equivalent dose of breviscapine liposomes or breviscapine solution calculated by their body surface area. Six beagle dogs were randomly divided into groups A and B. According to the crossover design, the groups A and B, respectively received breviscapine liposomes and commercial injection (breviscapine solution) (2.8 mg $kg^{-1}$ body weight) via cubitus vein. Blood samples (1 mL) were taken from cubitus vein. After 1-week washout period, group A was given commercial injection and group B breviscapine liposomes at the same dose. With the same dosing protocol, six rabbits were administered breviscapine liposomes and commercial injection at the dosage of 5 mg $kg^{-1}$ body weight via auricle vein of ears.

Twelve rats were randomly divided into two groups and injected with breviscapine liposomes or commercial injection at a single dose of 9.4 mg $kg^{-1}$ body weight. Rats were anaesthetized with ethyl carbamate (15%, w/w, 1 mL × 100 $mg^{-1}$ body weight) and securely positioned on a surgical table. Midline incisions in the neck and gaskin skin were performed, and then the carotid artery and femoral veins were separated. Before administration one carotid artery was ligated at the end near the brain and a polyethylene cannula was placed in it toward the heart for the blood sampling. Breviscapine liposomes or commercial injection were respectively injected into the femoral vein and blood samples were collected from the carotid arteries.

### 2.5. Blood samples pretreatment

The blood samples need to be pretreated before HPLC analysis according to the reported method (Wenli Lv et al., 2006). Fifty microliters of protocatechualdehyde methanol solution (68.2 μg $mL^{-1}$) and 100 μL of 1 M phosphoric acid was added to the each blood sample (0.5 mL). Plasma sample was extracted with 3 mL of ethyl acetate after being acidificated by 100 μL of 0.01 M HCl (Dafang Zhong et al., 2003). After the samples were vortexed for 3 min, the mixture was centrifuged (TGL-16C, Anke Co., China) for 10 min at 3000 rpm. The supernatant was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in mobile phase and centrifuged for 10 min at 10,000 rpm, and the supernatant was injected for the HPLC analysis.

### 2.6. Validation of analytical method

Stock solution of scutellarin (250 μg $mL^{-1}$) was prepared by dissolving 12.5 mg of scutellarin in a 50 mL volumetric flask. The stock was then diluted with methanol to obtain scutellarin standard solutions at different concentrations. 0.8 mL blank plasma was added into 20 μL of the standard solutions. The plasma samples were pretreated according to the procedure described in the Section 2.5. The calibration curves were then constructed using a $1/y^2$ weighted linear regression of the peak-area ratios versus the plasma concentration of the analyte.

At the same concentrations, the freeze–thaw stability was evaluated after three freeze (−20 °C)–thaw (ambient) cycles.

### 2.7. Data processing and statistical analysis

The plasma concentration–time data of the breviscapine liposomes and commercial injection were fitted by 3P97 pharmacokinetics program (the Section of Mathematical Pharmacology of Chinese Mathematical Pharmacological Society). The pharmacokinetic parameters were calculated. The area under the concentration–time curve (AUC) was calculated with trapezium method. The one-way analysis of variance (ANOVA) was conducted to compare the major pharmacokinetic parameters.

## 3. Results and discussions

### 3.1. Validation of analytical method

The calibration curves of scutellarin plasma concentrations in Beagle dogs, rabbits or rats, all had good linearity. The regression equations were listed in Table 1. The lowest limit of quantitation (LLOQ) was 5 $ng^{-1}$, defined as the peak-area ratios of scutellarin to noise at 10:1.

At the low, medium and high concentration, the relative recoveries were ($100.8 \pm 14.1$)%, ($98.96 \pm 3.91$)% and ($95.60 \pm 1.74$)%. The absolute recoveries were ($71.18 \pm 5.04$)%, ($78.7 \pm 9.69$)%, and ($84.25 \pm 0.54$)%. The within-day RSD was less than 7.34%, and that of the between-day was less than 9.62%. The method was precise and reproducible.

### 3.2. Pharmacokinetics of breviscapine liposomes

The semilogarithmic plasma concentration–time profiles of the breviscapine liposomes and breviscapine solution in each mammal species were shown in Fig. 1. In all of the mammal species, the breviscapine liposomes and breviscapine solution were well described as an open two-compartment model. The major pharmacokinetic parameters after intravenous administration in beagle dogs, rabbits and rats are presented in Table 2, The $CL_{(s)}$ for the breviscapine liposomes increased as a function of body weight in Beagle dogs, rabbits and rats. Likewise, the apparent $V_{(c)}$ also increased with the body weight. The decrease in AUC as a function of body weight might be due to the increase of $CL_{(s)}$ and $V_{(c)}$.

Even though the similar physiology of mammals to human makes it feasible to use animal pharmacokinetics parameters to

**Table 1**
The regression equations of scutellarin plasma concentrations in Beagle dogs, rabbits and rats ($n = 5$)

| Sorts of animal | Regression equations | $r$ | Linearity ranges (μg/mL) |
|---|---|---|---|
| Beagle dogs | $y = 0.2595 - 0.00069$ | 0.9998 | 0.025–20.0 |
| Rabbits | $y = 0.2250C - 0.01411$ | 0.9997 | 0.020–20.0 |
| Rats | $y = 0.139x - 0.0406$ | 0.9995 | 0.025–40.0 |

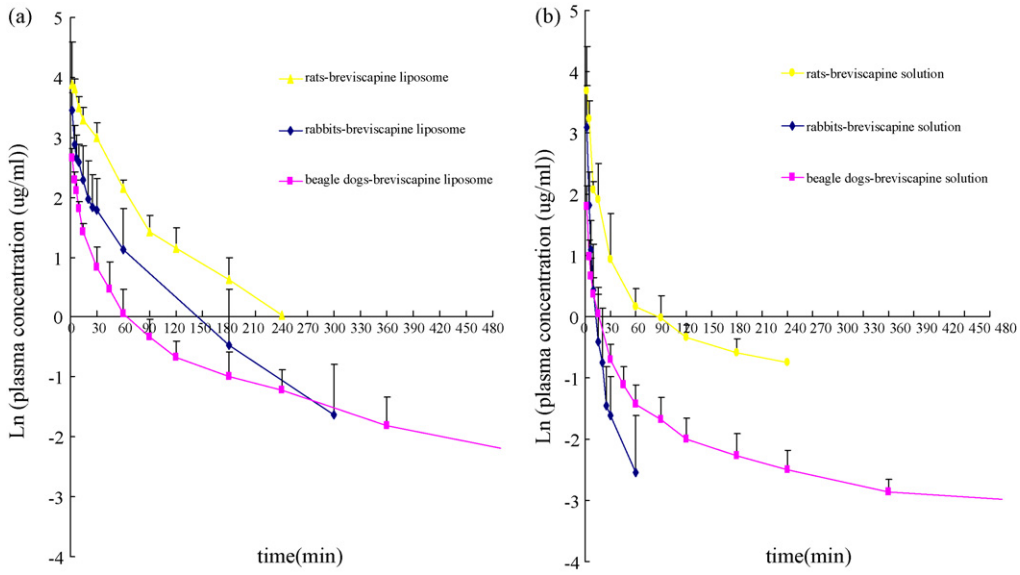


**Fig. 1.** Mean semilogarithmic plasma concentration–time profiles of (a) breviscapine liposomes and (b) breviscapine solution after a single equivalent dose in Beagle dogs (2.8 mg/kg), rabbits (5.0 mg/kg) and rats (9.4 mg/kg), respectively ($n = 6$).

**Table 2**
Pharmacokinetic parameters of breviscapine lipsomes and breviscapine solution after a single equivalent dose in Beagle dogs (2.8 mg/kg), rabbits (5.0 mg/kg) and rats (9.4 mg/kg), respectively ($n = 6$)

| Parameters of breviscapine liposomes | Breviscapine liposomes | | | Breviscapine solution | | |
|---|---|---|---|---|---|---|
| | Beagle dogs | Rabbits | Rats | Beagle dogs | Rabbits | Rats |
| $V_{(c)}$ (mL) | 1580 ± 265 | 248 ± 199 | 37.84 ± 17.35 | 2460 ± 2200 | 152 ± 134 | 17.65 ± 0.66 |
| $CL_{(s)}$ (mL min$^{-1}$) | 87.6 ± 9.63 | 15.3 ± 9.03 | 0.92 ± 0.17 | 324 ± 69.1 | 84.6 ± 40.6 | 2.97 ± 0.62 |
| AUC (ug min mL$^{-1}$) | 363 ± 42 | 1267 ± 1083 | 1691 ± 384 | 102 ± 19 | 196 ± 107 | 452 ± 103 |
| Animal weight (kg) | 10.0 ± 1.2 | 2.0 ± 0.2 | 0.2 ± 0.02 | 10.0 ± 1.2 | 2.0 ± 0.2 | 0.2 ± 0.02 |

The equivalent dose was calculated according to the animal body surface area.

assess human pharmacokinetic behavior, cross-species comparison of pharmacokinetic parameters requires appropriate scaling by the allometric approach. Three important parameters were AUC, $CL_{(s)}$ and $V_{(c)}$. These data were analyzed to determine the allometric relationship with body weight. The logarithm values of AUC, $CL_{(s)}$ and $V_{(c)}$ were linear with the logarithm value of body weight by the allometric equation: $Y = a \times W^b$. The function '$Y$' represents the parameter of concern (area-under-curve, clearance or volume of distribution), '$a$' is the allometric coefficient which is different for each drug, '$W$' is the species average body weight and '$b$' is the scaling exponent (Riviere et al., 1997). The allometric coefficient '$a$' describes the y-intercept. Different chemicals or drugs have differ-

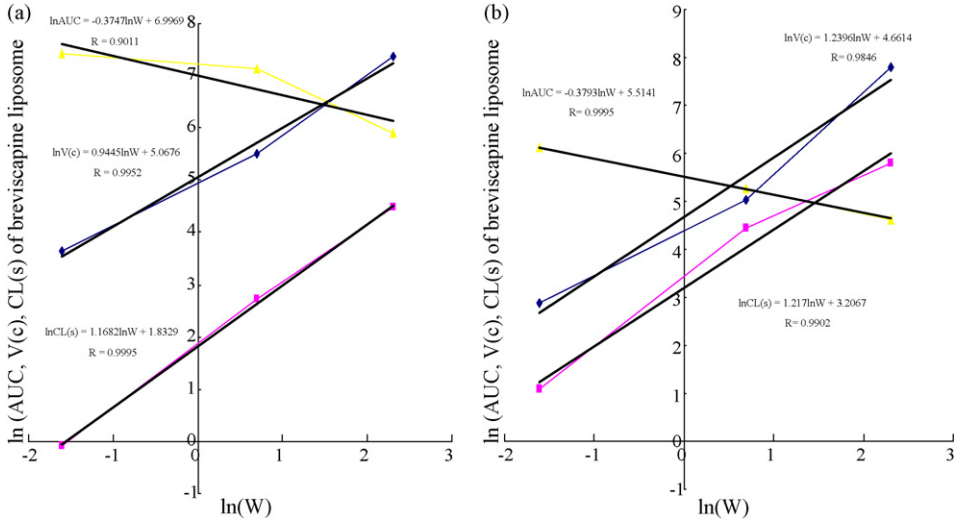


**Fig. 2.** Allometric plots obtained for breviscapine liposomes (a) and breviscapine solution (b) after a single equivalent i.v. dose in Beagle dogs (2.8 mg/kg), rabbits (5.0 mg/kg) and rats (9.4 mg/kg), respectively ($n = 6$).

**Table 3**
Allometric equations of breviscapine liposomes and breviscapine solution after a single equivalent dose in Beagle dogs (2.8 mg/kg), rabbits (5.0 mg/kg) and rats (9.4 mg/kg), respectively ($n = 6$)

| | $\ln Y = b \ln W + \ln a$ | Allometric equation $Y = a W^b$ |
|---|---|---|
| Breviscapine liposomes | $\ln AUC = -0.3747 \ln W + 6.9969$ ($r = 0.9011$) | $AUC = 1093\ W^{-0.3747}$ |
| | $\ln V_{(c)} = 0.9445 \ln W + 5.0676$ ($r = 0.9952$) | $V_{(s)} = 158.9\ W^{0.9445}$ |
| | $\ln CL_{(s)} = 1.1682 \ln W + 1.8329$ ($r = 0.9995$) | $CL_{(s)} = 6.252\ W^{1.1682}$ |
| Breviscapine solution | $\ln AUC = -0.3793 \ln W + 5.5141$ ($r = 0.9995$) | $AUC = 248.2\ W^{-0.3793}$ |
| | $\ln V_{(c)} = 1.2396 \ln W + 4.6614$ ($r = 0.9846$) | $V_{(s)} = 105.8\ W^{1.2396}$ |
| | $\ln CL_{(s)} = 1.217 \ln W + 3.2067$ ($r = 0.9902$) | $CL_{(s)} = 24.70\ W^{1.217}$ |

ent a values. The allometric exponent '*b*' represents the slopes of the lines. The value of b reflects the functional relationship between a given pharmacokinetic parameter and animal species. If the *b* value is greater than zero, there is a positive correlation between a given pharmacokinetic parameter and animal body weight. And the negative correlation exits for the negative *b*. Allometric scaling allows for direct comparison of pharmacokinetic parameters between different species based on the goodness-of-fit of allometric equation. The allometric relationship between body weight ($W$) and area-under-curve (AUC), volume of distribution ($V_{(c)}$) and clearance ($CL_{(s)}$) were shown in Fig. 2. Fig. 2a represented the allometric information of breviscapine liposomes and Fig. 2b showed that of breviscapine solution. The allometric equations of breviscapine liposomes and solution were listed in Table 3. The parameters of AUC, $CL_{(s)}$ and $V_{(c)}$ showed good correlation with the body weight by the allometric equation. Especially the relative coefficient ($r$) of AUC for the breviscapine liposomes was 0.9972. The results could be used for extrapolating dosage between human and animals and also for dosage adjustment of breviscapine liposomes in order to achieve same AUC as commercial injection. The average body weight of human is 60 kg and the dosage of commercial injection is 100 mg/day. In order to achieve same AUC as commercial injection, the dose calculated from the allometric equation for breviscapine liposomes is 24.2 mg.

Both $CL_{(s)}$ and $V_{(c)}$ showed a positive correlation with body weight. And AUC exhibited a negative correlation. Such results correlated well with the Dose equation: $AUC = F \times X/CL$. Here, the value of $F$ is 1.0 for the intravenous administration and $X$ is the dose.

### 3.3. Pharmacokinetics of commercial injection (breviscapine solution)

According to Fig. 1 and Table 2, it appeared that breviscapine was rapidly cleared from bloodstream in all of the mammals. The result accorded well with the study of $^3$H-scutellarin in vivo distribution (Cai, 1981). Large amount of radioactivity was recovered in cholecyst and intestine 1 hr after i.v. injection. 41.2% of $^3$H-scutellarin was found in the excrement and urine in 24 h. The allometric analysis of AUC, $V_{(c)}$ or $CL_{(s)}$ versus body weight in the mammal species for commercial injection are shown in Fig. 2. According to the allometric equations listed in Table 3, the $CL_{(s)}$ for commercial injection increased as a function of body weight. Likewise, the apparent $V_{(c)}$ also increased with the body weight and was only slightly greater than the blood volume. These results also suggested that most of the scutellarin remained in the blood. The decrease in AUC as a function of body weight may be attributed to the increase in $CL_{(s)}$ and $V_{(c)}$. The values of AUC, $CL_{(s)}$ and $V_{(c)}$ showed good correlation with the body weight by the allometric equation.

### 3.4. Comparison of pharmacokinetic parameters between breviscapine liposomes and commercial injection

Comparative pharmacokinetic parameters of the breviscapine liposomes and commercial injection in different mammals were summarized in Table 4. The AUC, $V_{(c)}$ and $CL_{(s)}$ ratios of the breviscapine liposomes to commercial injection were calculated. The mean plasma AUC ratios of the breviscapine liposomes to commercial injection in all of the mammals were all above 300%, with the maximum value of $(646 \pm 39)$% in rabbits. The mean $CL_{(s)}$ ratios in all of the mammals were all less than 57%, with the maximum value of $(18 \pm 6)$% in rabbits. Compared with the commercial injection, the liposomes did increase the concentrations of scutellarin in plasma and retard the clearance of scutellarin from circulation system.

**Table 4**
Pharmacokinetic parameters ratios of breviscapine liposomes to breviscapine solution after a single equivalent dose in Beagle dogs (2.8 mg/kg), rabbits (5.0 mg/kg) and rats (9.4 mg/kg), respectively ($n = 6$)

| Parameters ratio of liposomes to reference | Beagle dogs | Rabbits | Rats |
|---|---|---|---|
| $AUC_{lip}/AUC_{ref}$ | 3.56 ± 0.26 | 6.46 ± 0.39 | 3.07 ± 0.58 |
| $V_{(c)lip}/V_{(c)ref}$ | 0.64 ± 0.21 | 1.63 ± 0.52 | 0.90 ± 0.23 |
| $CL_{(s)lip}/CL_{(s)ref}$ | 0.27 ± 0.15 | 0.18 ± 0.06 | 0.57 ± 0.18 |

## 4. Conclusions

The allometric equation might be applied to extrapolate dose in human from the mammal data and also for dosage adjustment of breviscapine liposomes in order to achieve same AUC as commercial injection. A more precise dose extrapolation between human and mammals can be made if more detailed pharmacodynamic information is available. Compared with the commercial injection, the breviscapine liposomes delivered more scutellarin in plasma and the mean residence time was longer. These properties apparently improved the in vivo pharmacokinetic behavior of scutellarin.

## Acknowledgements

This work was financially supported by the Chinese National Key foundation of Science (Grant No. 2004AA2Z3230) and the Natural Science Foundation of Jiangsu province, China (Grant No. BK2001 21 0).

## References

Cai, X.L., 1981. Absorption, distribution and metabolism of $^3$H-breviscapine in mice. Chin. Trad. Herb. Drugs 12, 26–27.
Zhong, D.-F., Yang, B.-H., Chen, X.-Y., Li, K., Xu, J.-H., 2003. Determination of scutellarin in rat plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B 796, 439–444.
Hong, H., Liu, G.-Q., 2004. Protection against hydrogen peroxide-induced cytotoxicity in PC12 cells by scutellarin. Life Sci. 74, 2959–2973.
Li, S.-H., Jiang, X.-H., Lan, K., Yang, J.-Y., Zhou, J., 2003. Pharmacokinetics of scutellarin in dogs. J. Chin. Pharm. Sci. 12, 127–130.
Liu, H., Yang, X.-L., Wang, Y., Tang, X.-Q., Jiang, D.-Y., Xu, H.-B., 2003. Protective effects of scutellarin on superoxide-induced oxidative stress in rat cortical synaptosomes. Acta Pharmacol. Sin. 24, 1113–1117.
Liu, Y.-M., Lin, A.-H., Chen, H., Zeng, F.-D., 2003. Study on pharmacokinetics of scutellarin in rabbits. Acta Pharm. Sci. 38, 775–778.

Riviere, J.E., Martin-Jimenez, T., Sundlof, S.F., Craigmill, A.L., 1997. Interspecies allometric analysis of the comparative pharmacokinetics of 44 drugs across veterinary and laboratory animal species. J. Vet. Pharmacol. Therap. 20, 453–463.

Shi, S.G., Shao, S.Q., Chen, K.N., 1998. Study of therapeutic effects of *Erigeron breviscapus* preparation on cerebral ischemia-perfusion damage in rats. Acta Academiae Medicinae Militaris Tertiae 20, 320–322.

Shuai, J., Dong, W.W., 1998. Experimental research of PKC inhibitor, *Erigeron breviscapus* on the ischemic/reperfusional brain injury. Chin. Pharmacol. Bull. 14, 75–77.

Lv, W.-L., Guo, J.-X., Li, J., Huang, L-S., Ping, Q.-N, 2005. Distribution of liposomal breviscapine in brain following intravenous injection in rats. Int. J. Pharm. 306, 99–106.

Lv, W.-L., Guo, J.-X., Li, J., Zhao, C.-W., Zhao, L., Ping, Q.-N., 2006. Study on the pharmacokinetics of breviscapine liposomes following intravenous injection in Beagle dogs. Acta Pharm. Sci. 40, 24–29.

Zhang, W.D., Chen, W.S., Wang, Y.H., Yang, G.J., Kong, D.Y., Lu, H.T., 2000. Studies on the flavone glycosides from the extract of *Erigeron breviscapus*. Chin. Trad. Herb. Drugs 31, 565–568.

Zhang, J., Li, X.S., Zhang, W.D., 2002. The advance in studies on chemical component and pharmacology of *Erigeron breviscapus*. J. Pharm. Pract. 20, 103–107.

Zhou, Q.S., Zhao, Y.M., Bai, X., Li, P.X., Ruan, C.G., 1992. Effect of new-breviscapine on fibrinolysis and anticoagulation of human vascular endothelial cells. Acta Pharmcol. Sin. 13, 239–242.