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ORIGINAL ARTICLE

Comparative pharmacokinetics of five saponins after intravenous administration of TSFS injection and TSFS injection plus TFFG in rats under different physiological states

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Blood stasis syndrome;
Ultra-HPLC–MS/MS

Abstract Sanqi is a popular traditional Chinese medicine and commonly used for promoting blood circulation and removing blood stasis. Notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd are the major active constituents of Sanqi. The purpose of the study was to investigate the pharmacokinetic behavior of the five active constituents from total saponin from Sanqi when it was used in the blood stasis animals or in combination with Gegen. The concentrations of the five active constituents in rat plasma were determined by an ultra-HPLC–ESI–MS/MS method. The main pharmacokinetic parameters were calculated and statistically analyzed using the unpaired student's *t*-test. It was found that the pharmacokinetic parameters of notoginsenoside R1, ginsenoside Rg1 and Rb1 represented a statistically significant difference ($P<0.05$) between the normal rats and the blood stasis rats after administration of total saponin from Sanqi (TSFS). And there were statistically significant differences ($P<0.05$) in the pharmacokinetic parameters of all the five constituents between administration of TSFS alone and combined with total flavonoid from Gegen (TFFG) in blood stasis rats. It suggested that the pharmacokinetic behavior of the active constituents from TSFS could be changed when it was used in blood stasis animals or in combination with TFFG.

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1. Introduction

Sanqi, the roots of *Panax notoginseng* (Burk) F. H. Chen, is one of the widely used traditional Chinese medicinal herbs (TCM) in China for promoting blood circulation and removing blood stasis [1]. The dammarane-type saponins, are considered as the major active

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constituents in Sanqi. According to the structure of dammarane skeleton, the saponins from Sanqi can be divided into two groups, 20 (S)—protopanaxadiol and 20 (S)—protopanaxatriol [2]. 20 (S)—protopanaxadiol group saponins, such as ginsenoside Rb1, show anti-inflammatory action, vasodilating effect and sedative effect on central nervous system. 20 (S)—protopanaxatrial group saponins, such as ginsenoside Rg1, possess the properties of exciting central nervous system, anti-fatigue and hemolysis [3]. However, notoginsenoside R1, ginsenoside Rg1, Rd, Re and Rb1 (Fig. 1(A)) are regarded as the principal active constituents of Sanqi [4].

Hematological disorders such as thrombosis, hemorrhage, congestion, local ischemia and tissue changes were considered to be related to the blood stasis syndrome [5]. Blood stasis syndrome has the status of platelet activation. As soon as blood stasis developed, the blood circulation and the pharmacokinetic behavior of some active constituents will be affected [6,7]. Sanqi could inhibit platelet activation [4,8]. In recent years, some pharmacokinetic studies on the active constituents from total saponin from Sanqi (TSFS) have been investigated [9–15]. However, few of them were investigated with the consideration of the pathological condition [6,7]. Drug is used to treat diseases while patient is the ultimate consumer of drug; therefore, it is necessary to study the pharmacokinetics of TSFS in animals with blood stasis syndrome for its rational clinical application. On the other hand, Sanqi is often used in combination with Gegen in clinical application in order to obtain synergistic effects and reduce possible side effect [16,17]. Gegen, the roots of *Pueraria lobata* (Willd.) Ohwi, is another TCM which has been proved effective for promoting blood circulation and removing blood stasis [18]. Therefore, it is also important to perform the pharmacokinetic studies to evaluate the rationality and compatibility of combined treatment [19,20].

In this paper, an ultra-performance liquid chromatography–tandem mass spectrometry (ultra-HPLC–MS/MS) method for the simultaneous determination of notoginsenoside R1 and ginsenoside Rg1, Re, Rb1 and Rd in rat plasma using digoxin as an internal standard (IS, Fig. 1(B)) was developed and validated, and applied to investigate the pharmacokinetic behavior of the five active constituents from TSFS when it was used in the blood stasis animals or in combination with Gegen.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards of notoginsenoside R1, ginsenoside Re, ginsenoside Rd and digoxin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ginsenoside Rg1 and ginsenoside Rb1 were purchased from Victory Bio-technology Company Limited (Sichuan, China). Xuesaitong injection (consisting of 50.0 mg/mL TSFS) was obtained from Yunnan Phytopharmaceutical Company Limited (Yunnan, China). Total flavonoid from Gegen (TFFG) was obtained from Lvyuan Health-Care Products Company Limited (Guangdong, China). Adrenaline hydrochloride injection was procured from Tianjin Jinyao Amino Acid Company Limited (Tianjin, China). Acetonitrile and methanol of HPLC grade were purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was purified by redistillation and passed through a 0.22 μm membrane filter before use.

2.2. Animals

A total of 24 male Sprague-Dawley rats (200–240 g) with certificate number SCXK 2010-0001 were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were kept in our laboratory for at least 3 days before use and had access to the standard laboratory food and water ad libitum. Animal experiments were carried out according to the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University and the protocol of the study was approved by the Animal Ethics Committee of this institution.

2.3. Preparation of solution for intravenous administration

The TSFS injection solution used in pharmacokinetic study was prepared by diluting Xuesaitong injection to a concentration of 5.40 mg/mL with saline. The TFFG injection solution was prepared as we reported previously [21]. The combined injection consisted of 5.40 mg/mL TSFS and 16.4 mg/mL TFFG. Both the injection solutions passed through a 0.22 μm membrane filter for sterilization before use.

2.4. Preparation of standard solutions and quality control samples

Stock solutions of notoginsenoside R1 (0.750 mg/mL), ginsenoside Rg1 (1.97 mg/mL), Re (1.23 mg/mL), Rb1 (1.97 mg/mL) and Rd (1.95 mg/mL) were separately prepared by dissolving the reference substances in methanol:water (1:1, *v/v*). A series of working solutions were prepared by diluting the stock solution with methanol:water (1:1, *v/v*). Calibration curves were prepared

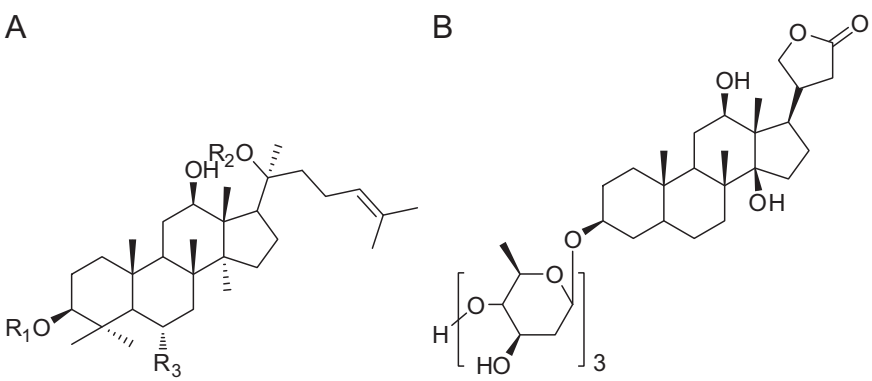

Fig. 1 Chemical structures of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd (A) and internal standard digoxin (B).

by spiking blank rat plasma with working solutions of analytes to obtain concentrations ranging from 0.050 to 5.00 μg/mL for notoginsenoside R1, 0.10 to 10.0 μg/mL for ginsenoside Rg1, 0.050 to 5.00 μg/mL for ginsenoside Re, 0.20 to 50.0 μg/mL for ginsenoside Rb1, and 0.20 to 10.0 μg/mL for ginsenoside Rd. The IS solution was prepared at a concentration of 100 μg/mL in methanol. All the solutions were stored at 4 °C and were brought to room temperature before use. For the validation of the method, four different concentration levels of quality control (QC) plasma samples were prepared containing notoginsenoside R1 (0.10, 1.00, 4.00, and 20.0 μg/mL), ginsenoside Rg1 (0.20, 2.00, 8.00, and 40.0 μg/mL), ginsenoside Re (0.10, 1.00, 4.00, and 20.0 μg/mL), ginsenoside Rb1 (0.50, 5.00, 40.0, and 200 μg/mL) and ginsenoside Rd (0.50, 5.00, 8.00, and 40.0 μg/mL).

2.5. Method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, matrix effect, dilution integrity and stability.

The selectivity of the method was validated by analyzing blank rat plasma from six different rats, QC plasma samples and plasma samples after drug administration.

The linearity of the method was validated by preparing different concentrations of standard samples with blank plasma and assayed in duplicate on three consecutive days. The lower limit of quantitation (LLOQ) was defined as the lowest concentration on the calibration curve, in accordance with an accuracy within a 20% deviation and a precision below 20%.

The accuracy and precision were calculated by determination of QC plasma samples at three different concentrations on three different validation days. The accuracy was expressed as the relative error (RE) and the precision as the relative standard deviation (RSD).

The extraction recoveries of analytes at three different concentrations were calculated by comparing the peak areas of the extracted (pre-spiked) QC samples with those of the post-spiked standard plasma samples at an equivalent concentration.

The matrix effects of analytes at three different concentrations were determined by comparing the peak areas of the post-spiked standard plasma samples with those of the neat standard samples at an equivalent concentration.

The dilution integrity was investigated at 20.0 μg/mL for notoginsenoside R1, 40.0 μg/mL for ginsenoside Rg1, 20.0 μg/mL for ginsenoside Re, 200 μg/mL for ginsenoside Rb1 and 40.0 μg/mL for ginsenoside Rd of QC samples in order to validate the dilution procedure. The plasma samples were prepared by 5-fold dilution with blank plasma.

The stability of analytes in plasma was determined by using QC plasma samples at three different concentrations in five replicates, including (a) the long-term stability of the QC plasma samples at −80 °C for 30 days, (b) the freeze-thaw stability of the QC plasma samples after three freeze-thaw cycles (−80 °C to room temperature), (c) the stability of the fresh QC plasma samples at room temperature for 4 h. The stability of the processed QC samples in the sample injector at 10 °C for 12 h was carried out with the QC samples used in the validation of intra-day precision.

2.6. Sample preparation

The 100 μL aliquot of plasma sample was spiked with 10 μL IS (100 μg/mL) and 50 μL methanol:water (1:1, *v/v*) in a 1.5 mL EP tube. The mixture was vortexed for 30 s and 0.4 mL acetonitrile was added, then vortexed for 3 min. After centrifugation at 15,000 rpm at 4 °C for 15 min, a certain volume of supernatant was removed to another EP tube and evaporated to dryness under nitrogen at 35 °C. The residue was reconstituted in 100 μL incipient mobile phase, vortexed for 2 min and centrifuged at 15,000 rpm at 4 °C for 10 min. 5 μL aliquot of the supernatant was injected into the ultra-HPLC–MS/MS system. The plasma samples, which concentrations were greater than the upper limit of the calibration curve, could be re-analyzed by appropriate dilution.

2.7. Instrumentation and analytical conditions

Analyses were performed on a Waters Acquity™ UPLC system (Waters Corp., Milford, MA, USA) coupled with a Micromass Quattro Micro API mass spectrometer (Waters Corp., Milford, MA, USA). Chromatographic separations were performed on a Thermo Syncronis C18 column (10 cm × 2.1 mm, 1.7 μm), maintained at 35 °C, with water (solvent A) and acetonitrile (solvent B) as the mobile phase at a flow rate of 250 μL/min. The gradient elution program was as follows: 0–8 min, 19–35% B; 8–12 min, 35–65% B.

An electrospray ionization (ESI) source interface operated in the positive ionization mode was used. Nitrogen was used as both desolvation gas and cone gas, with a flow rate of 50 L/h and 600 L/h, respectively. The desolvation and source temperatures were set at 450 and 120 °C, respectively. The capillary voltage was set at 3.2 kV. Argon was employed as the collision gas at a pressure of approximately 0.3 Pa. Quantitation was performed using time-dependent selected reaction monitoring (SRM) by the $[M+Na]^+$ molecular ions of the analytes. The corresponding operational parameters are shown in Table 1. The full scan and daughter scan mass spectra of notoginsenoside R1, ginsenoside Rg1, Re, Rb1, Rd and IS are shown in Fig. 2.

2.8. In vivo pharmacokinetic study

The rats were randomly divided into the following three groups ($n=8$): Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg. The blood stasis model rats were established as described by Tian et al. [7] with a little modification: the blood stasis rats were injected with adrenaline hydrochloride injection (0.8 mg/kg). After waiting for 2 h, the rats were soaked in ice-water for 5 min keeping their heads outside surface. Then, those rats were injected with the same injection again after 2 h.

Rats were fasted for 12 h and administered via the caudal vein. Blood samples (0.2 mL) were collected from the suborbital veniplex and transferred to heparinized tubes at 0.033, 0.083, 0.167, 0.333, 0.667, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 h after administration, and then centrifuged at 3500 rpm for 5 min to obtain plasma. The plasma was frozen at −80 °C until analysis. The plasma concentrations of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd at different time points were measured and expressed as mean ± standard deviation (SD), the mean concentration–time curve was plotted.

The pharmacokinetic parameters were calculated by a DAS (Drug and Statistics for Windows) software package (version 2.1, Chinese Pharmacological Society), relating to area under the plasma concentration-time curve (AUC), clearance (CL), half-life

Table 1 Parameters of SRM mode used for determination.

Constituents	*m/z* of precursor ion (Da)	*m/z* of production (Da)	Dwell time (s)	Cone voltage (eV)	Collision voltage (eV)	Acquisition time (min)
Notoginsenoside R1	955.9	775.7	0.5	65	55	4.5–5.5
Ginsenoside Rg1	823.8	643.5	0.5	62	42	5.0–6.5
Ginsenoside Re	969.8	789.6	0.5	60	48	5.0–6.5
Ginsenoside Rb1	1132.0	789.7	0.8	65	55	9.0–10.5
Ginsenoside Rd	969.9	789.6	0.8	65	55	10.0–11.0
Digoxin	803.7	803.7	0.5	60	15	8.5–9.5

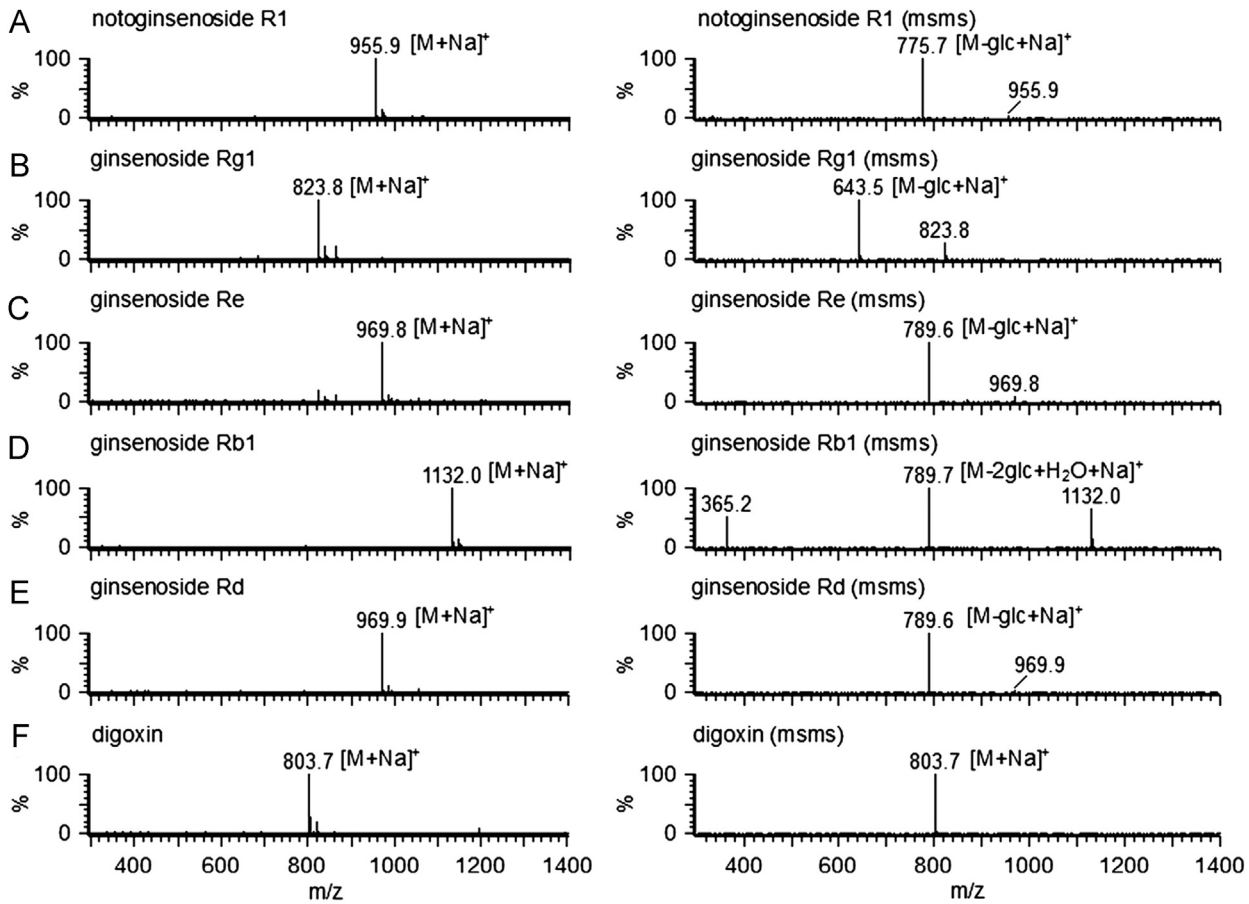

Fig. 2 MS and MS/MS spectra of notoginsenoside R1 (A), ginsenoside Rg1 (B), Re (C), Rb1 (D), Rd (E) and digoxin (F).

($t_{1/2}$), mean residence time (MRT) and apparent volume of distribution (Vd). The pharmacokinetic parameters were given as mean ± SD. The statistical analysis was performed with SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA) by the unpaired Student's *t*-test. All statistical tests were performed at the two sided 5% level of significance.

3. Results

3.1. Method validation

3.1.1. Selectivity

The typical chromatograms of notoginsenoside R1, ginsenoside Rg1, Re, Rb1, Rd and IS are shown in Fig. 3. The retention times were 5.01, 5.61, 5.68, 9.72, 10.57 and 9.24 min, respectively. The peak shapes were good under the described conditions without interference peaks.

3.1.2. Linearity and LLOQ

The calibration curves were linear with correlation coefficients (*r*) exceeding 0.99. Regression equations and LLOQs for notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd are listed in Table 2.

3.1.3. Precision and accuracy

The intra-day and inter-day precision and accuracy of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd at three different concentrations are summarized in Table 3. The acceptance criteria for intra-day and inter-day precision and accuracy are 15% for the QC concentrations.

3.1.4. Extraction recovery and matrix effect

The extraction recoveries and matrix effects of notoginsenoside R1, ginsenoside Rg1, Re, Rb1, Rd and IS are shown in Table 3. These data indicated that the extraction recovery and matrix effect of the method were acceptable.

3.1.5. Stability

The results of the stability data of QC samples study are summarized in Table 4. It showed no significant degradation when QC samples were stored at −80 °C for 30 days, at −80 °C for three freeze-thaw cycles (−80 °C to room temperature) and at room temperature for 4 h. In addition, the processed samples in the sample injector at 10 °C for 12 h were stable, and the accuracy (RE, %) and precision (RSD, %) were within 10%.

3.1.6. Dilution integrity

For notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd, the accuracy (RE) and precision (RSD) of the diluted QC samples were in the range from −8.0% to 3.7% and 1.8% to 7.9%. The results showed that it was appropriate to dilute the plasma samples when the plasma concentrations were greater than the upper limit of the calibration curve for quantitation.

3.2. Pharmacokinetic study

The validated method was successfully applied to the pharmacokinetic study of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd in rat plasma after intravenous administration of TSFS and TSFS combined with TFFG. The mean plasma concentration–time curves are illustrated in Figs. 4 and 5, respectively. The main pharmacokinetic parameters of the five constituents including AUC_{0-n}, MRT_{0-t}, $t_{1/2}$, CL, and, Vd are presented in Tables 5–9.

There were statistically significant differences ($P<0.05$) in the pharmacokinetic parameters of notoginsenoside R1, ginsenoside Rg1 and Rb1 between Group I and Group II, which were both administered of TSFS alone. In comparison with Group I, the AUC_{0-t} of notoginsenoside R1 and ginsenoside Rg1 in the blood stasis rats were remarkably increased ($P<0.05$), the $t_{1/2}$ values were increased ($P<0.05$), the CL values were decreased ($P<0.05$), on the contrary, the AUC_{0-t} of ginsenoside Rb1 was

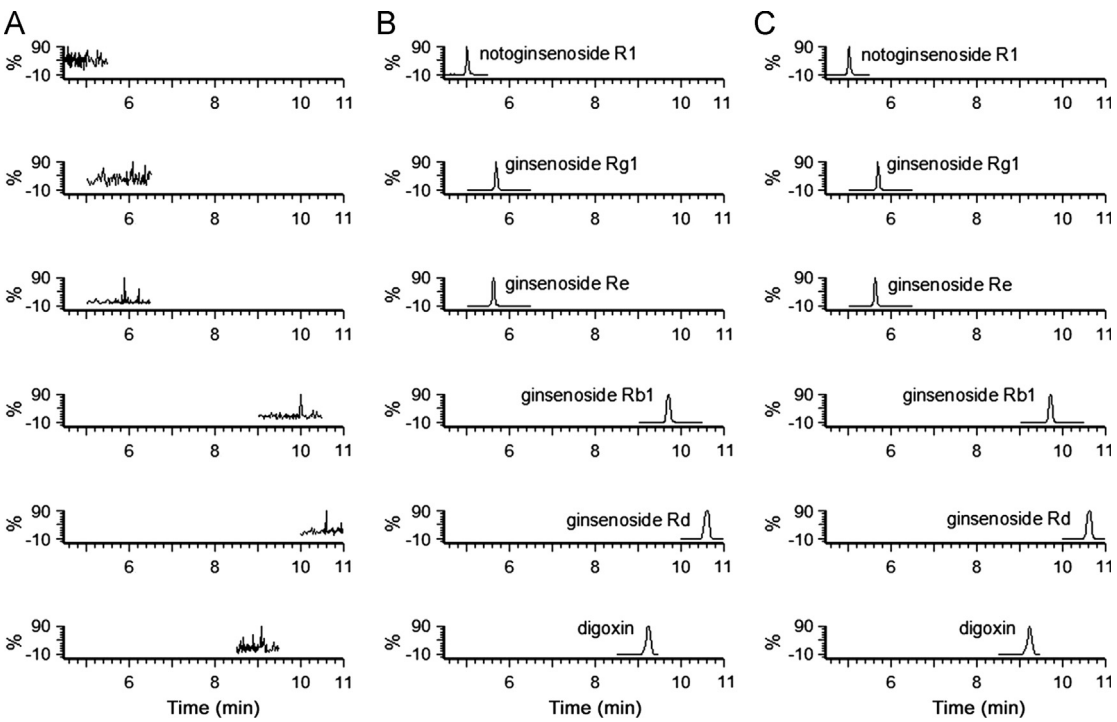

Fig. 3 Typical chromatograms of blank plasma (A), blank plasma spiked with notoginsenoside R1, ginsenoside Rg1, Rd, Re, Rb1 and digoxin (B), and plasma sample obtained 1 h after intravenous administration of TSFS combined with TFFG (C).

Table 2 Regression data and LLOQ for the five constituents determined.

Constituents	Regression equation	Correlation coefficient (r)	Linear range (μg/mL)	LLOQ (μg/mL)
Notoginsenoside R1	$y=0.02487x-0.0004$	0.9950	0.050–5.00	0.050
Ginsenoside Rg1	$y=0.04590x-0.0017$	0.9944	0.10–10.0	0.10
Ginsenoside Re	$y=0.04011x+0.0002$	0.9919	0.050–5.00	0.050
Ginsenoside Rb1	$y=0.02171x+0.0011$	0.9991	0.20–50.0	0.20
Ginsenoside Rd	$y=0.07144x+0.0059$	0.9958	0.20–10.0	0.20

Table 3 Precision, accuracy, extraction recovery and matrix effect of the five constituents determined in rat plasma.

Constituents and concentration (μg/mL)	Intra-day		Inter-day		Extraction recovery		Matrix effect	
	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Notoginsenoside R1								
0.10	4.2	−6.8	6.3	−4.8	90.6	4.2	96.8	3.1
1.00	3.6	−3.8	2.7	−2.6	80.5	6.5	95.6	6.5
4.00	5.8	−1.9	0.9	−1.6	83.7	4.0	97.6	1.6
Ginsenoside Rg1								
0.20	3.2	2.2	3.2	1.0	93.1	3.7	94.0	2.6
2.00	5.6	3.7	3.8	2.1	88.0	3.3	88.6	5.7
8.00	5.4	0.2	1.3	0.5	93.4	1.0	90.8	1.8
Ginsenoside Re								
0.10	3.6	9.0	2.3	3.6	89.8	4.7	93.1	3.1
1.00	4.3	2.3	2.3	1.6	87.3	7.1	89.2	7.0
4.00	5.7	−1.4	1.4	−0.8	93.6	1.3	91.2	1.3
Ginsenoside Rb1								
0.50	4.2	−5.1	5.7	−3.1	90.5	3.6	96.2	6.0
5.00	5.4	−4.9	4.1	−3.4	91.7	6.6	102.4	9.8
40.0	3.9	−4.3	7.2	−1.1	86.6	4.4	90.7	5.4
Ginsenoside Rd								
0.50	3.8	−3.7	3.2	−2.8	85.1	3.6	92.6	6.2
5.00	5.0	−3.5	3.1	−2.1	86.7	5.7	96.9	3.8
8.00	3.9	0.6	1.0	0.2	86.7	5.2	90.7	8.2
Digoxin (I.S.)								
10.00	–	–	–	–	90.2	5.8	98.9	3.9

Table 4 The stability of the five constituents in plasma sample ($n=5$).

Constituents and concentration (μg/mL)	Frozen for 30 days		Three freeze-thaw cycles		Room temperature for 4 h	
	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
Notoginsenoside R1						
0.10	1.0	−8.4	5.0	−2.9	5.7	−1.9
4.00	4.9	−0.7	2.9	−1.7	6.8	−1.0
20.0	7.1	−0.5	2.9	−2.2	7.2	−0.5
Ginsenoside Rg1						
0.20	1.0	−1.2	10.0	0.1	4.8	4.7
8.00	6.0	−0.9	7.0	−0.4	4.8	−0.1
40.0	11.4	−0.7	5.9	−0.7	6.4	−0.1
Ginsenoside Re						
0.10	4.4	−1.0	4.3	−0.6	3.8	−1.5
4.00	6.3	−2.1	6.1	−0.2	5.6	−0.8
20.0	6.6	−2.3	5.0	−1.4	4.9	−1.4
Ginsenoside Rb1						
0.50	5.6	−3.8	2.1	−6.2	4.3	0.8
40.0	3.2	3.8	4.3	5.9	1.9	5.6
200	6.3	1.1	8.3	2.3	8.0	2.8
Ginsenoside Rd						
0.50	2.8	−4.9	4.6	−1.4	6.1	−0.8
8.00	4.7	−0.4	2.9	−2.2	5.6	−4.0
40.0	4.3	−1.3	4.4	−0.1	2.7	−3.2

remarkably decreased ($P<0.05$), the CL was increased ($P<0.05$). There were no statistically significant differences in the pharmacokinetic parameters of ginsenoside Re and Rd between the two groups mentioned above.

There were statistically significant differences ($P<0.05$) in the pharmacokinetic parameters of all the five constituents between Group II and Group III. In comparison with Group II, the AUC_{0-t} values of notoginsenoside R1 and ginsenoside Rg1 in Group III

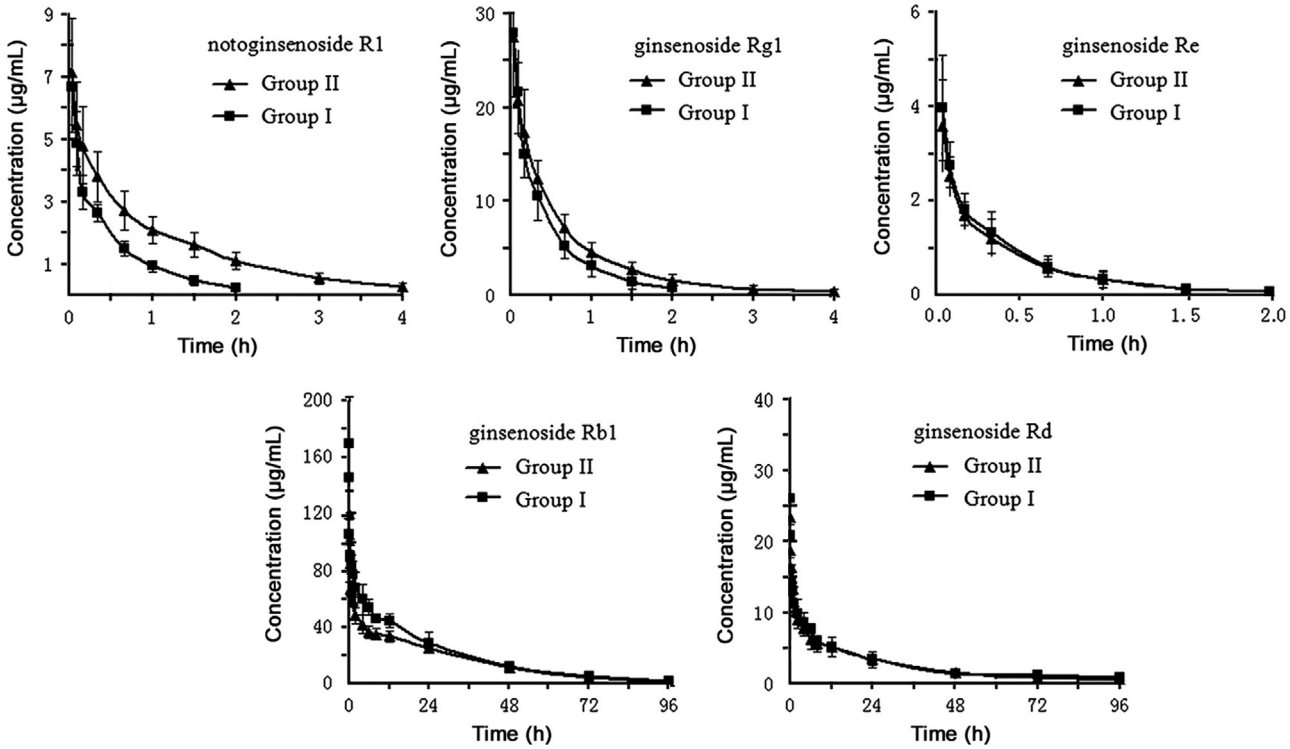

Fig. 4 Plasma concentration–time curves of notoginsenoside R1, ginsenoside Rg1, Rd, Re and Rb1 after intravenous administration of TSFS in normal rats (Group I) and blood stasis rats (Group II).

rats were remarkably decreased ($P<0.05$) and the CL values were increased ($P<0.05$), on the contrary, the AUC_{0-t} values of ginsenoside Re, Rb1 and Rd were remarkably increased ($P<0.05$) and the CL values were decreased ($P<0.05$).

4. Discussion

4.1. Method development

4.1.1. Optimization of LC–MS/MS conditions

The reference standards of the five constituents and IS were analyzed by direct injection to optimize the MS/MS conditions. The MS spectra of the five constituents and IS were investigated in both positive and negative ion modes. In the positive full scan mode, the five constituents and IS could form adduct ions of $[M+Na]^+$. Although the five constituents and IS could form stable de-protonated ions of $[M-H]^-$ in the negative full scan mode, the response in positive mode was much higher than that in negative mode. Therefore, we selected $[M+Na]^+$ in the positive full scan mode as the precursor ions for SRM detection of the five constituents and IS. The MS parameters were optimized according to the MS responses of the five constituents and IS. The product ion spectra of the five constituents and IS were produced using argon as collision gas, and the collision energy was optimized for a stable response and highest intensity as shown in Table 1. It was found that acetonitrile produced better resolution and peak shapes than methanol. When a small amount (0.05%, 0.1% and 0.5%) of acid (formic acid or acetic acid) was added into the mobile phase, the MS responses did not change significantly. Therefore, we selected water–acetontrile as the mobile phase. In order to separate the analytes completely from the endogenous substances and reduce the matrix effect, a gradient elution mode was applied for the separation and simultaneous determination of the five constituents and IS in rat plasma samples.

4.1.2. Optimization of sample preparation

Protein precipitation and liquid–liquid extraction were investigated in order to extract the five constituents and IS from plasma. However, the liquid–liquid extraction with ethyl acetate or *n*-butanol showed limited extraction recovery efficiency of the analytes. So protein precipitation was finally selected. Different precipitation organic solvents (methanol, acetonitrile or acetone) were evaluated in order to improve the extraction recovery efficiency of the analytes and IS. Acetonitrile was finally selected because of its high extraction recovery efficiency and perfect peak shape.

4.2. Pharmacokinetic parameters

An ultra-HPLC–ESI–MS/MS method had been developed and validated for determination of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd after administration of TSFS alone and TSFS combined with TFFG in rats under different physiological states. In comparison with the normal rats, the blood stasis rats had a significant extension of the half-life time and MRT_{0-t} ($P<0.05$), and increase in the AUC ($p<0.05$) of notoginsenoside R1 and ginsenoside Rg1 after administration of TSFS. The following reasons may explain why the pharmacokinetic parameters change. The blood circulation of the blood stasis rats will be sluggish, leading to the increase of whole blood viscosity, plasma viscosity, fibrinogen and hematocrit [7]. Notoginsenoside R1 and ginsenoside Rg1 (20(S)-protopanaxatriol) were absorbed rapidly into plasma and metabolized quickly, so the poor blood

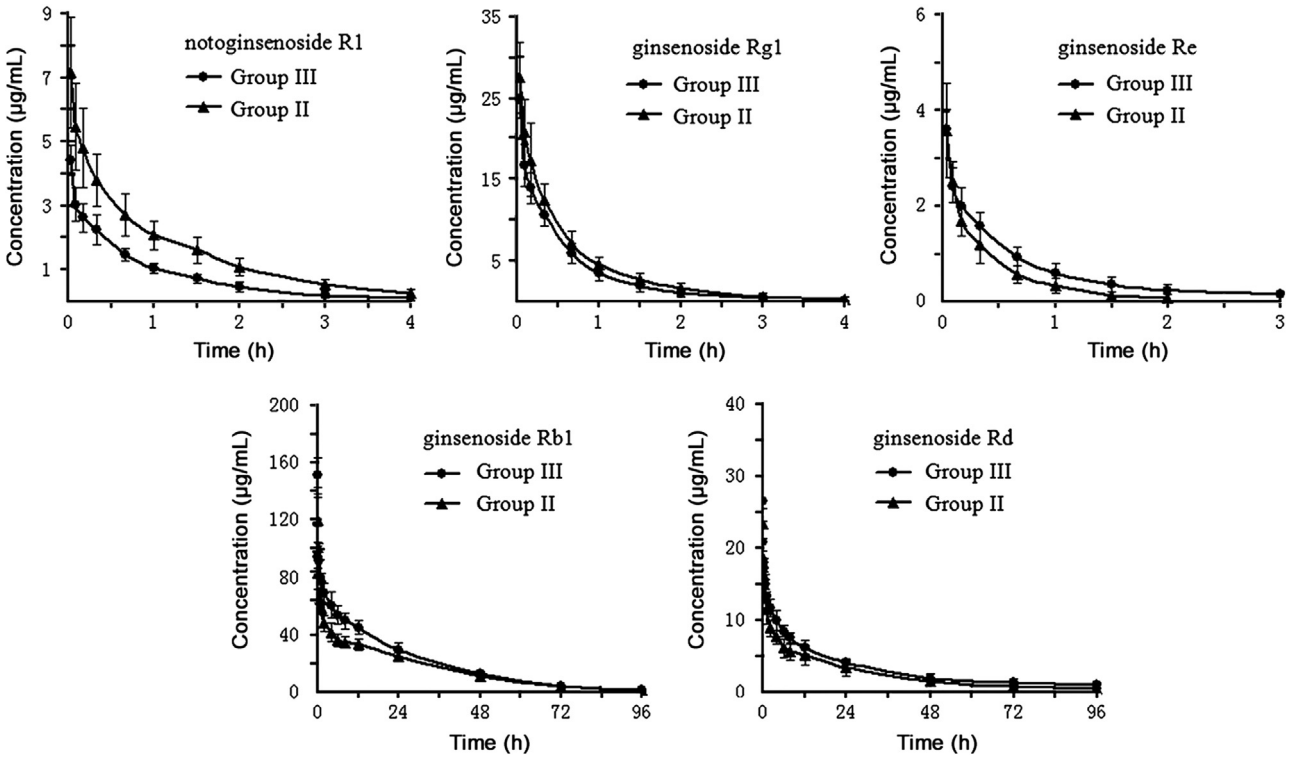

Fig. 5 Plasma concentration–time curves of notoginsenoside R1, ginsenoside Rg1, Rd, Re and Rb1 after intravenous administration of TSFS alone (Group II) and TSFS combined with TFFG in blood stasis rats (Group III).

Table 5 Pharmacokinetic parameters of notoginsenoside R1 in three group rats after intravenous administration of drugs ($n=6$).

Parameter	Group I	Group II	Group III
$AUC_{(0\to t)}$ (μg h/mL)	2.99±0.43	6.41±1.36*	3.25±0.47Δ
$AUC_{(0\to\infty)}$ (μg h/mL)	3.18±0.52	6.86±1.41*	3.36±0.54Δ
$MRT_{(0\to t)}$ (h)	0.51±0.04	1.08±0.13*	0.94±0.11
$t_{1/2}$ (h)	0.50±0.13	1.03±0.23*	0.80±0.14
CL (L/h/kg)	0.75±0.14	0.35±0.08*	0.70±0.10Δ
Vd (L/kg)	0.53±0.09	0.52±0.14	0.80±0.08Δ

Data represent Mean±SD. Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg.

*$P<0.05$, compared with normal rats.

Δ$P<0.05$, compared with acute blood stasis rats administered with TSFS.

circulation was more likely to influence the pharmacokinetic behavior of the two constituents. Meanwhile, in blood stasis rats, the elimination of ginsenoside Rb1 (20(S)-protopanaxadiol) from rat plasma was rapid in comparison with normal rats. It was noticed that the blood stasis rats need the supply of ginsenoside Rb1 to remove the blood stasis. It is coincident with the anti-platelet aggregation effect of ginsenoside Rb1 [22]. On the other hand, the major metabolic pathways of saponins in rat were found to be oxygenation and deglycosylation, which were catalyzed mainly by enzymes in rat liver [23]. Tian et al. [7] reported that the blood stasis syndrome had effect on rat liver cytochrome P450-catalyzed drug metabolism, owing to this reason, blood stasis syndrome will have effect on the pharmacokinetic behavior of some saponins from Sanqi.

The interaction between TSFS and TFFG was further investigated by comparing the pharmacokinetic parameters after administration of TSFS alone and TSFS combined with TFFG in blood stasis rats. Blood stasis rat has the status of platelet activation, which is a complex and multifactor process, including platelet adhesion, aggregation and reaction release. TSFS and TFFG have common mechanisms of inhibiting platelet activation [24]. Meanwhile, there are some differences in metabolic pathway due to the complexity of the constituents in TSFS and TFFG. The major metabolic pathways of saponins in TSFS were found to be oxygenation and deglycosylation [23], while the flavonoids *C*-glycosides in TFFG such as puerarin were glucurnidation and sulfation [25]. Therefore, there were limited competitive metabolism and excretion between TSFS and TFFG. In comparison with administration of TSFS alone, the AUC ($P<0.05$) of

Table 6 Pharmacokinetic parameters of ginsenoside Rg1 in three group rats after intravenous administration of drugs ($n=6$).

Parameter	Group I	Group II	Group III
$AUC_{(0\rightarrow t)}$ (μg h/mL)	11.62±2.14	15.99±2.84*	12.51±1.76Δ
$AUC_{(0\rightarrow \infty)}$ (μg h/mL)	12.00±2.34	16.39±2.98*	12.96±2.10Δ
$MRT_{(0\rightarrow t)}$ (h)	0.44±0.08	0.74±0.14*	0.63±0.11Δ
$t_{1/2}$ (h)	0.40±0.08	0.74±0.22*	0.62±0.18
CL (L/h/kg)	0.73±0.16	0.53±0.11*	0.66±0.11
Vd (L/kg)	0.41±0.08	0.56±0.14	0.61±0.12

Data represent Mean±SD. Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg.

*$P<0.05$, compared with normal rats.

Δ$P<0.05$, compared with acute blood stasis rats administered with TSFS.

Table 7 Pharmacokinetic parameters of ginsenoside Re in three group rats after intravenous administration of drugs ($n=6$).

Parameter	Group I	Group II	Group III
$AUC_{(0\rightarrow t)}$ (μg h/mL)	1.42±0.35	1.32±0.31	1.98±0.45Δ
$AUC_{(0\rightarrow \infty)}$ (μg h/mL)	1.48±0.42	1.37±0.37	2.11±0.51Δ
$MRT_{(0\rightarrow t)}$ (h)	0.40±0.10	0.41±0.09	0.67±0.18Δ
$t_{1/2}$ (h)	0.42±0.14	0.42±0.15	0.68±0.22Δ
CL (L/h/kg)	0.84±0.25	0.90±0.25	0.58±0.14Δ
Vd (L/kg)	0.47±0.05	0.50±0.08	0.54±0.09

Data represent Mean±SD. Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg.

*$P<0.05$, compared with normal rats.

Δ$P<0.05$, compared with acute blood stasis rats administered with TSFS.

Table 8 Pharmacokinetic parameters of ginsenoside Rb1 in three group rats after intravenous administration of drugs ($n=6$).

Parameter	Group I	Group II	Group III
$AUC_{(0\rightarrow t)}$ (μg h/mL)	1906.7±169.6	1547.4±142.9*	1936.9±212.0Δ
$AUC_{(0\rightarrow \infty)}$ (μg h/mL)	1953.4±178.9	1606.0±131.0*	1986.5±228.2Δ
$MRT_{(0\rightarrow t)}$ (h)	22.94±1.21	24.80±1.34*	22.59±1.48Δ
$t_{1/2}$ (h)	17.33±1.88	19.67±3.88	17.96±1.62
CL (L/h/kg)	0.005±0.001	0.006±0.001*	0.005±0.001Δ
Vd (L/kg)	0.12±0.01	0.17±0.04*	0.12±0.01Δ

Data represent Mean±SD. Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg.

*$P<0.05$, compared with normal rats.

Δ$P<0.05$, compared with acute blood stasis rats administered with TSFS.

notoginsenoside R1 and ginsenoside Rg1 decreased after administration of TSFS combined with TFFG. It indicated that administration of TSFS combined with TFFG in blood stasis rats would effectively promote the blood circulation, leading to the elimination of notoginsenoside R1 and ginsenoside Rg1 faster than that of TSFS alone. The differences in the AUC of ginsenoside Re, Rb1 and Rd in blood stasis rats were significant between TSFS alone and TFSF combined with TFFG. It could be inferred that TFFG might have effects on the absorption and bioavailability of ginsenoside Re, Rb1 and Rd in blood stasis rats: the elimination stepped down and AUC increased, which might have a synergistic effect on anti-platelet activation.

5. Conclusion

A simple and validated ultra-HPLC–MS/MS method was developed for the determination of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd in rat plasma. The method was then successfully applied to the pharmacokinetic comparisons of notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd in normal rats after intravenous administration of TSFS and in blood stasis rats after intravenous administration of TSFS alone and TSFS combined with TFFG. The results suggested that the pharmacokinetic behavior of the active constituents from TSFS could be changed when it was used in the blood stasis animals or in combination with TFFG. The results are

Table 9 Pharmacokinetic parameters of ginsenoside Rd in three group rats after intravenous administration of drugs ($n=6$).

Parameter	Group I	Group II	Group III
$AUC_{(0\to t)}$ (μg h/mL)	260.0±50.7	248.7±49.2	315.7±41.0$^{\Delta}$
$AUC_{(0\to\infty)}$ (μg h/mL)	298.0±76.7	271.5±48.2	360.5±76.8$^{\Delta}$
$MRT_{(0\to t)}$ (h)	26.42±3.30	25.80±1.63	26.68±3.90
$t_{1/2}$ (h)	31.75±9.44	29.74±9.55	31.87±12.30
CL (L/h/kg)	0.008±0.003	0.009±0.001	0.006±0.001$^{\Delta}$
Vd (L/kg)	0.34±0.06	0.37±0.17	0.28±0.06

Data represent Mean±SD. Group I, normal rats administered of TSFS 27 mg/kg; Group II, blood stasis model rats administered of TSFS 27 mg/kg; Group III, blood stasis model rats administered of TSFS 27 mg/kg combined with TFFG 82 mg/kg.
* $P<0.05$, compared with normal rats.
$^{\Delta}P<0.05$, compared with acute blood stasis rats administered with TSFS.

useful for the further study of the rational clinical application of TSFS.

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