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Original Article

Effects of *Evodiae Fructus* on targeted distribution of berberine in rats

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Abstract: According to Traditional Chinese Medicine (TCM) theories, Evodiae Fructus (EF) was considered as a meridian guiding drug (MGD) in Zuojin Pill and could enhance the effectiveness of prescription. However, the scientific evidence for this effect is still unclear. In this study, the meridian guiding mechanism of EF was investigated. Rats were randomly divided into four groups, control group (water), low dose of EF (250 mg/kg), medium dose of EF (500 mg/kg) and high dose of EF (1000 mg/kg). Water or EF was given to the rats by intragastric administration for 7 days. After the last administration, berberine (80 mg/kg) was administered to rats by gavage. Then the blood and tissues of interest were collected at predetermined time points. Concentrations of berberine in different tissues were analysed by ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS) and the target parameters including relative targeting efficiency (RTE), relative uptake efficiency (RUE) and maximum relative concentration (RCmax) were calculated. Compared with control group, various doses of EF could improve the targeting efficiency of berberine in liver. The value of RUE was 1.10, 1.21, 1.37 and the RTE was 4.33%, 10.78% and 18.73% for the low, medium and high dose of EF, respectively. Moreover, it appreciably reduced the distribution of berberine in spleen and lung. EF increased the Cmax of berberine in nearly all tissues except for lung and spleen, with the value range from 2% to 36%. In a word, these results suggested that EF could increase the distribution of berberine in liver and reduce the concentration in spleen and lung, which indicated that the meridian guiding action of EF was credible.

Keywords: *Evodiae Fructus*, berberine, distribution, rat, UPLC-MS/MS

Introduction

Meridian guiding theory, an important principle of Traditional Chinese Medicine (TCM) theory, was recorded initially in "Shennong Bencao Jing", which was the source of Chinese herbal medicine. Based on meridian guiding theory, meridian guiding drug (MGD) similar to targeted vector was commonly combined with other herbs to enhance the effectiveness of the prescription in target organs [1]. Meridian guiding theory, which acted as a bridge which linked theory and clinical medication, was one of the characteristics of TCM [2]. Therefore, it was necessary to investigate the mechanism of meridian guiding theory.

Promoting absorption of drugs [3], physiologic barrier theory [4, 5] and receptortheory [6] had been applied to explain meridian guiding theory in the past. Based on dose-effect relationship, the main effect of the whole prescription was related to the concentration of drug in target tissues. At present, study of targeting distribution of active compound in vivo was considered as the most popular experimental method to expound the mechanism of MGD [7, 8].

Zuojin Pill, a classical formula in traditional Chinese medicine, consists of *Coptis chinensis* Franch. and EF at the weight ratio of 6 to 1, and it was found initially in "Danxi's experiential therapy" for treating liver-fire invading the stomach. *Coptis chinensis* Franch. has been widely used for the treatment of gastroenteritis, diarrhea, cancer [9], inflammation-related diseases [10] and diabetes mellitus [11]. Berberine was considered as the primary bioactive constituent and chemical marker for the quality control of *Rhizoma coptidis* [12, 13]. EF was considered

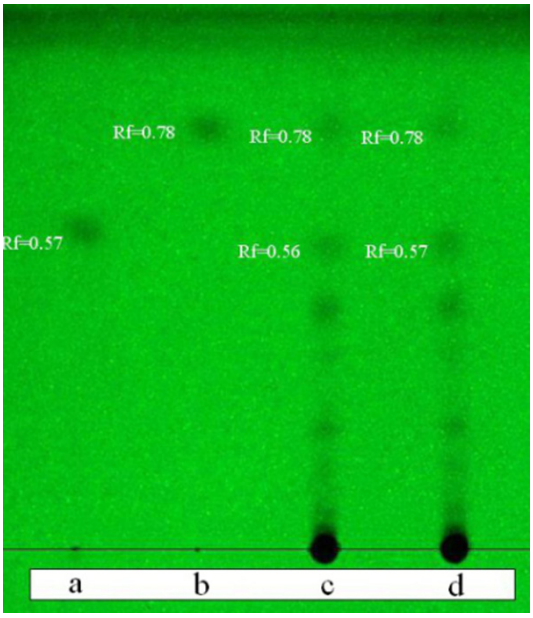

Figure 1. Thin-layer chromatog analysis of EF. Spot a was evodiamine, spot b was rutecarpine, spot c and d were EF in different batch drug.

as MGD in Zuojin Pill and could enhance the effectiveness of the prescription. Although there were a lot of previous studies focused on the pharmacological effects of EF, few reports involved in the meridian guiding effect besides its clinical application.

In order to investigate the meridian guiding action of EF, the concentrations of berberine in rat tissues were analysed by UPLC-MS/MS after oral administration of berberine with different doses of EF. The meridian guiding action was estimated by relative targeting efficiency (RTE), relative uptake efficiency (RUE) and maximum relative concentration (RCmax). So far, this is the first investigation to use targeting parameters to study EF on meridian guiding theory in rats.

Materials and methods

Chemicals and reagents

Evodia rutaecarpa was provided by Shunkang Medical Company (Zhengzhou, China). Berberine (purity >98%) was obtained from Henan Xinyi Phytochemistry Company, (Xinxiang, China). Evodiamine and rutecarpine were provided by National Institutes for Food and Drug Control (Beijing, China). Paracetamol was purchased from Sigma (CA, USA). Acetonitrile used for UPLC-MS/MS was of chromatographic grade, and obtained from Tedia Company Inc (Beijing, China). Other solutions were analytical reagent grade.

Extraction of Evodiae Fructus (EF)

EF (200 g) was socked in eight times of water for 45 min, then boiled the mixture and keep boiling for 60 min, filtered the extraction. The dregs were disposed via the same procedure for a second time. The filtrate was combined and concentrated to 200 mL, and then stored at -20°C until use.

Thin-layer chromatography was adopted for qualitative identification of EF. The development for the extract was hexane chloroform acetone methanol (24:10:5.5:1), using evodiamine and rutecarpine as the reference compounds. The picture was taken at 254 nm and the photograph was shown in **Figure 1**.

Animals

All experimental procedures were conducted in conformity with NIH guidelines for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Experimental Animals of Henan Provincial Academy of TCM. Male Sprague Dawley (SD) rats (8-10 weeks of age) were purchased from Beijing Vital River Laboratory Animal Technology Company Limited (Beijing, China). Animals were housed under standard conditions and fed with free diet.

Tissue distribution studies

After five days adaptation Sprague Dawley rats were randomly divided into four groups, 60 rats in each group. The divided experimental groups were as follows: control group (water), low dose of EF (250 mg/kg), medium dose of EF (500 mg/kg), high dose of EF (1000 mg/kg). Water or EF was given to the rats by intragastric administration daily for 7 consecutive days. Five minutes after the last administration at day 7, berberine (80 mg/kg) was administered in rats by gavage.

Blood and tissues of interest (heart, liver, lung, stomach and kidney) were collected after administration of berberine at the predetermined time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h). Six rats were sacrificed from every group for

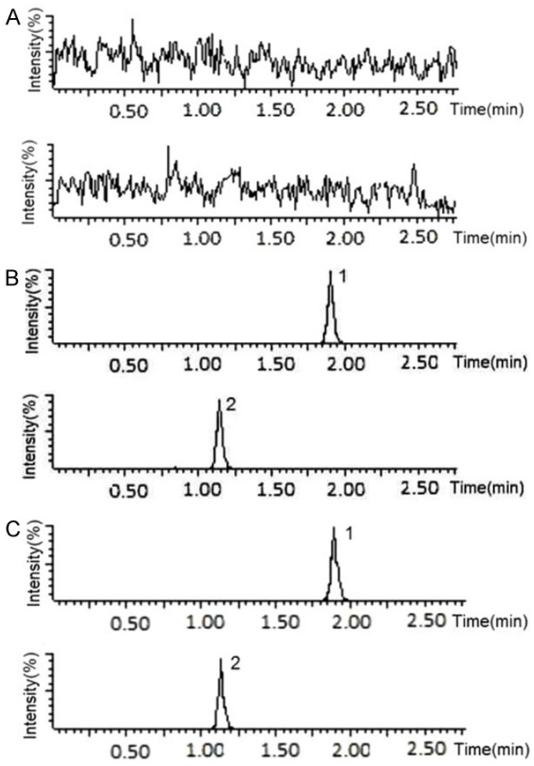

Figure 2. Chromatograms for berberine and IS in rat liver samples. 1. Berberine; 2. Internal standard (IS). A. Blank liver homogenate sample; B. Blank liver homogenate sample spiked with berberine and IS; C. Liver homogenate sample obtained at 2 h after oral administration of berberine.

each time point. Plasma was separated by centrifugation and tissues were blotted dry with filter parer after washed with 0.9% normal saline. Plasma and tissue samples were stored at -80°C for further detection.

Sample preparation for UPLC-MS/MS analysis

Plasma samples (200 μL) were placed in Eppendorf tubes. Thereafter 20 μL of the internal standard (paracetamol) was added to samples to get a final concentration of 100 ng/mL. After shaking for 10 s, 1000 μL of acetonitrile was added to each tube, shake-mixed for 5 min and ultrasonic extracted for 20 min. After centrifugation at 12000 rpm for 10 min, the supernatant (900 μL) was pipette-transferred to another Eppendorf tube. Then, the residue was extracted via the same procedure for a second time. The supernatant was combined for each sample and dried by nitrogen at 40°C. The residue was redissolved in 80 μL of the mobile phase and the solution was filtrated through a microporous filter membrane (0.22 μm) prior to analysis. 5 μL of the sample was analysed by UPLC-MS/MS.

Before extracting tissue, the collected samples were homogenized in equal weight of 0.9% normal saline, then 200 μL homogenate was processed in the same way as described above for plasma and then detected by UPLC-MS/MS.

Chromatography conditions

The quantitation of berberine was carried out on a Waters Acquity ultra-performance liquid chromatography equipped with an automatic sampler, a quaternionic pump and a column compartment. All chromatographic separation was achieved on the Waters ACQUITY UPLC™ BEH C_{18} chromatography column (50 mm × 2.1 mm, 1.7 μm). The column temperature was maintained at 30°C. The flow rate was 0.3 mL/min. The mobile phase consisted of acetonitrile and water containing 0.1% formic acid (20:80, v/v). The sample injection volume was 5 μL.

In order to achieve high sensitivity, a triple quadrupole mass spectrometer (Xevo-TQ-S mass spectrometer, Waters Corporation) was employed for quantification of berberine and the internal standard (IS). The mass spectrometer was set in the positive electrospray ionization (ESI) mode and the quantification mode was multiple reaction monitoring (MRM). The capillary voltage was 3.20 kV, desolvation gas (N_2) temperature was maintained at 500°C at a flow rate of 700 L/h, and the collision gas (N_2) flow rate was 150 L/h. The precursor-to-product ion transitions were monitored at m/z 336.2>320.2 for berberine and m/z 152.2>110.1 for the IS.

Method validation

The selectivity of the analytical method was assessed by comparing the chromatograms of six different batches of blank samples with the corresponding spiked samples with berberine and the IS. Each blank sample was investigated to ensure that it had no potential interference at the retention times of berberine and IS.

Linearity was assessed by method of three independent calibration curves on three different days by weighted linear regression of the analyte-IS peak area ratios. The lower limit of quantification (LLOQ) was defined as the lowest con-

Table 1. Regression equation, linear range and lower limit of quantification (LLOQ) of berberine

Sample	Regression equation	Correlation coefficient	Linear range (ng/g)	LLOQ (ng/g)
Blood	Y=0.7917X+0.2240	0.9993	2-160	2
Heart	Y=0.8263X+0.1941	0.9986	2-80	2
Liver	Y=0.6481X+0.2527	0.9997	5-320	5
Spleen	Y=0.7369X+0.2334	0.9991	2-160	2
Lung	Y=0.7180X+0.2470	0.9982	2-160	2
Kidney	Y=0.7608X+0.2294	0.9990	2-160	2

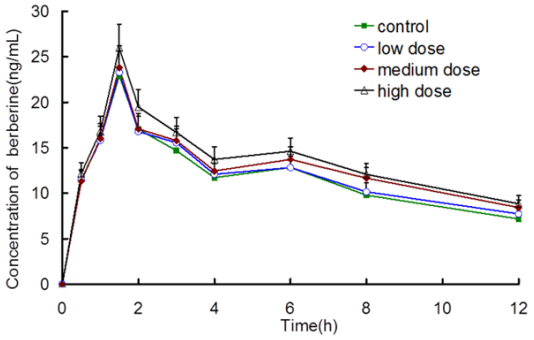

Figure 3. The concentration-time curves of berberine in plasma after peroral administration of 80 mg/kg.

centration on the calibration curve with that was quantitatively determined with acceptable precision of ± 20% and accuracy of ± 20%.

Accuracy and precision were determined by analyzing six replicates of the quality control (QC) samples at three different concentrations (5, 20, 80 ng/g). The intra-day precision and accuracy were tested within a single day, while the inter-day over three consecutive days.

The extraction recovery of berberine was determined at low, medium and high QC concentrations and the peak area ratios were compared with peak area. Freeze-thaw stability was evaluated after three freeze (-80°C) and thaw (room temperature) cycles before sample analyzed. Short-term stability was assessed by analyzing QC samples kept at room temperature for 24 h. Long-term stability was tested by assaying QC samples after storage at -80°C for 30 days.

Targeting efficiency evaluation

The role of MGD is somewhat similar to the goal of modern drug target delivery system. In view of this, the targeting efficiency was utilized to evaluate the meridian guiding effect of EF in this study. Three targeting parameters, RUE, RTE and RCmax were calculated to assess the influence of EF on the distribution of berberine in rats and the relevant parameters were calculated according to the following equations [14, 15]:

$$RUE = \frac{AUC_{sample}}{AUC_{control}},\ RCmax = \frac{Cmax_{sample}}{Cmax_{control}},$$

$$RTE = \frac{(AUC_{tissue}/AUC_{sum})_{sample} - (AUC_{tissue}/AUC_{sum})_{control}}{(AUC_{tissue}/AUC_{sum})_{control}}$$

$$AUC_{sum}=AUC_{heart}+AUC_{liver}+AUC_{lung}+AUC_{spleen}+AUC_{kidney}+AUC_{plasma}$$

In the equations, AUC_{sum} involves the sum of area under curve ($AUC_{0-\infty}$) of six tissues in control group and coadministration group, respectively.

Date analysis

The pharmacokinetic parameters were calculated using DAS 2.0 pharmacokinetic software. All date were analyzed using one-way analysis of variance (ANOVA) followed by the Student test through SPSS 17.0 for Windows. Probability value P<0.05 was considered significant.

Results

Specificity

Chromatograms of the blank liver homogenate sample, and blank liver homogenate sample spiked with berberine and IS, and liver homogenate sample obtained at 2 h were shown in **Figure 2**. As shown in **Figure 2B**, there was no endogenous peak observed at the retention time of the two compounds, and the retention times for berberine and IS were 1.87 and 1.13 min, respectively.

Linearity and LLOQ

The regression equation, linear range and LLOQ of berberine are demonstrated in **Table 1**. There was an excellent linear relationship in different tissues with correlation coefficient

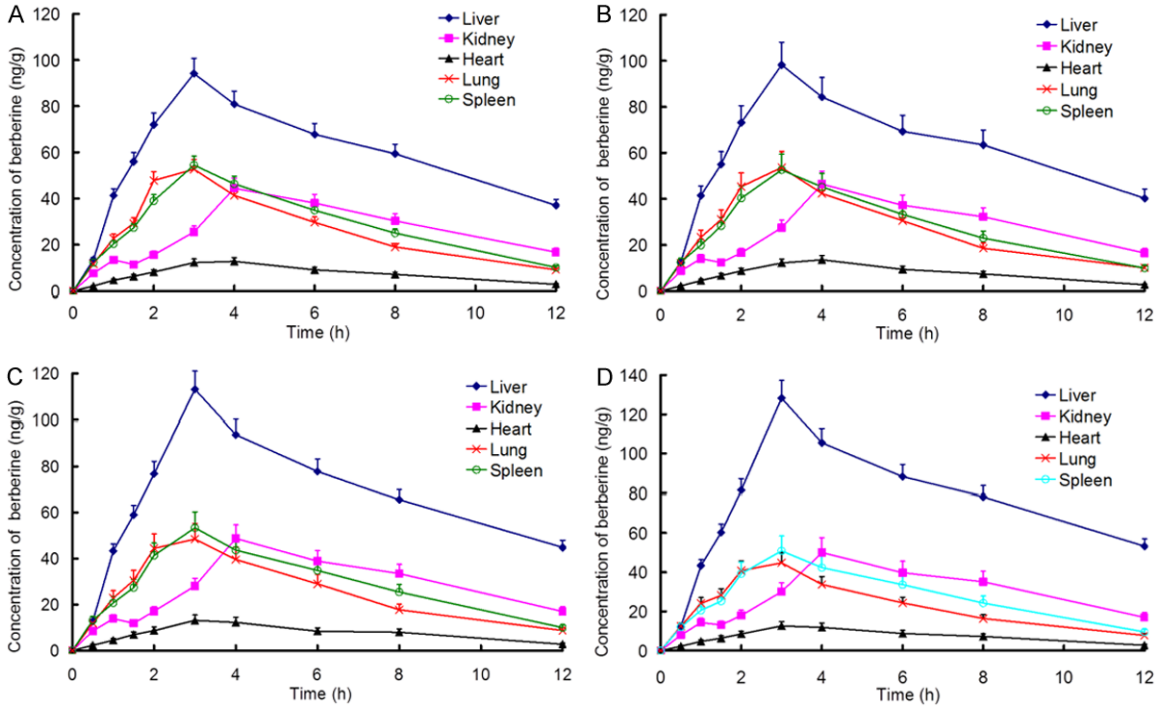

Figure 4. The profiles of berberine in different tissue after peroral administration of 80 mg/kg. A. Control group; B. Low dose of EF (250 mg/kg); C. Medium dose of EF (500 mg/kg); D. High dose of EF (1000 mg/kg).

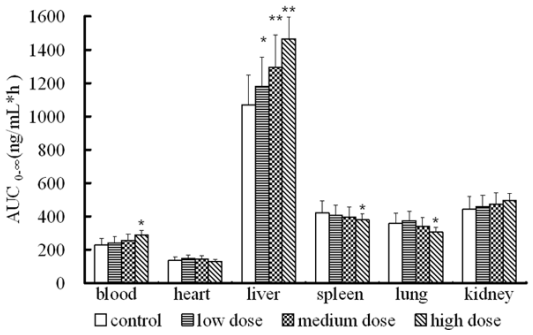

Figure 5. The $AUC_{(0-\infty)}$ of berberine in different tissue. Significant differences ($^{*}P<0.05$ or $^{**}P<0.01$) were indicated between control and EF groups.

≥0.9981. The ranges of concentrations and LLOQ could satisfy the requirements of tissue distribution study of berberine.

Precision and accuracy

The results of precision and accuracy were obtained based on the analysis of QC samples (n=6) at concentrations of 5, 20 and 80 ng/g. The intra-day accuracy (RE) of berberine ranged from -4.2% to 6.1%, and the inter-day accuracy ranged from -3.3% to 6.5%. The intra- and inter-precision (RSD) of berberine were less than 10.5% and 8.1%. These data indicated the acceptable accuracy and precision of the present method for the determination of berberine in rat tissues.

Recovery and stability

The recovery of berberine at low, medium and high QC concentrations was within 75.8%-86.3%. The results of stability experiments showed that there was no significant degradation occurred when stored at room temperature for 24 h, -80°C for 30 days and after three freeze-thaw cycles at three different concentrations. It indicated the samples were stable under various conditions.

Pharmacokinetics and tissue distribution of berberine

As a control, the pharmacokinetics of berberine was studied. The distribution of berberine at a series of time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h) were quantified in the liver, heart, lung, spleen and kidney.

The mean concentration-time profile of berberine in plasma was illustrated in **Figure 3**. Berberine peak in plasma was observed at 1.5 h

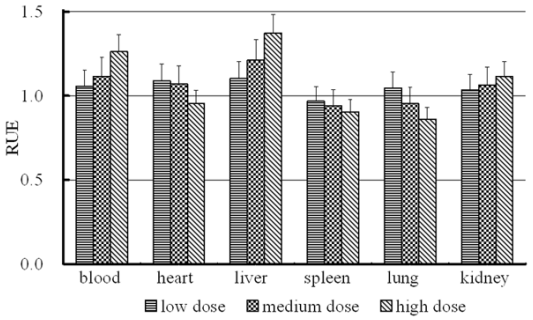

Figure 6. RUE in different tissues after coadministration of berberine (80 mg/kg) with EF.

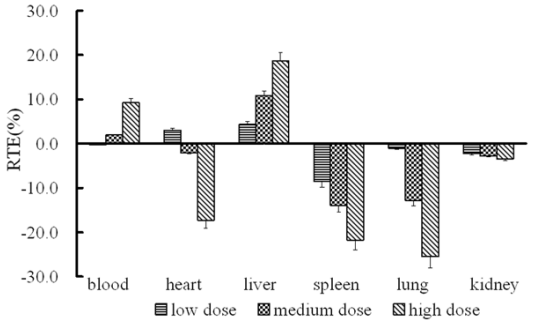

Figure 7. The effect of EF on the RTE of berberine (80 mg/kg) in different tissues after coadministrated with different doses of EF in rats.

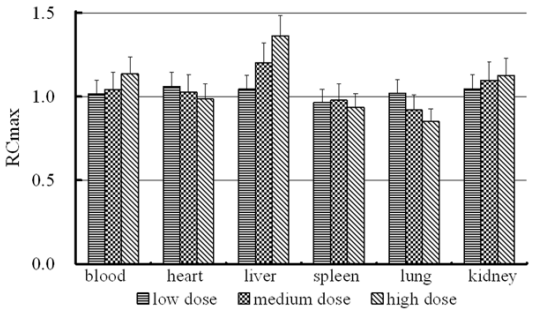

Figure 8. Effect of three different doses of EF on the RCmax of berberine (80 mg/kg) in rats.

and 6 h respectively, and the higher concentration (22.88 ng/mL) was presented at 1.5 h. According to the calculations by pharmacokinetics software, the values of $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were 138.61 ng/mL*h and 228.66 ng/mL*h, respectively. The concentration reached a maximum with an average Cmax of 22.88 ng/mL.

Results of tissue distribution of berberine indicated that berberine could distribute widely throughout the body, but there were significant differences in the distribution of different tissues. The concentration-time profile indicated that berberine reached the tissues within 0.5 h after dosing. Three hours after administration to rats, peak concentration of berberine was observed in all collected tissues except kindey. As shown in **Figure 4**, the ranking of maximum concentrations in tissues was as followed: liver > spleen ≈ lung > kidney > plasma > heart.

The $AUC_{(0-\infty)}$ of berberine in different tissues had changed in varying degrees after combination with EF, the result was shown in **Figure 5**. Compared with control group, the $AUC_{(0-\infty)}$ of each doses group of EF increased significantly in liver ($P<0.01$ or $P<0.05$), but decline in spleen and lung.

Targeting efficiency evaluation

Normally, targeting efficiency was evaluated by comparing the drug concentration in the target tissue with those of the non-target organ, and the RUE, RCmax and RTE could be used for targeting evaluation.

RUE could indicate the effect of EF on the distribution of berberine in different tissues as an index for targeting evaluation. The RUE in different tissues was shown in **Figure 6**. In each dose group of EF, RUE in liver and kidney were greater than 1 and exhibited dose-effect relationship. On the contrary, with the increase of EF dose, the values of RUE were smaller and smaller in lung, spleen and heart. RUE in all the three tissues was less than 1 in high dose group of EF, the value was 0.86, 0.90 and 0.96, respectively.

RTE is an important parameter to estimate whether the drug has targeting effect or not. It indicates the distribution of the drug in the target organ was enhanced when the value of RTE was greater than zero. On the contrary, it implied the drug hardly distributed to the tissue when RTE was less than zero. The RTE of berberine in each experimental group after combination with EF was shown in **Figure 7**. The result revealed that each dose of EF enhanced the targeting distribution of berberine in liver, with the RTE values 4.33%, 10.78% and 18.73%, respectively. However, EF was able to prevent the distribution in spleen, lung and kidney, and

the RTEs were between -1.09% and -25.50%. Although low dose of EF exhibited targeting effect in heart with RTE 4.32%, medium and high dose of EF decreased berberine distribution with RTE -2.06% and -17.29%, respectively.

RCmax which can reflect the distribution change after co-administrated with EF is a key indicator to evaluate targeting efficiency. As shown in **Figure 8**, each dose of EF could increase the maximum concentration of berberine in blood, liver and kidney, and the RCmax was between 1.02 and 1.36, meanwhile, it decreased the Cmax of berberine in spleen. High dose of EF increased the Cmax of berberine in heart and lung. However, low dose of EF decreased the Cmax of berberine in heart and lung, the RCmax was 0.96 and 0.86.

Discussion

Chinese herbs have been used in China based on theories of TCM for a long time, including four properties (cool, cold, warm, and hot), five tastes (sour, bitter, sweet, spicy, and salty), meridian tropism and meridian guiding theory. The meridian guiding drug (MGD) guided the selective therapeutic effects of a prescription on a certain part of the body and was very valuable for clinical treatment in China [2]. The research of meridian guiding theory will contribute to the intelligibility and popularization of TCM. For example, *Evodiae Fructus*, considered as a MGD of liver, was usually used to enhance the effectiveness of other drug.

Evodiae Fructus, the dried, nearly ripe fruit of *Evodia rutaecarpa* (Juss.) Benth., *Evodia rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang or *Evodia rutaecarpa* (Juss.) Benth. var. bodinieri (Dode) Huang, is listed in the China Pharmacopoeia (2015 edition). EF had a long history of application not only in China but also in Japan and other Asian countries [16, 17]. It was used in traditional Chinese medicine to cure for gastrointestinal disorders, analgesia, soothing liver, dispelling colds, amenorrhea as well as other pharmacological effects [18-20]. In phytochemical studies, alkaloids, such as evodiamine, rutaecarpine and limonin, are the main active ingredients of EF. Rutaecarpine and evodiamine exhibit cardiotonic effects via activation of the vanilloid receptor [21]. Rutaecarpine plays an anti-inflammatory role through inhibiting COX-2 activity, while evodiamine suppresses COX-2 gene expression [22, 23]. The alkaloids of EF has shown the inhibit effect of cellular growth, invasion, and metastasis of a wide variety of tumor cells [24]. Although there were a lot of previous studies focused on the pharmacological effects of EF, few reports were involved in the meridian guiding effect besides its clinical application. In order to investigate the meridian guiding action of EF, the concentrations of berberine in rat tissues were analysed by UPLC-MS/MS. The meridian guiding action was estimated by RTE, RUE and RCmax.

The distribution research would be helpful to understand and explain meridian guiding theory. From the $AUC_{(0-\infty)}$ figures, the distribution of berberine increased in liver and blood while it declined in spleen and lung after combination with EF. As the $AUC_{(0-\infty)}$ of blood raised, the reason of this phenomenon maybe that EF promoted the absorption of berberine. But this is not the only reason because the distribution in some tissues reduced rather than improved in spleen and lung. EF may influence the disposition of berberine in other aspects, such as drug transporters, metabolic enzymes.

The results of targeting efficiency evaluation demonstrated that berberine exhibited targeting effect to liver after co-administered with EF, accompanied by a decreased spleen and lung distribution. This is in accordance with the meridian guiding theory of TCM, in which the EF is usually used to focus the effect of other drug on the liver as a MGD of liver. According to TCM theory, meridian tropism of an herb may involve several relative organs. MGD may enhance the activity of other herbs to act on single organ or several relevant organs simultaneously. The findings of this study suggest that it might be a simple and efficient method to enhance the therapeutic concentrations in target tissues combined with MGD, and the meridian guiding theory of TCM was credible.

Conclusion

In this study, it was the first time to reveal the fact that EF play a meridian guiding role through promoting the liver targeting distribution of berberine and decreasing the concentrations in other tissues. MGD may be an efficient method to enhance therapeutic concentrations in tar-

get tissue. The results in this research provide a strong foundation for elucidating the mechanism of meridian guiding theory.

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Disclosure of conflict of interest

None.

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