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Original Article

Metabolic and pharmacokinetic studies of scutellarin in rat plasma, urine, and feces

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Aim: To study the metabolic and pharmacokinetic profile of scutellarin, an active component from the medical plant *Erigeron breviscapus* (Vant) Hand-Mazz, and to investigate the mechanisms underlying the low bioavailability of scutellarin though oral or intravenous administration in rats.
Methods: HPLC method was developed for simultaneous detection of scutellarin and scutellarein (the aglycone of scutellarin) in rat plasma, urine and feces. The *in vitro* metabolic stability study was carried out in rat liver microsomes from different genders.
Results: After a single oral dose of scutellarin (400 mg/kg), the plasma concentrations of scutellarin and scutellarein in female rats were significantly higher than in male ones. Between the female and male rats, significant differences in AUC, t_{max2} and C_{max2} for scutellarin were found. The pharmacokinetic parameters of scutellarin in the urine also showed significant gender differences. After a single oral dose of scutellarin (400 mg/kg), the total percentage excretion of scutellarein in male and female rats was 16.5% and 8.61%, respectively. The total percentage excretion of scutellarin and scutellarein in the feces was higher with oral administration than with intravenous administration. The *in vitro* $t_{1/2}$ and CL_{int} value for scutellarin in male rats was significantly higher than that in female rats.
Conclusion: The results suggest that a large amount of ingested scutellarin was metabolized into scutellarein in the gastrointestinal tract and then excreted with the feces, leading to the extremely low oral bioavailability of scutellarin. The gender differences of pharmacokinetic parameters of scutellarin and scutellarein are due to the higher CL_{int} and lower absorption in male rats.

Keywords: scutellarin; scutellarein; metabolism; pharmacokinetics; excretions; gender differences

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Introduction

Breviscapines are flavones extracted from the medical plant *Erigeron breviscapus* (Vant) Hand-Mazz. One of the active components of breviscapine is scutellarin ($C_{21}H_{18}O_{12}$; 4′,5,6-hydroxyl-flavone-7-glucuronide). Scutellarin is hydrolyzed *in vivo* to form scutellarein (4′,5,6,7-hydroxyl-flavone), an aglycone of scutellarin, and it is further metabolized to form conjugated metabolites. Scutellarin has been extensively used for the clinical treatment of cardiovascular and cerebrovascular diseases in China for many years. Pharmacological research indicates that scutellarin is associated with anti-hypertrophic effects[1], fibrinolysis and anticoagulant effects[2], protective effects in the brain and heart[3], hypercholesterolemia suppression[4], and endothelium-independent relaxation induction[5]. Scutellarin produces an anti-inflammatory effect via actions on arachidonic acid metabolism[6]. The compound has also been shown to have some anti-HIV effects[7] and to attenuate hepatocellular damage[8]. It is interesting that scutellarin exhibits weak estrogenic properties[9, 10], similar to genistein and apigenin. Many phytoestrogen compounds, such as genistein, apigenin, daidzein, glycitein, and their metabolites, have been demonstrated pharmacokinetic differences between male and female in humans and rats.

Gender differences affect the metabolism, excretion, and bioavailability of soy isoflavones in humans and rats[11]. To date, the pharmacokinetic profiles of scutellarin and scutellarein in different genders are unknown. Therefore, this study represents to investigate preliminary pharmacokinetics in male and female rats following administration of single oral and intravenous doses of scutellarin.

There are increasing benefits for scutellarin use in patients; however, problems with its analysis in biological samples have been reported in many studies. Pharmacological profiles of the parent drug and its metabolites in plasma[12, 13], tissues[14],

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urine[15], and bile[16] in humans and rats have been evaluated using high-performance liquid chromatography (HPLC) with ultraviolet detector or HPLC with mass spectrometer. After oral administration of scutellarin, only a minute amount of the compound was detected in human blood[13], and its bioavailability was estimated to be only 10.67%[17]. However, substantial amounts of scutellarein were present in blood and urine in humans[18]. Scutellarein has similar pharmacological effects to scutellarin, but it is more potent than scutellarin. Previous studies have shown that scutellarin consistently displays double peaks in its plasma concentration-time curves[14]. Although the pharmacokinetics and metabolism of scutellarin have been extensively studied in humans and animals, the reasons for its extremely low oral bioavailability have not been elucidated. Prior to this study, there was very limited information available on the excretion dynamics of scutellarin. Most studies focused on excretion through the urine[19] with less emphasis on excretion through the feces. Scutellarin and scutellarein were found to be negligible in the urine in this present study. Hao *et al*[20] asserted that the gastrointestinal tract played an important role in scutellarin's bioavailability. Accordingly, fecal excretion of scutellarin and scutellarein was investigated in this study, and this is the first report on the fecal excretion process and the pharmacokinetics in different genders of rats for these compounds. This study provides evidence that improving the formulation design of scutellarin in a drug delivery system would result in better bioavailability and efficacy.

Materials and methods

Chemicals and reagents

Scutellarin (95% purity) was obtained from Gejiu Bio-Medicine Industry Ltd (Yunnan, China). Quercetin, the internal standard (batch No 100081-200406; 97.3% purity by HPLC) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Scutellarein (98% purity) was obtained from DELTA Medicine Science Ltd (Anhui, China). β-NADPH was purchased from Sigma Chemical Co (St Louis, Mo, USA). HPLC-grade methanol was purchased from Fisher Scientific Co (Somerville, NJ, USA), and acetic ether was purchased from the National Medicine Corporation (Shanghai, China). Triple-distilled water was used throughout the experiments.

Animals

Sprague-Dawley rats were obtained from the Experimental Animal Center of Xi-an Jiaotong University (Xi-an, China). All rats were maintained under standard conditions with access to food and water *ad libitum*. They were housed in stainless steel cages in standard laboratory conditions (a regular 12 h day-night cycle in a well-ventilated room with an average temperature of 25–28 °C and a relative humidity of 40%–60%). The animal experimental protocol was approved by the University Ethics Review Committee for Animal Experimentation.

Intravenous and oral administrations

Pharmacokinetics

Twenty Sprague-Dawley rats (weighing 280 to 320 g; 10 males and 10 females, 4–6 months old) were fasted for 16 h but allowed water *ad libitum* the day before drug administration. Scutellarin was resuspended in 0.5% carboxymethylcellulose sodium, and it was administered to rats (400 mg/kg) by oral gavage. Blood samples (0.2 mL) were collected from the tail vein of each rat before dosing and at 5, 10, 20, 40, and 80 min and 2, 4, 6, 8, 11, 16, 24, and 36 h post-dosing. Plasma samples (0.1 mL) were obtained by centrifugation immediately after blood collection and were stored at -20 °C until analysis.

Excretion

Forty Sprague-Dawley rats (weighing 200 to 220 g; 20 males and 20 females, 3–4 months old) were housed in metabolic cages and used to study the fecal and urinary excretions of scutellarin. They were fasted for 16 h but allowed water *ad libitum* the day before drug administration. The urine and feces samples were collected 0–4, 4–8, 8–12, 12–16, 16–24, 24–32, 32–40, 40–48, 48–60, and 60–72 h after intragastric or intravenous administration of scutellarin (400 or 40 mg/kg, respectively). The volume of urine and weight of the dried feces were recorded.

Instruments

The Waters HPLC system (Milford, MA, USA) consisted of a Waters 515 pump, a Waters 717 autosampler, and a Waters 2996 diode-array-detector. The HPLC system was interfaced to a computer through Empower Pro software. A refrigerated centrifuge (Beckman Coulter™, Allegra™ X-ZZR), the Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and a pressurized gas blowing evaporator (HBC-12) were used to process the samples.

Chromatographic conditions

An ODS-2 HYPERSIL C_{18} analytical column (250 mm×4.6 mm, 5 μm) (Thermo Scientific Corporation, USA) with a C_{18} guard column (4 mm×3 mm, 5 μm) (Phenomenex Corporation, Torrance, CA, USA) was used in chromotography. The mobile phase consisted of methanol and water at a ratio of 1:1 (*v*/*v*), and the pH was adjusted to 2.5 with phosphoric acid (1 mol/L). The mobile phase was delivered at a flow rate of 1 mL/min. The diode array detector was operated at 335 nm.

Sample preparation

Preparation of standard solutions

Stock solutions of scutellarin (250 μg/mL), scutellarein (50 μg/mL), and quercetin (the internal standard (190 μg/mL)) were prepared in methanol. A series of standard working solutions (scutellarin 20, 10, 5, 2.5, 1.25, 0.625 μg/mL; scutellarein 5, 2.5, 1.25, 0.625, 0.312, 0.156 μg/mL) were prepared by further diluting the standard stock solution in the mobile phase (pH 2.5). Appropriate amounts of the working solution were diluted in the pooled blank plasma, urine, and fecal

samples to cover the respective calibration standard ranges. All samples were stored at 4 °C and brought to room temperature before use.

Preparation of plasma samples

Quercetin stock solution (10 μL; 2.5 μg/mL) was transferred to a 10 mL tapered glass tube and evaporated to dryness under a stream of nitrogen. Plasma (100 μL) and phosphoric acid (50 μL; 1 mol/L) solutions were then added to the glass tube and mixed for 1 min before being extracted with acetic ether (1 mL) by vortex mixing for another 3 min. The glass tubes were then centrifuged at 3000 revolutions per minute for 10 min. The upper organic phase was transferred to a clean tube and evaporated to dryness at 35 °C under a stream of nitrogen. The residue was immediately reconstituted in the mobile phase (100 μL) and centrifuged at 10 000 revolutions per minute at 4 °C for 10 min. The supernatant (80 μL) was transferred into an autosampler vial, and 50 μL of it was injected into the HPLC system.

Preparation of the urine and feces samples

The blank urine and feces samples were collected from rats housed in metabolic cages. Quercetin (10 μL; 2.5 μg/mL) was transferred to a 10 mL tapered glass tube and evaporated to dryness under a stream of nitrogen. The collected urine samples were centrifuged at 10 000 revolutions per minute for 10 min, and the urine sample (200 μL) and phosphoric acid solution (100 μL; 1 mol/L) were added to the glass tube. This mixture was sonicated for 1 min, vortexed for 5 min, and centrifuged at 10 000 revolutions per minute for 10 min. Then the mixture (20 μL) was injected into the HPLC system for analysis.

The rat feces were first dried at room temperature and ground to powder with a mortar. The dry feces powder (0.2 g) was then sonicated in methanol (2 mL) for 10 min. Distilled water (1 mL) was then added to the sample, and another sonication step was performed. The samples were centrifuged at 10 000 revolutions per minute for 10 min, and the supernatant was filtered through a 0.45 μm membrane and transferred into an autosampler vial. Samples (20 μL) were injected into the HPLC system for analysis.

Mechanistic study on gender differences

Liver microsomal preparation

Pooled rat liver microsomes from both genders were prepared according to the method reported previously[21]. Three male and three female rats (weighing 200 to 220 g; 3–4 months old) were euthanized with CO_2 after a 24-h fasting period. The pooled, extracted liver microsomes were suspended in Tris buffer (0.1 mol/L; pH 7.4) and stored at -80 °C before use. Rat microsomal protein contents were determined by a Nanodrop 1000.

Liver microsomal incubations

The scutellarin stock solution in dimethyl sulfoxide was added to Tris buffer (0.1 mol/L; pH 7.4) with the rat liver microsomes. The mixture was first shaken for 5 min to equilibrate in a shaking water bath at 37 °C. Incubation was then initiated by adding a β-NADPH solution. The final concentrations of scutellarin, NADPH and the microsomal protein in the incubation mixture (1.5 mL) were 30 μmol/L, 1 mmol/L and 2 mg/mL, respectively. The percentage of dimethyl sulfoxide in the incubation mixture was kept less than 0.5% (*v*/*v*). Incubation sample mixtures (50 μL) were collected at 0, 10, 20, 30, 40, 50, 60, and 90 min. The reactions were terminated with ice-cold methanol (100 μL) to precipitate the proteins, and the samples were subsequently centrifuged at 16 000×*g* for 15 min. Negative controls were prepared by adding NADPH, which was followed by immediate termination using ice-cold methanol and considered the 0 min aliquots. Supernatants (50 μL) were collected and analyzed by HPLC.

Pharmacokinetic and statistical analyses

The pharmacokinetic parameters in plasma and urine were estimated using the DAS2.0 software (Drug and Statistics, version 2.0, Mathematical Pharmacology Professional Committee of China, Shanghai)[22]. Differences in the plasma scutellarin concentrations at each time point and differences in the pharmacokinetic parameters (AUC, $t_{1/2}$, t_{max}, C_{max}) in the plasma and urine between male and female rats were analyzed using ANOVA and a Student's *t*-test with the SSPS 11.5 software pack. $P<0.05$ was considered statistically significant. Data are presented as the mean±SD.

Results

Selectivity

Six individual, blank rat plasma, urine, and feces samples were analyzed to determine if anything in the matrix interfered with the analytes. Under the optimized HPLC conditions, scutellarin, an unknown metabolite, scutellarein, and quercetin were separated chromatographically with retention times of 5.1, 6.4, 8.9, and 11 min, respectively. No interference was observed at these retention times (Figure 1).

Linearity of the calibration curves and lower limit of quantification

Calibration curves were constructed based on the peak area ratios of the analytes to the internal standard *versus* the concentrations. These were made using a weighted (1/c) liner regression analysis. Good linear relationships were established for both scutellarin and scutellarein in the plasma (0.1 mL), urine (0.2 mL), and fecal (0.2 mL) homogenates. The lower limits of quantification (LLOQ) were defined as signal-to-noise ratios (S/N) greater than 10, and they were evaluated by analyzing six replicates of the biological samples spiked with scutellarin and scutellarein. The results of the calibration curves and LLOQ are summarized in Table 1.

Precision and accuracy

Intra-day accuracy and precision were evaluated by analyzing the quality control (QC) samples (n=5) at different time points in the same day. They were determined by repeated

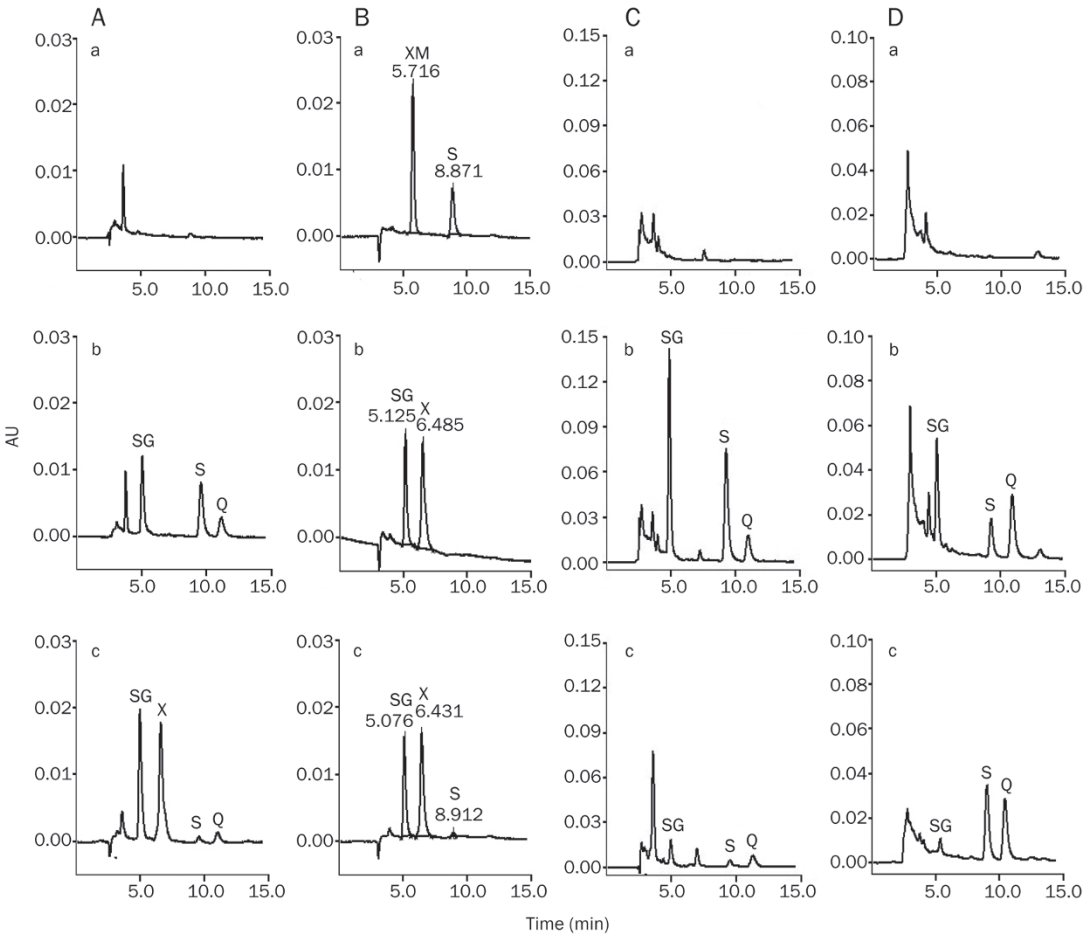

Figure 1. HPLC chromatograms of scutellarin and scutellarein in biological samples. Panel A: (a) Blank plasma sample; (b) Blank plasma spiked with scutellarin, scutellarein and internal standard; (c) Plasma sample at 8 h after an oral administration of scutellarin (400 mg/kg). Panel B: (a) Plasma sample collected at 8 h after treatment with β-glucuronidase; (b) Plasma sample at 8 h after treatment with sulfatase; (c) Plasma sample at 8 h without any enzyme treatment and no internal standard added to the sample. Panel C: (a) Blank urine sample; (b) Blank urine spiked with scutellarin, scutellarein and internal standard; (c) Urine sample at 0–4 h after oral administration of scutellarin (400 mg/kg). Panel D: (a) Blank feces solution; (b) Blank feces solution spiked with scutellarin, scutellarein and internal standard; (c) Blank feces solution sample collected at 8–12 h after oral administration of scutellarin (400 mg/kg). SG, scutellarin; X, scutellarin metabolite; XM: compound formed after treatment with β-glucuronidase; S, scutellarein; Q, quercetin.

Table 1. The calibration curves and LLOQ of scutellarin and scutellarein in the respective matrix of plasma, urine, and feces.

Samples	Calibration curve	Linear range (μg/mL)	*r*	LLOQ (μg/mL)
Plasma	*y*(scutellarin)=0.0009684 *x*+0.0228	0.06–2.0	0.9980	0.06
Plasma	*y*(scutellarein)=0.007873 *x*+0.2151	0.02–0.50	0.9977	0.02
Urine	*y*(scutellarin)=0.0955 *x*+0.0658	0.64–41.0	0.9997	0.64
Urine	*y*(scutellarein)= 0.3320 *x*+0.0355	0.16–10.4	0.9996	0.16
Feces	*y*(scutellarin)=0.0831 *x*+0.0203	0.64–41.0	0.9999	0.64
Feces	*y*(scutellarein)= 0.2418 *x*–0.0157	0.16–10.4	0.9998	0.16

analyses of the QC samples on three different days (n=5). The concentration of each sample was determined with newly prepared calibration standards. To determine the absolute recovery, non-extracted samples (pure sample freshly prepared in methanol) and a set of post-extracted spiked samples were analyzed in the same run. These post-extracted spiked samples were at three concentrations in the plasma (2, 0.5, and 0.12 μg/mL for scutellarin and 0.5, 0.12, and 0.03 μg/mL for

scutellarein, respectively) and in the urine and feces (20.5, 5.13, and 1.28 μg/mL for scutellarin and 5.2, 1.3, and 0.32 μg/mL for scutellarein, respectively). Absolute recovery was determined by measuring the peak-area ratio of a post-extracted sample against the non-extracted samples. The results of these analyses are shown in Table 2.

Stability

Scutellarin has a structure almost identical to baicalin and is stable in acidified biological samples[23]; however, baicalin lacks the 4′-hydroxyl group that scutellarin has. Oxidation-reduction reactions, which are mediated by phenol radicals, are the major cause for the degradation of these compounds in biological samples. Previous studies have shown that acidic conditions can stabilize these flavonoids in solutions and biological samples[24]. The pH is responsible for degrading baicalin[24] but not the matrix. Therefore, in this study, scutellarin and scutellarein were stabilized by adjusting the pH to 2.5 in all samples with phosphoric acid (1 mol/L).

The stability of scutellarin and scutellarein during sample storage and processing was evaluated by analyzing the QC samples. At room temperature for 8 h, the scutellarin and scutellarein concentrations in the biological samples varied by less than ±9.90%, and the responses did not vary by more than 7.74% after 24 h of storage at 4 °C. The concentration variations were within ±11.13% of the nominal concentrations after three cycles of freezing at -20 °C, and thawing at 20 °C showed no significant loss of scutellarin or scutellarein. The processed samples were reconstituted in the mobile phase and placed at room temperature (25 °C) in the autosampler for 8 h, and scutellarin and scutellarein showed very good post-preparation stabilities with RSDs less than 10.43%. When the processed samples were stored at -20 °C for two weeks, scutellarin and scutellarein showed good stabilities at the concentrations studied with RSDs of ±11.44%.

Pharmacokinetic and excretion studies in rats

Pharmacokinetics

The mean plasma concentration-time curves exhibited double peaks in both male and female rats after oral administration of scutellarin (400 mg/kg) (Figures 2 and 3). Significantly higher plasma concentrations of scutellarin were measured in females

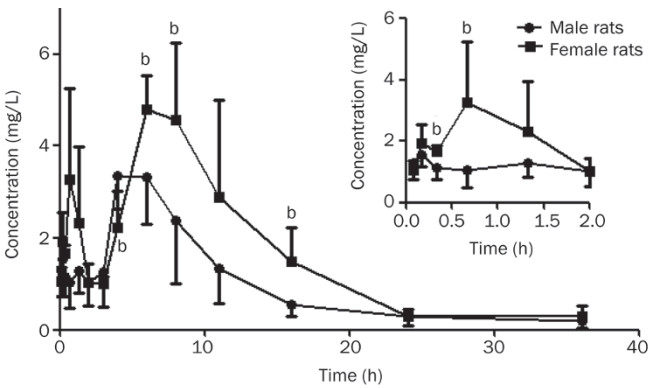

Figure 2. Plasma concentration-time curve of scutellarin after a single oral dose of 400 mg/kg scutellarin in both male and female rats (n=10). The smaller figure enlarged the original one whose data started to from 0 h to 2.0 h. Mean±SD. $^bP<0.05$ *vs* male rats.

Table 2. Precision of the assays and recoveries of scutellarin and scutellarein from the respective biological samples (mean±SD, n=5).

Samples	Added (μg/mL)	Intra-day Measured (μg/mL)	Intra-day RSD/%	Inter-day Measured (μg/mL)	Inter-day RSD/%	Relative recovery/%	Absolute recovery/%
Scutellarin in plasma	2.00	1.95±0.11	5.86	1.99±0.12	5.90	99.10±6.40	52.50±1.60
	0.50	0.47±0.04	7.70	0.49±0.04	8.80	95.60±8.10	62.10±1.80
	0.12	0.126±0.01	5.10	0.129±0.01	5.09	100.90±5.20	57.30±3.50
Scutellarein in plasma	0.50	0.50±0.01	2.00	0.525±0.02	3.81	100.52±2.12	59.81±2.78
	0.12	0.137±0.00	2.30	0.125±0.01	0.81	105.71±9.01	65.03±7.13
	0.03	0.029±0.00	7.20	0.029±0.00	7.80	94.73±8.41	59.82±2.70
Scutellarin in urine	20.50	20.15±0.19	0.94	20.37±0.60	2.93	98.29±0.92	77.64±0.37
	5.13	4.74±0.11	2.29	4.80±0.32	6.62	92.42±2.11	78.42±1.26
	1.28	1.12±0.01	1.12	1.12±0.01	0.96	87.45±0.98	87.23±1.30
Scutellarein in urine	5.20	5.31±0.05	0.92	5.35±0.07	1.37	102.04±0.94	73.22±0.23
	1.30	1.21±0.03	2.87	1.21±0.05	3.87	93.31±2.68	69.46±0.86
	0.32	0.33±0.01	2.45	0.30±0.02	5.41	100.10±2.45	70.01±2.25
Scutellarin in feces	20.50	20.61±0.41	2.01	20.38±0.63	3.09	100.53±2.02	94.56±5.16
	5.13	5.35±0.08	1.50	4.91±0.36	7.30	104.31±1.56	105.50±9.22
	1.28	1.17±0.05	4.37	1.19±0.09	7.25	90.94±3.97	92.55±6.33
Scutellarein in feces	5.20	5.11±0.45	8.82	5.53±0.40	7.14	98.34±8.67	66.17±2.80
	1.30	1.29±0.16	12.41	1.31±0.15	11.34	98.91±12.27	67.52±8.34
	0.32	0.34±0.80	0.83	0.33±0.02	6.09	106.01±0.88	66.48±6.77

RSD: relative standard deviation.

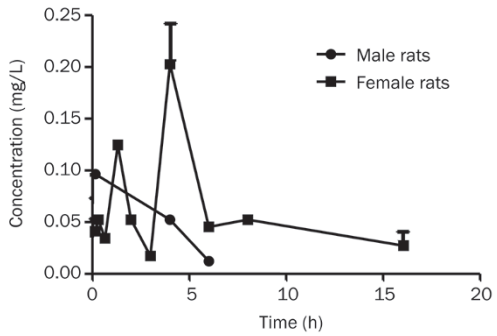

Figure 3. Plasma concentration-time curve of scutellarein after a single oral dose of 400 mg/kg scutellarin in both male and female rats. Mean±SD. *n*=10.

(P<0.05) at 20 and 40 min and at 4, 6, 8, and 16 h (Figure 2). Similar results were also noted for scutellarein in female rats (Figure 3). Significant differences for scutellarin in AUC, t_{max2}, and C_{max2} were noted between the male and female rats (P<0.05 or P<0.01) (Table 3).

Excretion

The urinary pharmacokinetic parameters of scutellarin and suctellarein after the oral administration of 400 mg/kg scutellarin and intravenous administration of 40 mg/kg scutellarin are shown in Table 4 and Table 5, and the urinary excretion rates of scutellarin and scutellarein are shown in Figure 4 and Figure 5. The percentages of the accumulated excretion of scutellarin and scutellarein in urine and feces are shown in Figure 6–9.

Table 4 and Table 5 show that the excretion percentage of scutellarein was lower than that of scutellarin. Scutellarin was excreted quickly, but scutellarein was excreted slowly from 0

Table 3. Parmacoknetics parameters for scutellarin after oral administration in both male and female rats (mean±SD, *n*=10). [b]P<0.05, [c]P<0.01 *vs* male rats.

Pharmaco-kenetics parameters	Units	Scutellarin	
		Male	Female
$AUC_{(0-t)}$	mg/L·h	34.71±9.88	56.08±9.28[c]
$AUC_{(0-\infty)}$	mg/L·h	35.71±9.72	57.30±10.46[c]
$t_{1/2}$	h	6.14±2.79	5.26±1.55
t_{max1}	h	0.18±0.09	0.43±0.23
t_{max2}	h	5.60±1.67	8.80±2.17[b]
C_{max1}	mg/L	1.78±0.37	3.56±1.86
C_{max2}	mg/L	3.96±0.77	5.29±0.97[b]

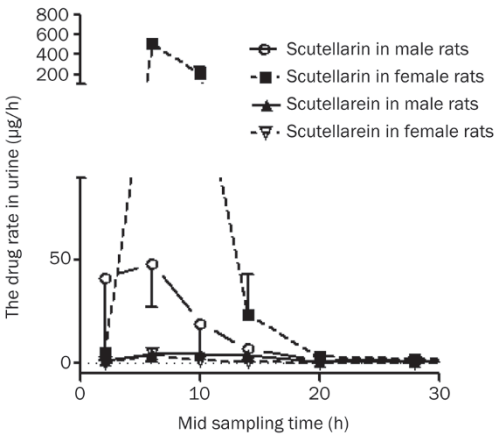

Figure 4. The mean excretion rate-time curve in male and female rats urine for both scutellarin and scutellarein after oral administration of 400 mg/kg scutellarin (*n*=10). Mean±SD.

Table 4. The pharmacokinetic parameters scutellarin and scutellarein in urine after oral administration of 400 mg/kg scutellarin in rats (mean±SD, *n*=10). [b]P<0.05, [c]P<0.01 *vs* male rats.

Pharmacokenetics parameters	Units	Male		Female	
		Scutellarin	Scutellarein	Scutellarin	Scutellarein
$t_{1/2}$	h	4.46±1.99	19.52±9.55	7.52±1.10[b]	13.89±2.81
*K*e	1/h	0.18±0.06	0.04±0.02	0.09±0.01[b]	0.05±0.01
The drug accumulation excretion in urine	%	0.53±0.21	0.11±0.05	0.96±0.20	0.02±0.00[c]

Table 5. The pharmacokinetic parameters scutellarin and scutellarein in urine after intravenous administration of 40 mg/kg scutellarin in rats (mean±SD, *n*=10). [b]P<0.05, [c]P<0.01 *vs* male rats.

Pharmacokenetics parameters	Units	Male		Female	
		Scutellarin	Scutellarein	Scutellarin	Scutellarein
$t_{1/2}$	h	3.37±0.16	5.29±0.48	3.42±1.26	1.65±0.37[c]
*K*e	1/h	0.21±0.01	0.13±0.01	0.22±0.07	0.43±0.09[c]
The drug accumulation excretion in urine	%	3.72±0.33	0.58±0.20	6.42±1.89	0.67±0.15[b]

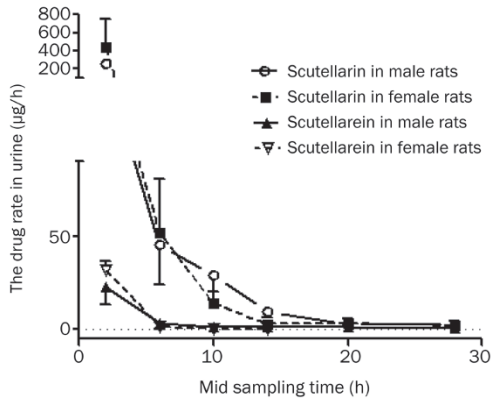

Figure 5. The mean excretion rate-time curve in male and female rats urine for both scutellarin and scutellarein after intravenous administration of 40 mg/kg scutellarin (n=10). Mean±SD.

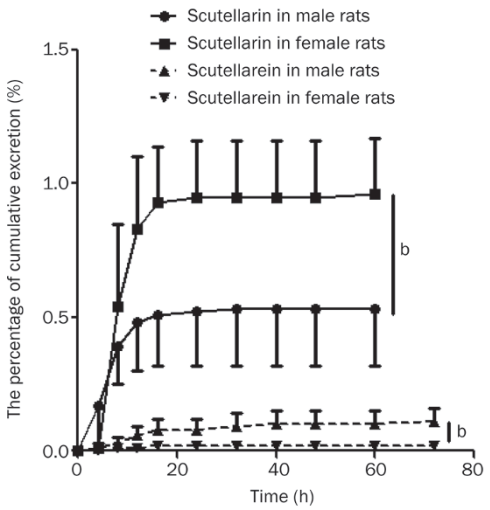

Figure 6. The percentage of cumulative excretion of scutellarin and scutellarein in male and female rats urine after administration of a single oral dose of 400 mg/kg scutellarin (n=10). Mean±SD. ^bP<0.05 *vs* male rats.

to 16 h (Figures 4 and 5). The elimination half-life of scutellarein was much longer than scutellarin with oral administration. The cumulative amounts of scutellarin recovered in the urine were 0.53% in male rats and 0.96% in female rats (P<0.05), and they were 0.11% and 0.02% in male and female rats, respectively, for scutellarein (P<0.05, Figure 6). After intravenous administration of scutellarin (40 mg/kg), the cumulative amounts of scutellarin recovered in the urine were 3.72% and 6.42% in male and female rats, respectively (P<0.05, Figure 7).

The amounts of scutellarin and scutellarein recovered in the feces were much higher than those recovered from the urine after oral scutellarin administration (Figure 8); however, less amounts were recovered by the intravenous route than the oral route (Figure 9). These results indicate that most of the

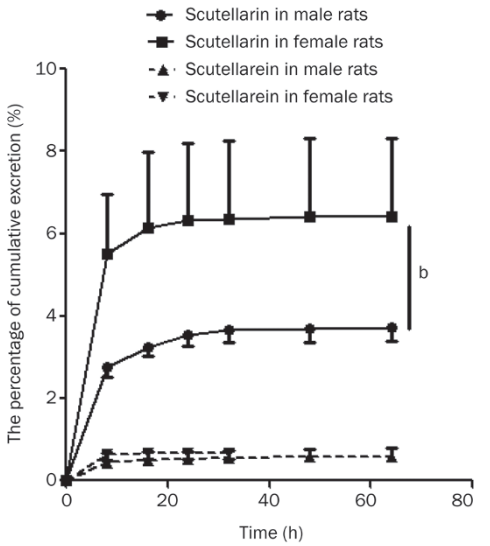

Figure 7. The percentage of cumulative excretion of scutellarin and scutellarein in male and female rats urine after administration of a single intravenous dose of 40 mg/kg scutellarin (n=10). ^bP<0.05 *vs* male rats. Mean±SD.

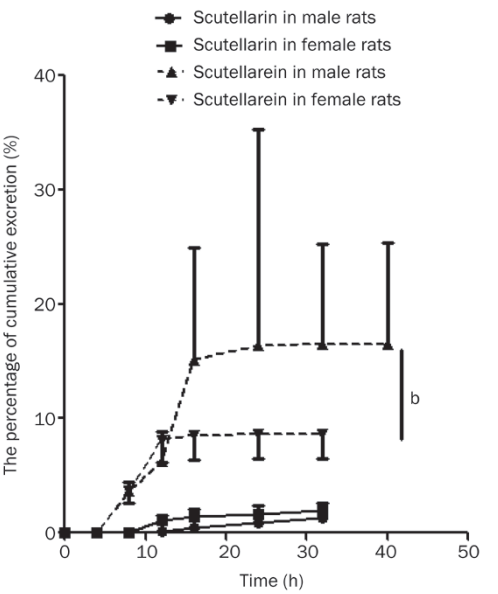

Figure 8. The percentage of cumulative excretion of scutellarin and scutellarein in male and female rats feces after administration of a single oral dose of 400 mg/kg scutellarin (n=10). Mean±SD. ^bP<0.05 *vs* male rats.

scutellarein that was excreted in the feces was unabsorbed after oral administration of scutellarin. Although the plasma level of scutellarein was very low, a substantial amount of scutellarein was recovered in the feces after oral administration. Thus, we conclude that the bulk of scutellarin is hydrolyzed by the intestinal microflora or transformed by enzymes in the gastrointestinal tract into scutellarein, which leads to the low bioavailability. Hao *et al*[20] hypothesized that the site of

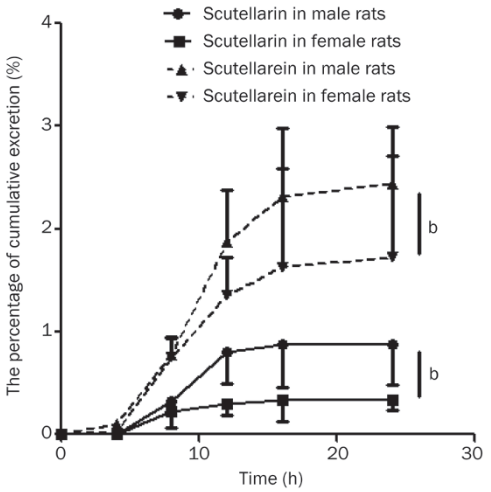

Figure 9. The percentage of cumulative excretion of scutellarin and scutellarein in male and female rats feces after adminstration of a single intravenous dose of 40 mg/kg scutellarin (n=10). Mean±SD. $^bP<0.05$ *vs* male rats.

the first-pass effect was not the liver but in the gastrointestinal tract. Therefore, scutellarein could be partially excreted in the feces without being absorbed. The percentages of scutellarin and scutellarein that were cumulatively excreted were different according to gender for both oral and intravenous administrations (Figures 8 and 9).

Mechanism study on gender differences

Gender-specific rat liver microsomes were used in an *in vitro* metabolic study of scutellarin. These microsome experiments were performed in triplicate at eight time points between 0 and 90 min. Scutellarin was rapidly metabolized in both genders, and the starting concentrations of scutellarin that remained in female and male rat liver microsomes were 16.16%±1.02% and 8.08%±1.90%, respectively. The calculated *in vitro* scutellarin $t_{1/2}$ in female and male rats were 75.5±1.78 min and 75.6±0.33 min, respectively. The CL_{int} values for scutellarin in male rats (5.56±0.11 mL·min^{-1}·kg^{-1}) was much higher than that in female rats (4.51±0.02 mL·min^{-1}·kg^{-1}, $P<0.01$).

Discussion

Scutellarin, an unknown metabolite and scutellarein were detected simultaneously in rat plasma 5 min after oral administration of scutellarin. This indicates that scutellarin is immediately absorbed and rapidly transformed into other metabolites *in vivo*. Scutellarin and the unknown metabolite can be hydrolyzed completely by β-glucuronidase from bovine liver (essentially sulfatase-free) but not by sulfatase from *Aerobacter aerogenes* (no β-glucuronidase activity at pH 7). Huang *et al*[25] reported that scutellarin was hydrolyzed and transformed into scutellarein. Therefore, after hydrolysis by β-glucuronidase, the scutellarin peak disappeared, but the scutellarein peak increased. Additionally, when the unknown metabolite disappeared, another compound formed after glucuronidase hydrolysis, and the retention time of the second unknown metabolite was 5.72 min [Figure 1, panel B (a)].

After oral administration of scutellarin, its plasma concentrations at 20 and 40 min and at 6, 8, and 16 h were significantly higher in female than in male rats (Figure 2), and the $AUC_{(0\text{-}t)}$ and C_{max2} for scutellarin were much higher in female than in male rats. Similar results were also noted for scutellarein in female rats (Figure 3). Due to the absence of calibration standards for the unknown metabolite, its plasma concentrations could not be quantified. It is interesting to note that the plasma levels, which were estimated by the peak ratios, appeared to be higher in females than in males. Identifying the structural and pharmacological effects of the unknown metabolite warrants further investigation. The plasma concentrations of scutellarein were very sporadic and low, and they were quantified at a few time points. However, the scutellarein concentrations still appeared to be higher in female than in male rats, which was similar to the findings for scutellarin and the unknown metabolite.

After oral administration, the plasma concentration–time profile for scutellarin in rats was present as double peaks (Figure 2). The first peak was at 0.18 and 0.43 h in male and female rats, respectively. This suggests that scutellarin is absorbed in the stomach or upper intestinal lumen and mucosa to produce the first concentration peak because the lower pH conditions favor the liposolubility of scutellarin and its absorption. The second peak emerged at 5.6 and 8.8 h in male and female rats, respectively, and its peak concentration was higher than the first peak. These results indicate that scutellarin is transformed in the inferior part of the intestines and the colon by bacterial enzymes into the corresponding aglycone, which is absorbed. The aglycone is then followed by a regioselective glucuronidation to transform scutellarin to enter the bloodstream[13].

The observed gender effect on the pharmacokinetics of scutellarin is similar to another flavonoid, genistein[26, 27]. It is suggested that these effects on genistein are due to higher CYP1A2 activity in male rats[27, 28]. Similar to other flavonoids, scutellarin could also be metabolized by CYP1A2 enzymes. The involvement of CYP1A2 in the metabolism of scutellarin will be studied in the future.

Conclusion

This study demonstrates that a substantial amount of scutellarin is converted to scutellarein before absorption. The presystemic transformation of scutellarin could be partially responsible for its low bioavailability. The gender effect was identified in the pharmacokinetics of scutellarin, and the plasma levels of scutellarin were much higher in female than in male rats. The observed differences in plasma, urine, and feces levels could be due to gender-related differences in enzyme activity.

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Author contribution

Jian-feng XING and Ya-lin DONG designed the study, performed the research and wrote the paper. Hai-sheng YOU, Jun LU, Si-ying CHEN, Hui-fang ZHU, Qian DONG, Mao-yi WANG, and Wei-hua DONG performed some of the research.

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