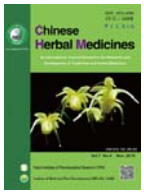

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Original article

Simultaneous Determination of Four Substances in Plasma and Dermal Microdialysates of Guinea Pig after Different Acupoints Administration of Fufang Baijiezi Gel

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ABSTRACT

Objective To study the pharmacokinetic properties of Fufang Baijiezi Gel (FBG) after different acupoints administration. **Methods** Sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin, which were four substances of FBG, were determined by a sensitive liquid chromatography tandem mass spectrometry method (LC-MS) both in plasma and dermal microdialysates of guinea pig simultaneously. Microdialysates were separated on an Ultimate® XB-Phenyl analytical column (150 mm × 2.1 mm, 5μm) and detected by electrospray ionization (ESI) in selected ion monitoring (SIM) mode. The method was validated in terms of selectivity, linearity, sensitivity, and recovery. **Result** A significant difference was observed in main pharmacokinetic parameters of C_{max}, t_{max}, and AUC between acupoints administration and nonacupoints administration. **Conclusion** Acupoints administration resulted in a more obvious increase in bioavailability of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin than nonacupoints administration.

Key words

acupoints administration; Fufang Baijiezi Gel; LC-MS; microdialysis; pharmacokinetics

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1. Introduction

Acupoint drug administration needs small dosage and little time to attain the same effects, so it has extensive use at home and abroad (Chen et al, 2005). It has been reported that the delivery coefficient of aminophylline, which was defined with the ratio of steady state plasma concentration to the skin resistense in the acupoints, was higher than that in the Sham-acupoints (Liu et al, 2006).

Fufang Baijiezi Gel (FBG) is a novel dosage form of Baijiezi Fufang, also called Sanfu Patch or Xiaochuangao Acupoint Paste, and is effective in the treatment of asthma (Fan et al, 2013; Chang 2013; Wu et al, 2012; Chen, 2005; Lv et al, 2009). FBG, made of *Sinapis alba* L., *Corydalis yanhusuo* W. T. Wang., *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag., is applied in Feishu, Xinshu, and Geshu acupoints traditionally. Sinapine thiocyanate is one of the representative components in *S. alba*, tetrahydropalmatine and 6-gingerol in *C. yanhusuo*, and asarinin in *A. heterotropoides* var. *mandshuricum* (Pharmacopoeia Committee of P. R. China. 2010). Permeation of sinapine thiocyanate and tetrahydropalmatine in acupoint skin is reportedly better than in non-accupoint skin (Liu et al, 2013; Guo et al, 2012). However, permeative properties of other components in FBG and their systemic pharmacokinetics are undocumented. Sinapine thiocyanate,

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tetrahydropalmatine, 6-gingerol, and asarinin are active components in FBG. In this study, microdialysis (Xu et al, 2012) coupled with LC-MS was developed, validated and used to study both the dermal and systemic pharmacokinetics of the four kinds of active components in FBG for different acupoint drug deliveries which provided an important advance of acupoints drug administration.

2. Materials and methods

2.1 Chemicals and materials

Sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were purchased from National Institutes for Food and Drug Control (Beijing, China). CMA 20 MD Elite probes (CMA 30, membrane 10 mm × 0.5 mm o.d., Microdialysis AB, Dalv.10, SE−16956 Solna, Sweden) were used. Acetonitrile was of HPLC grade (Merck KgaA, Germany). All other reagents were of analytical grade. De-ionized distilled water was used throughout the experiments.

2.2 Animals

Guinea pigs weighing (250 ± 20) g were obtained from West Hill Farm (Beijing, China) and housed with unlimited access to food and water except for 12 h fasting before the experiment. The animals were maintained on a 12-h light/12-h dark cycle (light on at 8:00) at ambient temperature (22–25 ^{o}C) and relative humidity of 60%. All experimental animal surgery procedures were reviewed and approved by Institutional Animal Experimentation Committee of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (Beijing, China).

2.3 LC-MS conditions

The LC-MS system consisted of the 1200 series HPLC system (Agilent Technologies, USA) and a single quadruple mass spectrometer (Product No. G2710BA, Agilent Corp, USA) equipped with electrospray ionization (ESI) source. The analytical column Ultimate® XB-Phenyl column (150 mm × 2.1 mm, 5 μm) was used. The mobile phase consisted of acetonitrile (solvent A) and 0.1% ammonium acetate buffer solution (pH 6.0, solvent B) at the flow rate of 0.3 mL/min. The chromatograph was developed with a 10 min linear gradient from 30% to 60% solvent A, then with an isocratic elution of 60% solvent A for 5 min followed by 5 min re-equilibration.

The MS operating conditions were optimized as follows: Dry air flow rate was 8.0 L/min capillary voltage 2.41 × 10^5 Pa, field voltage of 3.5 kV, ion source temperature of 150 ^{o}C, desolvation gas temperature of 350 ^{o}C. Quantification was obtained using the SIM mode of the transitions at *m/z* 368→310 for sinapine thiocyanate, *m/z* 391→356 for tetrahydropalmatine, *m/z* 294→277 for 6-gingerol, and *m/z* 354→372 for asarinin. Data acquisition and processing were accomplished using the ChemStation Software.

2.4 Stock and working solutions

Stock solutions of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin with concentration of 220, 50, 120, and 80 ng/mL were prepared in methanol with physiological saline, respectively, and stored at −20 ^{o}C away from light. Working solutions used for spiking plasma were all freshly prepared by diluting the stock solution with physiological saline to the appropriate concentration. All working solutions were refrigerated at 4 ^{o}C.

The calibration standard samples were prepared by freshly spiking the appropriate working solution into blank plasma at the concentration of 0.22−220 ng/mL for sinapine thiocyanate, 0.12−120 ng/mL for tetrahydropalmatine, 0.05−50 ng/mL for 6-gingerol, and 0.08−80 ng/mL for asarinin, and processed as described in the sample preparation. The quality control (QC) samples used for the intra- and inter-day accuracy and precision, extraction recovery, and stability study were prepared in the same way as the preparation of calibration standard samples.

2.5 Dialysate sample preparation

To 50 μL of each microdialysis sample, 50 μL of methanol was added and vortexed for 3 min. Five microliters aliquots of the mixture were injected onto LC-MS system.

2.6 Method validation

Calibration curves were generated by using the peak area of the analytes versus concentration. Accuracy and precision were assessed by determining QC samples of low, medium, and high concentration in five replicates on consecutive 3 d. The concentration of each sample was calculated by daily calibration curve. Accuracy was expressed as the relative error (RE) and precision as the coefficient of variation (CV). The extraction recoveries of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin at QC sample concentration were determined by comparing the peak areas of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin from blank microdialysates spiked prior to the extraction with those from blank microdialysates spiked post-extraction in five replicates. The total recovery was evaluated by comparing the peak areas obtained from the above extraction experiment with those from the corresponding neat standard solutions at QC sample concentration. The stability of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin in microdialysates were evaluated by QC samples placed at room temperature for 24 h and stored at −20 ^{o}C for three weeks in triplicate. The stability of reconstituted samples in autosampler vials was assessed at 20 ^{o}C for 24 h.

2.7 Drug administration

After 1 h of stabilization, FBG (1 g) was applied topically to the skin at a dose of 0.25 g/cm^2 at the microdialysis probe

cutaneous implantation point. Using an HPLC assay, the contentration of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were determined to be 628, 374, 65, and 77 mg/g of gel, respectively. The skin surface was covered with polyethylene film during the experiment (occlusive dressing technique). After 5 h of application, the remaining gel was gently removed with paper cloths without rubbing skin. Both the blood and dermal dialysis samples were collected in a 200 μL Eppendorff tube at 60 min intervals for 24 h and then stored at −20 °C until analysis.

2.8 In vivo microdialysis experiments

The Guinea pigs were ip anesthetized with 1 g/kg urethane and remained anesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. The body temperature of Guinea pigs was maintained at 37 °C during the experimental procedure. The fur in acupoints and non-acupoint regions of the Guinea pigs was removed with an electric rodent hair clipper 1 d before the study, respectively. Before the pharmacokinetic study, one blood microdialysis probe [10 mm membrane length and 5000 molecular mass (Mr) cut-off] was implanted in the femoral vein over a guide cannula and positioned toward the right atrium. Then, one dermal microdialysis probe (30 mm membrane length and 5000 molecular mass cut-off) was implanted in the dermis of the acupoint or non-acupoint region of the Guinea pigs. Both probes were connected to a micro injection pump and perfused with 0.9% saline at a flow rate of 1.5 μL/min (Shinkai et al, 2011; Tsai, 2009).

2.9 Recovery of microdialysate

For *in vivo* recovery, the blood and dermal probes were ip inserted into the femoral vein/right atrium and dermis of Guinea pig under anesthesia with urethane (1.0 g/kg). Perfusion solutions containing sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were passed through the microdialysis probe into blood and dermis of Guinea pig, respectively, at a constant flow rate of 1.5 μL/min using an infusion pump (BAS Microdialysis).

Following a stabilization period of 1 h post probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentration of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were determined by LC-MS. The *in vivo* relative recovery (R_{dial}) of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin across the microdialysis probe was calculated by the following equation: $R_{dial} = (C_{perf} - C_{dial}) / C_{perf}$ (Shinkai et al, 2011; Tsai et al, 2009). The microdialysate concentration (C_m) of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin was converted to unbound concentration (C_u) as follows: $C_u = C_m / R_{dial}$.

2.10 Pharmacokinetic analysis

Drug concentration in plasma of Guinea pig and dermal microdialysates were calculated from relative recovery and drug levels in dialysates. Pharmacokinetic parameters were calculated for each subject using non-compartmental methods by the Kinetica 5.1 software. The data were expressed as $\bar{x} \pm s$.

3. Results and discussion

3.1 Optimization of analytical condition

In this assay, sensitive and specific LC-MS was implemented with isocratic elution in run time of 30 min per sample. Small amounts of ammonium acetate were added to the mobile phase as a modifier that promotes the deprotonation in ESI negative mode. Acetonitrile was used to separate and elute analytes, and provided symmetric chromatographic peaks with lower background noise than methanol. The product ion spectrum of sinapine thiocyanate $[M - N{\equiv}S^-]^+$ at *m/z* 310.2, tetrahydropalmatine $[M - Cl^-]^+$at *m/z* 356.2, 6-gingerol $[M - OH^-]^+$ at *m/z* 277.2, and asarinin $[M + NH_4^+]^+$ at *m/z* 372.1 was shown in Figure 1.

3.2 Specificity

Specificity was assessed by screening analysis of six blank microdialysates of Guinea pig, comparing the chromatograms of blank microdialysates with the corresponding spiked ones to check interference. The retention time of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin was 2.3, 9.1, 10.2, and 14.0 min, respectively. The typical chromatogram (Figure 2) showed no significant interference peaks at the retention time of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin.

3.3 Linearity, precision, accuracy, and stability

In the present study, linear calibration curves were constructed over the concentration ranges of 0.22−220 ng/mL for sinapine thiocyanate, 0.12−120 ng/mL for tetrahydro- palmatine, 0.05−50 ng/mL for 6-gingerol, and 0.08−80 ng/mL for asarinin, respectively. The correlation coefficients (R^2) for all calibration curves were over 0.99. The precision of relative standard deviation (RSD) values for intra-day and inter-day were all within 7%, whilst accuracy was within (100 ± 7)% of the actual values at each QC level.

These results suggested that the present assay had acceptable accuracy and precision. The stability results (data not shown) showed that the concentration of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were between 93.9% and 102.1% of the initial values, indicating that the analytes were stable in Guinea pig plasma for at least 30 d storage at −80 °C and for three freeze-thaw cycle process, and in the reconstituted solutions for 24 h storage in the auto-sampler (−15 °C).

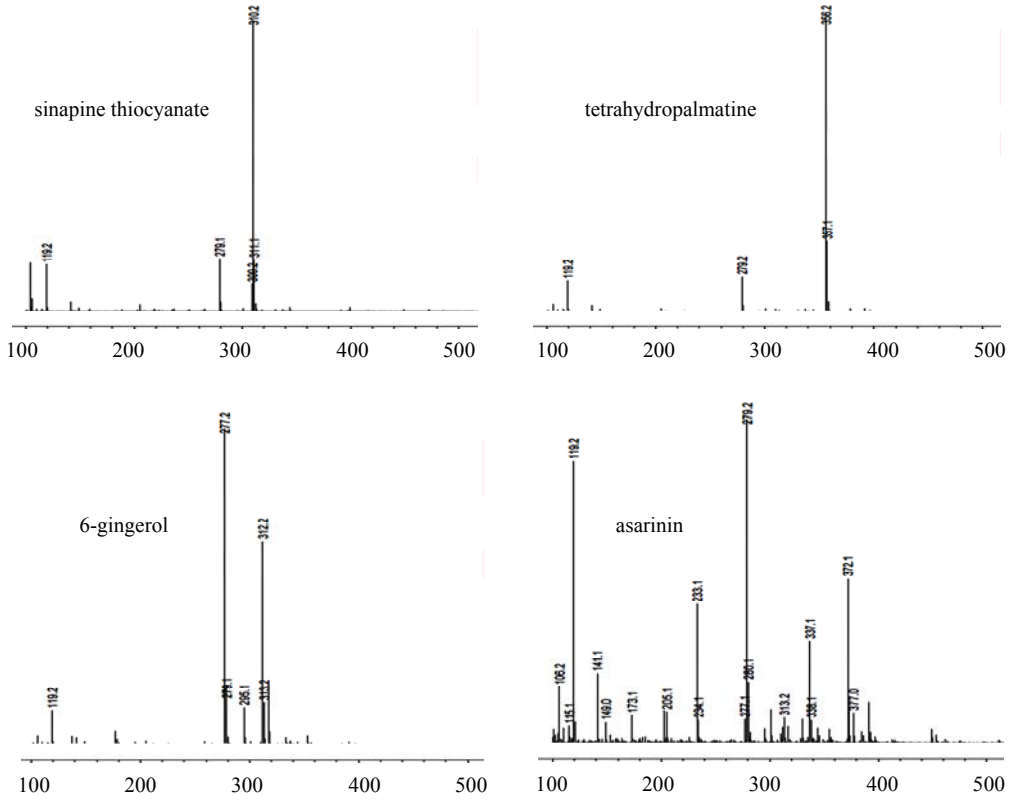

Figure 1 Ion scan of sinapine thiocyanate, 6-gingerol, tetrahydropalmatine, and asarinin

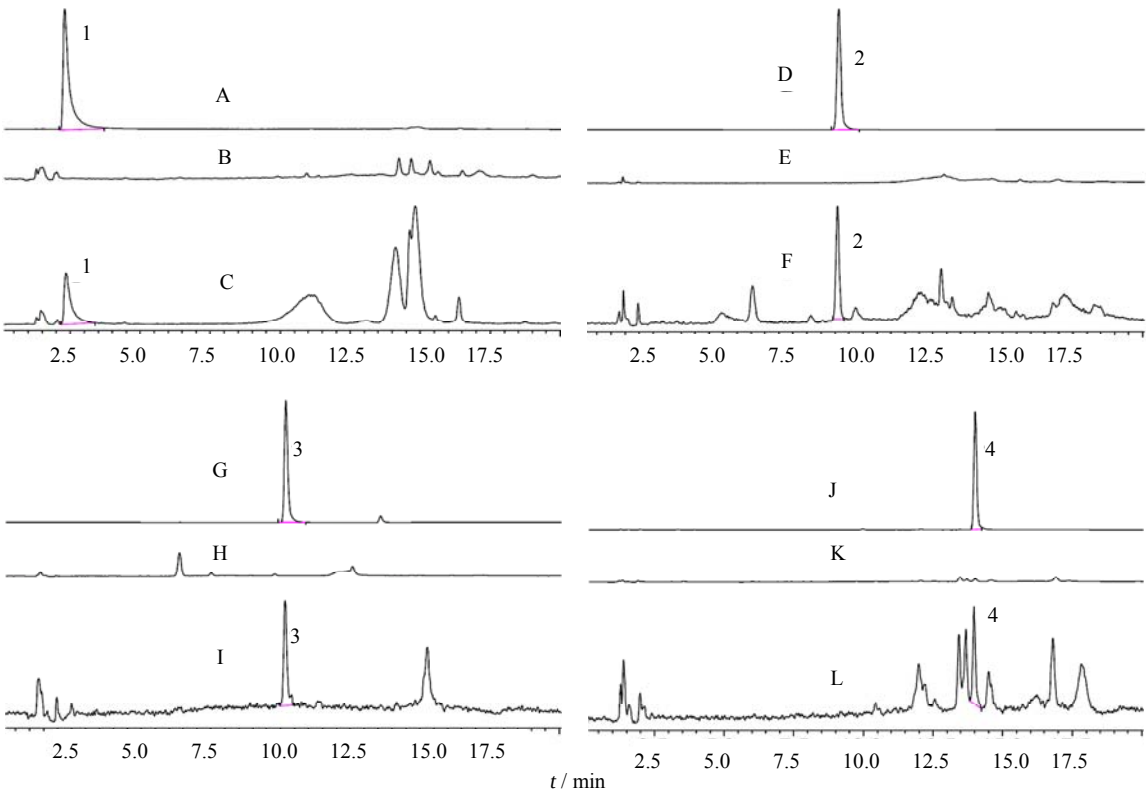

Figure 2 Determination of sinapine thiocyanate, 6-gingerol, tetrahydropalmatine, and asarinin in dialysis fluid by LC-MS

Channal-310.2: A−C; Channal-277.2: D−F; Channal-356.2: G−I; Channal-372.1: J−L.
standard substances: A, D, G, J; blank microdialysates: B, E, H, K; microdialysates: C, F, I, L.
1: sinapine thiocyanate; 2: 6-gingerol; 3: tetrahydropalmatine; 4: asarinin

3.4 In vivo recovery of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin from microdialysis probe

The average *in vivo* recovery levels of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin were between 40% and 45%. The sample concentration was corrected by the probe recovery before pharmacokinetic data analysis.

3.5 Pharmacokinetic study

The mean concentration-time profiles of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin in subcutaneous tissue fluid and plasma were illustrated in Figures 3 and 4, respectively, and their estimated pharmacokinetic parameters were presented in Tables 1 and 2, respectively.

4. Conclusion

Acupoint drug administration mechanism is unclear at present. Using microdialysis coupled with LC-MS, we have studied the pharmacokinetics of the four components in FBG for different acupoint drug delivery. An LC-MS assay method was successfully developed and validated to determine sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin in Guinea pig subcutaneous tissue fluid and plasma microdialysates simultaneously. Using this method, pharmacokinetics of the four active components in FBG is investigated after acupoints and nonacupoints administration. Acupoints administration results in a more obvious increase in bioavailability of sinapine thiocyanate, tetrahydropalmatine, 6-gingerol, and asarinin than nonacupoints administration according to the above LC-MS method. This study reveals the advantage of acupoint drug delivery in traditional Chinese medicine (TCM), which has certain reference significance for study on the acupoint drug delivery in TCM.

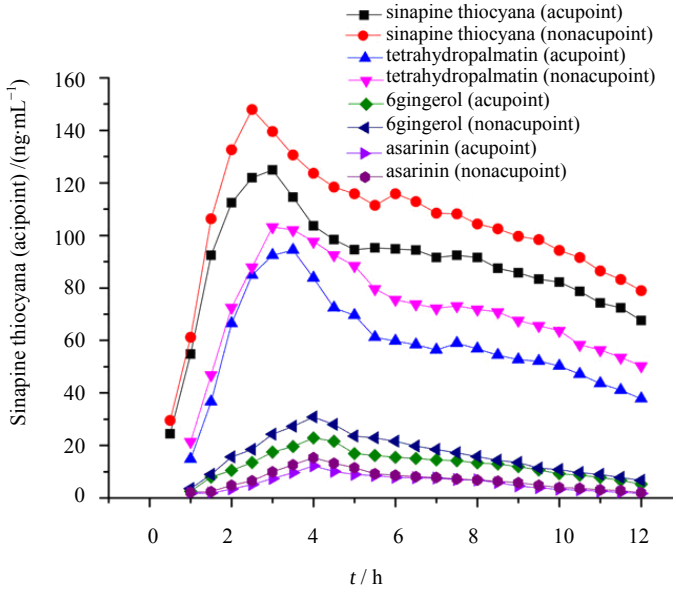

Figure 3 Mean concentration–time profiles of FBG in subcutaneous tissue fluid after different points administration (*n* = 5)

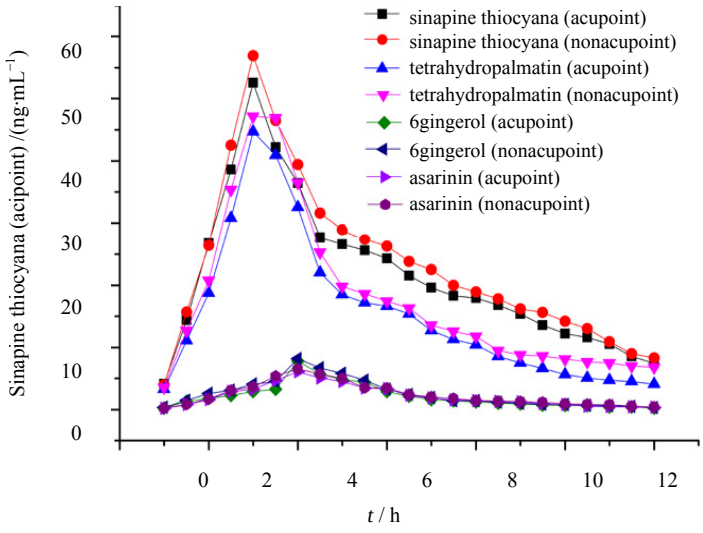

Figure 4 Mean concentration–time profiles of FBG in plasma after different points administration (*n* = 5)

Table 1 Topical transdermal pharmacokinetic parameters of FBG

Components	Area	C_{max} / (ng·mL^{-1})	t_{max} / h	AUC_{last} / (ng·mL^{-1}·h)	AUC_{tot} / (ng·mL^{-1}·h)	MRT / h
Sinapine thiocyanate	acupoint	147.9 ± 11.2	2.5	1238.0 ± 61.4	2142.0 ± 111.2	13.3 ± 0.72
	non-acupoint	125.0 ± 8.7	3.0	1056.0 ± 52.5	1775.0 ± 94.7	12.7 ± 0.66
tetrahydropalmatine	acupoint	103.2 ± 9.6	3.0	824.2 ± 46.3	1290.0 ± 72.3	11.5 ± 0.54
	non-acupoint	94.4 ± 10.3	3.5	674.9 ± 41.9	964.1 ± 51.4	10.0 ± 0.58
6-gingerol	acupoint	30.8 ± 2.4	4.0	188.7 ± 10.2	221.9 ± 11.9	7.5 ± 0.51
	non-acupoint	22.8 ± 2.1	4.0	147.7 ± 8.6	169.1 ± 8.8	7.2 ± 0.43
asarinin	acupoint	15.3 ± 1.4	4.0	80.6 ± 6.7	88.0 ± 6.8	6.5 ± 0.63
	non-acupoint	12.2 ± 1.1	4.0	66.9 ± 4.9	72.4 ± 5.9	6.5 ± 0.46

Table 2 Systemic pharmacokinetic parameters of FBG

Components	Area	C_{max} / (ng·mL^{-1})	t_{max} / h	AUC_{last} / (ng·mL^{-1}·h)	AUC_{tot} / (ng·mL^{-1}·h)	MRT / h
sinapine thiocyanate	acupoint	56.9 ± 6.6	3.0	268.4 ± 14.4	319.6 ± 16.5	7.3 ± 0.46
	non-acupoint	52.6 ± 7.1	3.0	247.0 ± 12.8	283.8 ± 15.4	6.8 ± 0.35
tetrahydropalmatine	acupoint	47.2 ± 4.2	3.0	201.8 ± 13.7	251.0 ± 13.8	7.9 ± 0.41
	non-acupoint	44.7 ± 4.2	3.0	177.3 ± 10.6	193.1 ± 9.82	6.8 ± 0.29
6-gingerol	acupoint	8.16 ± 0.72	4.0	28.82 ± 1.02	29.92 ± 1.64	5.3 ± 0.34
	non-acupoint	7.80 ± 0.83	4.0	24.31 ± 1.44	25.08 ± 1.94	5.4 ± 0.27
asarinin	acupoint	6.55 ± 0.79	4.0	26.96 ± 1.29	28.63 ± 1.68	5.8 ± 0.22
	non-acupoint	6.05 ± 0.65	4.0	24.23 ± 1.47	25.20 ± 1.57	5.5 ± 0.30

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