Research Article

Comparative Pharmacokinetic Studies of Four Ginsenosides in Rat Plasma by UPLC-MS/MS after Oral Administration of *Panax quinquefolius*-*Acorus gramineus* and *Panax quinquefolius* Extracts

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Panax quinquefolius (PQ) and *Acorus gramineus* (AG) are drug target pairs in traditional Chinese medicine (TCM), which are used to treat age-related diseases. In the present study, we simultaneously determined the contents of four main bioactive ginsenosides (Rb₁, Rb₂, Rd, and Re) in rat plasma using an ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Plasma specimens were purified by using the solid-phase extraction procedure, and separation was performed on Waters ACQUITY UPLC BEH C₁₈ (100 mm × 2.1 mm, 1.7 μm) in multiple reaction monitoring (MRM) mode and negative electrospray ionization (ESI) mode. The established UPLC-MS/MS method showed good linear correlation ($r \geq 0.9978$), stability (−11.93 to 12.11%), precision (RSD < 14.63%), and recovery (76.43%–95.20%). The lower limit of quantification was 3.6 ng/mL for Rb₁, 1.6 ng/mL for Rb₂, 1.2 ng/mL for Rd, and 2.5 ng/mL for Re. This validated method was successfully employed to investigate the pharmacokinetics of the four ginsenosides in rat plasma after oral administration of PQ-AG and PQ extracts. The results revealed the pharmacokinetic profiles of PQ-AG drug pair and clarified that AG played a critical role in stimulating the absorption of active ginsenosides in PQ. Collectively, our findings provided valid and reliable evidence for the rational use of PQ-AG in clinical practice.

1. Introduction

In multiple diseases caused by complex pathogenic factors, the advantages of using a single herb are absent or show weaker meaning. The drug target pairs are the combination of two relatively fixed drugs based on the theory of traditional Chinese medicine (TCM), showing important clinical significance in various diseases [1–4]. *Panax quinquefolius* (PQ) is used to restore vital energy and treat diabetes [5] and tumor [6] as Qi-tonifying agents in TCM. *Acorus gramineus* (AG) possesses the effects of stimulating appetite [7], resolving dampness [8], inducing resuscitation [9], improving learning and memory [10], and so on. Moreover, AG can promote drug absorption [11, 12]. As described in early publications and our previous studies, PQ-AG drug pair is

popular due to its remarkable and reliable therapeutic actions, such as antioxidant, antiaging, and blood circulation promoting properties, especially in treating diabetes [13–15].

A great progress has been made in pharmacological research of ginsenosides. However, only few pharmacokinetic studies have investigated drug pairs. In recent years, some methods have been used for the determination of ginsenosides. An LC-MS/MS method has been established to determine the concentrations of ginsenosides in plasma and brain [16]. Moreover, an HPLC-MS/MS method has been used to characterize and quantify ginsenosides in plant extracts from *Panax ginseng* and PQ [17]. An LC-MS fingerprint and a chemometric approach, in combination with multivariate statistics, have been used to distinguish between *P. quinquefolius* samples derived from the United States and China [18].

Because only few reports have assessed the pharmacokinetic profiles and the compatibility mechanism of the drug pair, we developed an ultra-performance liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry (UPLC-MS/MS) method to compare the pharmacokinetic profiles of four major bioactive components of ginsenosides, Rb₁, Rb₂, Rd, and Re (Figure 1) in rat plasma after oral administration of PQ-AG and single PQ extracts. Collectively, our findings provided scientific evidence on reasonable compatibility of the drug pair and laid the foundations for further investigation of the behavioral mechanism.

2. Materials and Methods

2.1. Chemicals and Reagents. Rb₁ (MUST-14032301), Rb₂ (MUST-14072210), Rd (MUST-16012503), Re (MUST-14091710), and dioscin (MUST-15012101) (IS, purity $\geq 98\%$) were purchased from Chengdu MANSITE Pharmaceutical Co., Ltd. (Chengdu, Sichuan, China).

PQ and AG were collected from Heilongjiang Ruixiang Pharmaceuticals Company (Harbin, Heilongjiang, China) and identified by Ruifeng Fan from the Heilongjiang University of Chinese Medicine. The plant species were deposited (specimen 2015093104) at the Pharmacy College. Acetonitrile and methanol were of HPLC grade and purchased from Fisher (Emerson, America). Formic acid was of HPLC grade and purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents were of analytical grade.

2.2. Animals. Male SD rats (230 ± 20 g) were obtained from the Laboratory Animal Center of Changchun Yisi (Changchun, Jilin, China). The animal experiments were performed in accordance with the Guide for the Care National Institutes of Health. Before the experiments, the rats were fed in an environmentally controlled room with temperature of $22 \pm 2^\circ\text{C}$, humidity of $50 \pm 10\%$, and 12 h dark-light cycle for 5 days.

2.3. Chromatographic Conditions. Chromatographic analysis was performed on a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA). An ACQUITY UPLC BEH C₁₈ (100×2.1 mm, $1.7 \mu\text{m}$) column was used for all analytes. The column temperature was at 35°C . The mobile phase was composed of formic acid aqueous solution (A, 0.1%) and acetonitrile (B) at a gradient elution of 25% B at 0–0.5 min, 25%–50% B at 0.5–2.0 min, 50–60% B at 2.0–5.0 min, 60–25% B at 5.0–5.5 min, and 25% B at 5.5–7.0 min, with a flow rate of 0.2 mL/min.

2.4. MS Conditions. MS detection was performed using the Xevo Triple Quadrupole MS (Waters Corp.) equipped with an ESI source in both positive and negative ionization modes. The experimental parameters were set as follows: CV 2.80 kV; desolvation temperature 350°C ; source temperature 650°C ; cone gas flow 50 L/h; and desolvation gas flow 650 L/h. The detection was carried out using the MRM scan mode. The CV and CE were optimized for each analyte, and selected values are listed in Table 1 and Figure 2. All

experimental data were collected in centroid mode using Masslynx 4.1 software (Waters Corp., Milford, USA), and postacquisition quantitative analysis was conducted using Target Lynx program (Waters Corp., Milford, USA).

2.5. Preparation of PQ-AG and PQ Extracts. The powder of PQ (0.5 kg) and PQ-AG (1.0 kg, with equal proportions of PQ and AG) was extracted for three times by refluxing with water (1 : 10, w/v) for 2 h of each time. Then, the extracts were filtered, and the filtrates were pooled and concentrated to dryness under reduced pressure by rotary evaporator.

2.6. Preparation of Calibration Standards and QC Samples. The standard stock solution was prepared in methanol with four ginsenosides at the final concentration of 1.14 mg/mL for Rb₁, 0.10 mg/mL for Rb₂, 0.78 mg/mL for Rd, and 1.58 mg/mL for Re. The working standard solution at required concentration was obtained by continuously diluting the stock solution. The IS stock solution was also prepared with methanol at a final concentration of 720 ng/mL. Calibration samples were prepared by mixing solutions of standard mixture, internal standard, and methanol with rat blank plasma at the final concentrations of 3.6, 7.2, 35.6, 71.2, 142.4, 284.8, 569.5, and 1, 139.0 ng/mL for Rb₁, 1.6, 3.2, 6.3, 31.3, 62.5, 125.0, 250.0, and 500.0 ng/mL for Rb₂, 1.2, 2.4, 4.9, 24.4, 48.8, 97.6, 195.1, and 390.2 ng/mL for Rd, and 2.5, 4.9, 9.9, 49.3, 98.6, 197.1, 394.3, and 788.5 ng/mL for Re. QC samples were also prepared with the same method at low, middle, and high concentrations (7.2, 142.4, and 911.4 ng/mL for Rb₁, 3.2, 62.5, and 400.0 ng/mL for Rb₂, 2.4, 48.8, and 312.2 ng/mL for Rd, 5.0, 98.6, and 630.8 ng/mL for Re). All solutions were stored at 4°C .

2.7. Preparation of Plasma Samples. Plasma specimens (200 μL) were mixed with IS solution (20 μL , 720 ng/mL) in an Eppendorf tube and centrifuged at 4,000 rpm for 15 min at 4°C . Then, the supernatant was removed from plasma sample. Hydrochloric acid (4 μL) was added into 200 μL plasma sample, and then, the mixture was vortexed for 1 min. Waters OASIS HLB SPE C18 columns (Waters, USA) were used to remove the proteins in plasma samples, and it was pre-activated by 3 mL methanol and 3 mL water. Then, 2 mL of 100% methanol was used to wash the column, and the eluate was collected and dried under nitrogen gas (45°C). Subsequently, 100 μL of 100% methanol was used to redissolve the residues, followed by centrifugation at 13,000 rpm for 15 min at 4°C . Before UPLC-MS/MS analysis, the sample was finally filtered through a $0.22 \mu\text{m}$ membrane filter.

2.8. Method Validation. The selectivity, linearity, precision, accuracy, extraction recover, matrix effects (ME), and stability were determined based on the FDA guidelines [19]. To verify whether there was endogenous interference, the specificity between the blank plasma and plasma samples after oral administration of PQ and PQ-AG extracts was evaluated by chromatographic comparisons. The precision and accuracy of analytical methods were expressed as %CV and the relative standard deviation (RSD) value for the QC samples. Recoveries

FIGURE 1: The chemical structures of Rb₁, Rb₂, Rd, Re, and dioscin (IS).

TABLE 1: Precursor/production pairs and parameters for MRM of ginsenosides.

Analytes	Ionization mode	[M-H] ⁻ (<i>m/z</i>)	MRM transitions (precursor-product)	Cone voltage (V)	Collision energy (eV)
Rb ₁	Negative	1107.5	1107.5 → 621.2	80	40
			1107.5 → 459.6*		45
Rb ₂	Negative	1077.7	1077.7 → 621.5	80	50
			1077.7 → 459.5*		60
Rd	Negative	945.5	945.5 → 621.5*	85	50
			945.5 → 459.5		45
Re	Negative	945.5	945.5 → 637.5	80	45
			945.5 → 475.5*		40
Dioscin	Negative	867.6	867.6 → 721.0	85	45

*Quantitative ion.

FIGURE 2: Continued.

FIGURE 2: Product ion mass spectra of Rb₁ (a), Rb₂ (b), Rd (c), Re (d), and dioscin (e).

were determined by the peak areas of the standard plasma and pure reference samples. The stability assays were carried out to demonstrate the experimental conditions of the samples.

2.8.1. Selectivity. Chromatograms of six different blank plasma samples were compared with those of the correspondingly spiked plasma and the plasma samples after oral administration of PQ-AG and PQ extracts to assess the selectivity.

2.8.2. Linearity and LLOQ. The calibration curves were constructed by plotting the peak area ratio versus the

concentrations of the four analytes with a weighted ($1/x^2$) least square linear regression using spiked plasma samples at six concentrations. The LLOQ was defined as the lowest analytical concentration of the calibration curve, at which the measured precision expressed as RSD was within $\pm 20\%$ and the accuracy was within $\pm 20\%$.

2.8.3. Precision and Accuracy. The intraday and interday precisions as well as accuracies were evaluated by determining the ginsenoside concentration in QC samples at three different levels (LQC, MQC, and HQC) in six

replicates on the same day and during three different days. The precision was defined as RSD of the measured concentration, and the accuracy (%) of the measured mean value was deviated from the nominal value. The intraday and interday precisions were within 15%. The accuracy was within 80–120%. The RSD of LLOQ samples should be within 20%.

2.8.4. Extraction Recovery and ME. Blank plasma samples from six rats were extracted and then spiked with analytes and IS to evaluate the ME. ME was evaluated by comparing the peak areas of QC samples at three concentration levels after sample preparation with those prepared in solution at the same concentration level. Recovery was calculated by comparing the peak areas of QC samples with those spiked after sample preparation at the same concentration level.

2.8.5. Stability. The stability of the four analytes was determined by exposing plasma samples to QC at three concentration levels in six replicates. Samples were stored at -80°C for 1 month to assess the short-term storage stability. Freeze-thaw stability was performed after three freeze-thaw cycles at -80°C during three consecutive days. To evaluate the postpreparative stability, extracted QC samples were kept in the autosampler (4°C) for 12 h before analysis. The stability assessment of these samples was calibrated with freshly prepared standard as previously described.

2.9. Statistical Analysis. DAS 3.2 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China, 2011) was applied for the analysis of concentration-time data to investigate the pharmacokinetic parameters of four ginsenosides in various groups. Data were presented as the mean $\pm$ SD (standard deviation) with triplicate measurements. A significance level was set at $P < 0.05$.

2.10. Pharmacokinetic Studies. Male SD rats were randomly and evenly divided into two groups ($n = 6$ per group) in pharmacokinetic studies. To evaluate the compatible effect of AG upon PQ treatment, rats were given PQ at a dosage of 0.54 g/kg (equivalent to the amount of raw drug 0.54 g) in the PQ group. PQ-AG was combined with an equal proportion to prepare the mixed extract. The oral dosage in rats was 1.08 g/kg (equivalent to the amount of the raw drug including 0.54 g AG and 0.54 g PQ) in the PQ-AG group. Blood samples were collected at specific time points of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, and 48.0 h. All the plasma samples were obtained by centrifuging the blood samples at 3,500 rpm for 10 min, then labeled and frozen at -80°C prior to analysis.

3. Results and Discussion

3.1. Optimization of UPLC-MS Conditions. In the optimization process, we found that acetonitrile/ H_2O system offered the best performance among different mobile phases, including methanol/water, acetonitrile/water, and methanol/acetonitrile/

water. Formic acid in water could provide the best peak shape among different modifiers. We also optimized additional UPLC conditions, such as column temperature (25, 30, 35, and 40°C) and flow rate (0.2, 0.3, and 0.4 mL/min). The highest selectivity and resolution were obtained for all tested compounds within 7.0 min under the optimized UPLC conditions as follows: Waters ACQUITY UPLC BEH C_{18} (100×2.1 mm, 1.7 μm) column at 35°C and 0.1% formic acid in water (A)-acetonitrile (B) mobile phase at a flow rate of 0.2 mL/min. The precursor ion was $[\text{M}-\text{H}]^{-}$ at m/z 1, 107.5 for Rb_1 , and the product ion peak was at m/z 459.6, which correspond to the loss of four molecules of Glc $[\text{M}-4\text{Glc}-\text{H}]^{-}$. The precursor ion was $[\text{M}-\text{H}]^{-}$ at m/z 1, 077.7 for Rb_2 , and the product ion peak was at m/z 459.5, which correspond to the loss of three molecules of Glc and one molecule of Ara $[\text{M}-3\text{Glc}-\text{Ara}-\text{H}]^{-}$. The precursor ion was $[\text{M}-\text{H}]^{-}$ at m/z 945.5 for Rd, and the product ion peak at m/z 621.5, which correspond to the loss of two molecules of Glc $[\text{M}-2\text{Glc}-\text{H}]^{-}$. The precursor ion was $[\text{M}-\text{H}]^{-}$ at m/z 945.5 for Re, and the product ion peak was at m/z 475.5, which correspond to the loss of two molecules of Glc and one molecule of Rha $[\text{M}-2\text{Glc}-\text{Rha}-\text{H}]^{-}$. The precursor ion was $[\text{M}-\text{H}]^{-}$ at m/z 867.6 for internal standard (IS), and the product ion peak was at m/z 721.0, which correspond to the loss of one molecule of Rha $[\text{M}-\text{Rha}-\text{H}]^{-}$. Table 1 summarizes the main MS parameters, including cone voltage (CV) and collision energy (CE).

Under the above-mentioned chromatographic conditions, saponin components showed more intensive deprotonated ions in the negative ion mode than those of protonated or sodiated precursors in the positive mode. Therefore, an UPLC-MRM-MS with an ESI interface in the negative mode was used for separation and detection of all compounds tested. Reference standards showed good peak shape and excellent resolution. Figure 2 shows the typical mass spectra and MS/MS spectra of Rb_1 , Rb_2 , Rd, Re, and dioscin. Dioscin was used as the IS. The chemical structure of IS was found to be similar to analytes, and it exhibited a stable response, indicating effective separation.

3.2. Method Validation

3.2.1. Selectivity. Figure 3 illustrates the typical multiple reaction monitoring (MRM) chromatograms from blank plasma sample and the sample at 8 h after oral administration of PQ and PQ-AG extracts. No interference peaks were detected during the retention time of IS as well as Rb_1 , Rb_2 , Rd, and Re.

3.2.2. Linearity and Lower Limit of Quantification (LLOQ). Table 2 displays the typical equations of calibration curves for the four analytes. The correlation coefficient (r) for each calibration curve was higher than 0.9978. The result indicated that the ratio of peak area strongly correlated with the concentration of each compound within the acceptable linearity ranges. The LLOQs of the four analytes are shown in Table 2.

3.2.3. Precision and Accuracy. The precision and accuracy of the assay were validated for the samples at LLOQ and three

(a)

FIGURE 3: Continued.

(b)

FIGURE 3: Continued.

(c)

FIGURE 3: Continued.

FIGURE 3: Representative MRM chromatograms of Rb₁, Rd, Re, Rb₂, and IS in rat plasma samples. Blank rat plasma (a), a blank plasma with IS and analytes at MQC levels (b), a plasma sample taken 8 h after oral administration of PQ (c), and PQ-AG extracts (d).

QC levels analyzed on the same day or during three consecutive days. The results are presented in Table 3. The intraday precision and interday precision ranged from 4.57% to 14.63%.

3.2.4. Extraction Recovery and ME. The recoveries were obtained at three concentration levels, ranging from 76.43% to 95.20%. The ME of all the analytes in blank plasma were within the acceptable range of 90.44%–107.11% (Table 4).

TABLE 2: The regression equations, linear ranges, and LLOQs of the four compounds.

Compound	Linear regression equation	Linear range (ng/mL)	r	LLOQ (ng/mL)
Rb ₁	$Y = 2.823 \times 10^{-3}X + 9.654 \times 10^{-2}$	3.6–1139	0.9987	3.6
Rb ₂	$Y = 7.729 \times 10^{-4}X + 2.824 \times 10^{-3}$	1.6–500.0	0.9989	1.6
Rd	$Y = 7.774 \times 10^{-3}X + 9.646 \times 10^{-2}$	1.2–390.2	0.9979	1.2
Re	$Y = 4.036 \times 10^{-4}X + 3.149 \times 10^{-3}$	2.5–788.5	0.9978	2.5

TABLE 3: Precision and accuracy for the determination of the ginsenosides in rat plasma.

Compounds	Spiked concentration (ng/mL)	Measured (ng/mL)	Intraday precision RSD (%)	Interday precision RSD (%)	Accuracy Re (%)
Rb ₁	3.6	3.3 ± 0.21	12.01	13.73	3.40
	7.2	7.1 ± 0.10	7.55	7.02	−1.22
	142.4	143.1 ± 1.05	6.83	4.57	4.39
	911.4	910.9 ± 0.05	9.57	5.44	6.01
Rb ₂	1.6	1.7 ± 0.13	13.54	14.01	−1.84
	3.2	3.2 ± 0.05	14.32	14.48	−5.32
	62.5	61.5 ± 0.18	6.82	8.07	3.76
	400.0	399.3 ± 0.25	9.97	9.19	5.37
Rd	1.2	1.3 ± 0.17	14.61	10.22	4.20
	2.4	2.7 ± 0.21	13.57	13.74	−2.10
	48.8	49.5 ± 0.17	10.83	7.22	−4.05
	312.2	311.9 ± 0.26	6.39	6.04	5.01
Re	2.5	2.5 ± 0.07	11.65	10.34	−6.94
	5.0	4.89 ± 0.26	14.63	14.57	3.29
	98.6	98.3 ± 0.04	8.77	11.22	7.35
	630.8	632.8 ± 0.31	12.08	7.58	−4.10

TABLE 4: Recoveries and matrix effects of the ginsenosides in rat plasma.

Compound	Concentration (ng/mL)	Extraction recovery		Matrix effect	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Rb ₁	7.2	77.51	6.31	93.37	8.12
	142.4	85.73	1.52	98.36	4.33
	911.4	76.43	13.15	90.44	8.79
Rb ₂	3.2	79.62	2.50	91.11	8.01
	62.5	86.89	10.74	97.83	7.52
	400.0	78.15	4.62	102.10	3.77
Rd	2.4	86.64	13.60	99.67	12.74
	48.8	95.20	10.17	100.29	8.88
	312.2	88.58	6.51	106.2	13.74
Re	5.0	82.34	6.74	102.56	11.12
	98.6	86.32	3.33	99.52	7.65
	630.8	85.79	12.10	107.11	15.02
IS	720	89.31	5.76	92.30	6.47

The ME of IS was 92.30%. Therefore, it demonstrated that ME of the plasma was negligible in the assay.

3.2.5. Stability. The sample stability during storage and processing procedures was evaluated by the analysis of QC samples (Table 5), showing that these analytes were stable after short-term storage (1-month storage at -80°C), post-preparative stability (12 h in the autosampler at 4°C), and three freeze-thaw cycles. All compounds remained stable for 2 weeks at -80°C .

3.3. Pharmacokinetics Study. Figure 4 shows the mean plasma concentration-time curves of Rb₁, Rb₂, Rd, and Re after oral administration of PQ-AG or PQ. Table 6 summarizes the pharmacokinetic parameters of noncompartment model, including maximum plasma concentration ($C_{\max}$), time to reach the maximum concentrations ($T_{\max}$), half-time ($t_{1/2}$), and area under concentration-time curve ($\text{AUC}_{0 \rightarrow t}$ and $\text{AUC}_{0 \rightarrow \infty}$). These results showed that UPLC-MS/MS could be used to detect Rb₁, Rb₂, Rd, and Re, revealing good absorption of four compounds. The significant differences among Rb₁, Rb₂, Rd, and Re were observed

TABLE 5: Stability of the ginsenosides in rat plasma.

Compound	Spiked concentration (ng/mL)	Mean concentration (ng/mL)	Short term	Re (%)		Recovery (%)		
				Freeze-thaw cycles	Postpreparation	Short term	Freeze-thaw cycles	Postpreparation
Rb ₁	7.2	7.1	-7.55	-6.90	6.86	96.32	97.66	101.85
	142.4	141.2	7.95	-4.48	9.35	100.48	95.35	101.65
	911.4	910.5	7.53	7.75	-6.47	101.65	101.95	96.10
Rb ₂	3.2	3.0	-9.70	-9.69	-9.72	93.67	94.21	93.37
	62.5	61.6	-2.12	6.77	2.39	95.33	100.41	99.94
	400.0	402.9	7.11	9.36	7.90	98.74	103.82	99.63
Rd	2.4	2.4	8.64	-11.93	4.43	102.66	97.24	100.10
	48.8	49.7	10.24	8.87	9.98	103.61	100.20	101.71
	312.2	310.2	7.35	8.12	-7.02	101.27	102.00	94.81
Re	5.0	5.1	-10.71	7.33	8.54	97.30	104.07	104.63
	98.6	95.5	12.11	7.53	8.92	99.25	94.12	97.21
	630.8	634.2	8.40	-9.85	9.76	101.73	97.39	102.50

FIGURE 4: Mean concentration-time profiles of Rb₁ (a), Rb₂ (b), Rd (c), and Re (d) after oral administration of PQ-AG and PQ extracts ($n = 6$, mean $\pm$ SD).

between PQ-AG and PQ groups after oral administration. For Rb₁ with certain bioavailability [20], the plasma $C_{\max}$ was 600.2 ± 53.6 ng/mL in the PQ-AG group, which was significantly higher than that (461.2 ± 15.9 ng/mL) of the PQ group ($P < 0.05$). Meanwhile, the $T_{\max}$ of Rb₁ was 6.67 ± 1.03 h for the PQ-AG group and 7.00 ± 1.10 h for the PQ group, illustrating Rb₁ with high uptake in rat plasma for

the PQ-AG group. The reason might be attributed to those other components in the PQ-AG group which promoted the absorption and metabolism of Rb₁. The $C_{\max}$ and $T_{\max}$ values of Rb₂ in the PQ-AG group were 264.1 ± 24.5 ng/mL and 7.67 ± 0.82 h, respectively. The $C_{\max}$ and $T_{\max}$ values of Rb₂ in the PQ group were 160.1 ± 18.8 ng/mL and 7.67 ± 0.82 h, respectively. Rd showed a good absorption in the PQ-AG

TABLE 6: Pharmacokinetic parameters of the ginsenosides after oral administration of PQ-AG and PQ extracts in rats ($n=6$, mean $\pm$ SD).

Group	Compounds	$C_{\max}$ (ng/mL)	$T_{\max}$ (h)	$t_{1/2}$ (h)	$AUC_{0\rightarrow t}$ (ng·h/mL)	$AUC_{0\rightarrow\infty}$ (ng·h/mL)
PQ-AG	Rb ₁	600.2 $\pm$ 53.6*	6.67 $\pm$ 1.03	18.52 $\pm$ 2.61*	8132.09 $\pm$ 454.40*	8414.43 $\pm$ 487.69*
	Rb ₂	264.1 $\pm$ 24.5*	7.67 $\pm$ 0.82	15.61 $\pm$ 5.21*	2458.74 $\pm$ 242.86*	2518.20 $\pm$ 288.99*
	Rd	283.4 $\pm$ 11.1*	6.33 $\pm$ 0.82*	14.21 $\pm$ 1.98*	3874.28 $\pm$ 356.39*	3955.23 $\pm$ 382.94*
	Re	460.4 $\pm$ 30.2*	0.58 $\pm$ 0.20*	9.81 $\pm$ 2.15*	3427.96 $\pm$ 293.76*	3582.89 $\pm$ 396.42*
PQ	Rb ₁	461.2 $\pm$ 15.9	7.00 $\pm$ 1.10	15.61 $\pm$ 1.52	5983.67 $\pm$ 313.47	6146.74 $\pm$ 328.31
	Rb ₂	160.1 $\pm$ 18.8	7.67 $\pm$ 0.82	13.34 $\pm$ 1.58	1214.41 $\pm$ 99.25	1229.91 $\pm$ 99.58
	Rd	200.9 $\pm$ 7.3	7.67 $\pm$ 0.82	13.11 $\pm$ 2.61	3005.92 $\pm$ 229.63	3324.67 $\pm$ 317.05
	Re	354.7 $\pm$ 26.6	1.08 $\pm$ 0.20	8.14 $\pm$ 1.20	2331.52 $\pm$ 214.10	2379.76 $\pm$ 223.68

* $P < 0.05$ compared with PQ group.

group, indicating that $C_{\max}$ was 283.4 ± 11.1 ng/mL and $T_{\max}$ was lower than that in the PQ group ($P < 0.05$), in which the $C_{\max}$ was 200.9 ± 7.3 ng/mL and $T_{\max}$ was 7.67 ± 0.82 h. The results also showed that the $T_{\max}$ of Rd and Re in the PQ-AG group was shorter than those in the PQ group ($P < 0.05$). Moreover, as shown from Figure 4 and Table 6, the $T_{\max}$ value of Re, which was different from the other three compounds between the two groups, was 0.58 ± 0.20 and 1.08 ± 0.20 h. Although the entire hypothesis needed to be further validated, Rd and Re had different absorptions compared with Rb₁ and Rb₂, and the double-peak phenomenon was detected at the same time, which could be probably attributed to the enterohepatic circulation in drug metabolism or the interactivity of PQ-AG drug pair [21, 22]. In addition, $C_{\max}$, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow\infty}$ of the four active ingredients in the PQ-AG group were higher than those in the PQ group. It suggested that four compounds in the PQ-AG group had synergistic effects with other compounds, leading to increased plasma contents of four analytes by promoting absorption. Compared with the PQ group ($P < 0.05$), the $t_{1/2}$ of four analytes in the PQ-AG group was 18.52 ± 2.61 , 15.61 ± 5.21 , 14.21 ± 1.98 , and 9.81 ± 2.15 h, respectively, indicating slow elimination. Moreover, the absorption was enhanced after compatibility, and the duration of drug action was prolonged for $t_{1/2}$ of the four compounds in the PQ-AG group. Furthermore, the bioavailability of bioactive components in the PQ-AG group showed an upward trend, indicating that the four bioactive compounds of Rb₁, Rb₂, Rd, and Re were well absorbed and gradually removed in the PQ-AG group, which might be attributed to the synergic action between PQ and AG. According to TCM theory, the compatibility mechanisms of drug pairs are not only arbitrary plus of two drugs but also the regularity of active components in vivo [23]. In the PQ-AG group, the dissolutions of various chemical constituents were increased due to the good absorption of ginsenosides in PQ [24]. We ventured to guess that the volatile components of AG probably enhanced the absorption of ginsenosides in PQ [25, 26]. In the PQ-AG group, the ginsenoside possessed a greater uptake and slower elimination, which was connected with the interaction between AG and PG, including the mediations of transport proteins, metabolic enzymes, and plasma protein binding. Definitely, the detailed mechanisms of PQ-AG drug pairs needed to be further investigated.

The results showed that AG enhanced the absorption and delayed the elimination of the four analytes in PQ. Moreover, it could result in a better bioavailability of ginsenosides. Some previous literatures have shown that certain compounds in AG may suppress P-glycoprotein functions in the intestinal membrane and the activity of enzyme CYP1A2 [27]. These findings could probably explain why the main components of PQ accumulated after PQ-AG was simultaneously administered. Although these hypotheses needed to be further investigated, we believed that AG and the compatibility of ingredients could result in a better absorption of ginsenosides in PQ.

4. Conclusions

In the present study, we successfully applied a simple, rapid, and sensitive UPLC-MS/MS method to simultaneously determine four bioactive ginsenosides in PQ and the combination of PQ and AG. We, for the first time, reported pharmacokinetic parameters of Rb₁, Rb₂, Rd, and Re in PQ-AG after oral administration. Our data showed that the four analytes had a better absorption and slower elimination after PQ and AG were combined. The changes of four main active substances in vivo provided valuable insights into the compatibility principles of PQ-AG. In addition, our current findings clarified the rational compatibility of TCM, suggesting better clinical application and research potentials.

Data Availability

All data contained in the manuscript will be made available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Ying Zhao conceived and designed the experiments. Hailong Xie performed the experiments. Dongxue Wang analyzed the data. Ying Zhao contributed reagents/materials/analysis tools. Hailong Xie and Ying Zhao wrote the paper. Ying Zhao acquired funding for the research. All authors approved the final manuscript.

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