Chinese Herbal Medicines 11 (2019) 308–313

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Effects of *Raphani Semen* on anti-fatigue and pharmacokinetics of *Panax ginseng*

Jian-bo Chen, Mei-jia Li, Li-xue Chen, Yin-shi Sun*

Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agriculture Sciences, Changchun 130112, China

ARTICLE INFO

Article history:
Received 4 June 2018
Revised 26 December 2018
Accepted 22 January 2019
Available online 14 May 2019

Keywords:
anti-fatigue
interactions
Panax ginseng C. A. Mey.
pharmacokinetics
Raphani Semen

ABSTRACT

Objective: To explain the phenomenon that *Panax ginseng* is not compatible with *Raphani Semen* based on pharmacodynamics and pharmacokinetics.

Methods: The forced swimming time and biochemical parameters such as blood lactate (BLA), serum urea nitrogen (SUN), and hepatic glycogen (GLU) were determined for anti-fatigue experiment. The UPLC-MS/MS was used to analyze the pharmacokinetics of Rg1, Re, Rb1, and Rd after orally administration of *P. ginseng* and *P. ginseng* combined with *Raphani Semen* to rats. Pharmacokinetic differences of four ginsenosides between single uses of *P. ginseng* and combined with *Raphani Semen* were investigated.

Results: The results showed that *Raphani Semen* tended to significantly reduce the anti-fatigue activity of *P. ginseng*. Co-administration of *P. ginseng* and *Raphani Semen* had significant effects on the pharmacokinetics of the four ginsenosides in rats compared to that observed with *P. ginseng* extract alone. The $AUC_{0-12\ h}$ values of the four ginsenosides in PG group were higher than the corresponding values in the PR group. It can be inferred that *Raphani Semen* decreased the blood exposure of the four ginsenosides in rats when it combined with *P. ginseng*.

Conclusion: The anti-fatigue activity and pharmacokinetic results showed that *Raphani Semen* may reduce the pharmacological actions of *P. ginseng*.

© 2019 Tianjin Press of Chinese Herbal Medicines. Published by Elsevier B.V. All rights reserved.

1. Introduction

Using two or more herbs together can decrease or limit their therapeutic actions or even generate toxic effects. This phenomenon has been recognized as “restraint” or “antagonism” since Song dynasty according to traditional Chinese medicine (TCM) theory (Wang et al., 2009; Dai, Luo, Wang, & Xia, 2005). “*Panax ginseng* (Renshen in Chinese) is not compatibility with *Raphani Semen* (Laifuzi in Chinese)” is a typical example.

Raphani Semen has been used to treat chronic intestinal disorder in TCM for more than 1400 years (Kim et al., 2015; Sham et al., 2013). *P. ginseng*, as one of the most famous botanical supplements, originates in oriental countries such as China, Japan, and Korea (Ang-Lee, Moss, & Yuan, 2001; Attele, Wu, & Yuan, 1999; Lars, Martin, & Ulla, 2006). It is not compatible with *Raphani Semen*, according to the “compatibility prohibition of TCM”. It is reported that ginsenosides were less in compatible decoction than in separate one during combination of *P. ginseng* with *Raphani Semen* (Zhang, Wang, & Song, 2007). In addition, there is no pharmacodynamics and pharmacokinetics research on the compatibility of the two herbs. We want to explain the phenomenon based on pharmacodynamics and pharmacokinetics.

In TCM theory, *P. ginseng* is normally used for developing physical strength of those suffering from severe fatigue (Saito, Yoshida, & Takagi, 1974). Fatigue is usually caused by decrease in glycemic level, consumption of liver glycogen and accumulation of fatigue metabolites (Tan et al., 2012). Pharmacokinetic researches are helpful for understanding herb-herb interactions and for assessing the herb-herb compatibility (Chen, Chou, Lin, & Yang, 2002; Wang et al., 2008). Given that *P. ginseng* contains a variety of chemicals, only some main representative compounds are selected as markers to analyze the pharmacokinetics of ginseng (Li et al., 2000; Liao et al., 2005). Rg1, Re, Rb1, and Rd are selected as the makers for pharmacokinetics study (Choi et al., 2015). These four compounds have been shown to have various biological activities (Mook-Jung et al., 2001; Yamaguchi, Higashi, & Kobayashi, 1996). Some studies have been reported on the pharmacokinetics of Rg1, Re, Rb1, and Rd in *P. ginseng* or other TCM formulas. However, the pharmacokinetic of these four active compounds in *P. ginseng* combined with *Raphani Semen* was not reported, the mechanism of decreasing or increasing therapeutic actions after co-administration with *Raphani Semen* was unknown.

* Corresponding author.
E-mail address: sunyinshi2015@163.com (Y.-s. Sun).

https://doi.org/10.1016/j.chmed.2019.05.001
1674-6384/© 2019 Tianjin Press of Chinese Herbal Medicines. Published by Elsevier B.V. All rights reserved.

This paper will study the anti-fatigue activity and pharmacokinetic of these four ginsenosides in *P. ginseng* after oral administration to rats either alone or in combination with *Raphani Semen*. Moreover, this paper reveals the assertion that *P. ginseng* is not compatible with *Raphani Semen* by carrying out pharmacokinetic experiment in rats for the first time.

2. Materials and methods

2.1. Chemicals and materials

Rg1, Re, Rb1, Rd and digoxin (Fig. 1) were purchased from Sigma Chemical Co. (St. Louis, MO). Assay kits for determining blood lactate (BLA), serum urea nitrogen (SUN) and hepatic glycogen (GLU) were purchased from Changchun Baiao Biotechnology Institute (Changchun, China). Chromatographic grade of acetonitrile, methanol and water were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Analytical methods

The UPLC-MS/MS (Waters Xeve TQ) with the analytical C_{18} column (Thermo Scientific, Syncronis Q, 2.1 × 50 mm, 1.7 um) was carried out at 35 °C. The elution water (A) and acetonitrile (B) was carried out under the condition of 20% B at 0–2.0 min, 20%→45% B at 2.0–4.5 min, 45%→60% B at 4.5–6.0 min, 60%→70% B at 6.0–7.0 min. Flow rate was set at 0.5 mL/min. The mass spectrometer was operated in negative ionization mode to evaluate the four ginsenosides and digoxin. The drying gas temperature was set to 380 °C, the drying gas flow was set to 80 L/h, the nebulizer pressure was set to 35 psi, and the capillary voltage was set to 3000 V.

2.3. Animals

Kunming mice with weight ranging from 20 to 24 g were collected for anti-fatigues study, and male Sprague-Dawley rats with weight ranging from 240 to 260 g were used for pharmacokinetic study. The rats were fed in a specific pathogen free cage, with free accesses to eating and drinking. The illumination condition was set to 12:12 h light-dark cycle, the temperature was set to (22 ± 2) °C and humidity was set to (50 ± 10)%.

2.4. Preparation of P. ginseng and Raphani Semen extract

P. ginseng (200 g) and *Raphani Semen* (200 g) were soaked separately in 70% ethanol (500 mL) and extracted using a reflux extraction method for 30 min. The extracting solution was filtered and evaporated under reduced pressure to obtain concentrates (100 mL). The contents of Rg1, Re, Rb1, and Rd in *P. ginseng* were measured to be 1.8 mg/mL, 2.5 mg/mL, 6.5 mg/mL, and 0.7 mg/mL, respectively. Finally, three drug solutions were prepared, namely *P. ginseng* extract (PG), *Raphani Semen* extract (RS), and *P. ginseng* extract mixed with *Raphani Semen* extract (PR). These solutions were stored at 4 °C.

2.5. Anti-fatigue assay

2.5.1. Experimental design

In this research, 40 mice were randomly divided into the control group (CG group, fed with distilled water), *P. ginseng* extract (PG) group, *P. ginseng* extract mixed with *Raphani Semen* extract (PR) group, and *Raphani Semen* extract (RS) group, respectively, each containing 10 mice. The PG group was orally administered with *P. ginseng* at a dose of 5 g/kg, PR group was applied with *P. ginseng* extract mixed with 5 g/kg *Raphani Semen* extract, and RS group was given with *Raphani Semen* extract at a dose of 5 g/kg, respectively. The administration was performed once a day for consecutive 4 weeks. On the 14th day (the last day for oral administration), forced swimming test (FST) was conducted for the four groups, and biochemical parameters such as serum urea nitrogen (SUN), blood lactate (BLA) and hepatic glycogen (GLU) were measured.

2.5.2. Forced swimming test

The mice in PG group, RP group and RS group were orally fed once a day for consecutive 4 weeks. At the last feeding day, the mice were fed and 30 min later placed in a swimming tank for FST. The FST was conducted in a swimming tank (50 cm × 40 cm × 40 cm), with water depth of (30 ± 2) cm at temperature of (25 ± 0.5) °C. A lead sinker accounting for 10% of mouse body weight was loaded on the tail root. The mice were thought exhausted when they failed to struggle above the water surface or failed to make necessary movements to breathe for 10 s.

2.5.3. Biochemical parameters analysis

Biochemical parameters were analyzed after 4 weeks of administration. From the 30th min after the last administration of PG, PR and RS, the mice were subjected to FST for 60 min without loads. After FST, the mice rested for an hour, followed by blood sample collection from eyeballs with 15 min of centrifugation at 4000 r/min at 4 °C to prepare serum. BLA and SUN were measured in accordance with the recommended procedures prescribed in kits. GLU level was tested by commercially available kits.

2.6. Pharmacokinetics study

The rats were randomly separated into two groups, each containing six rats. One group was orally administered with PG, and

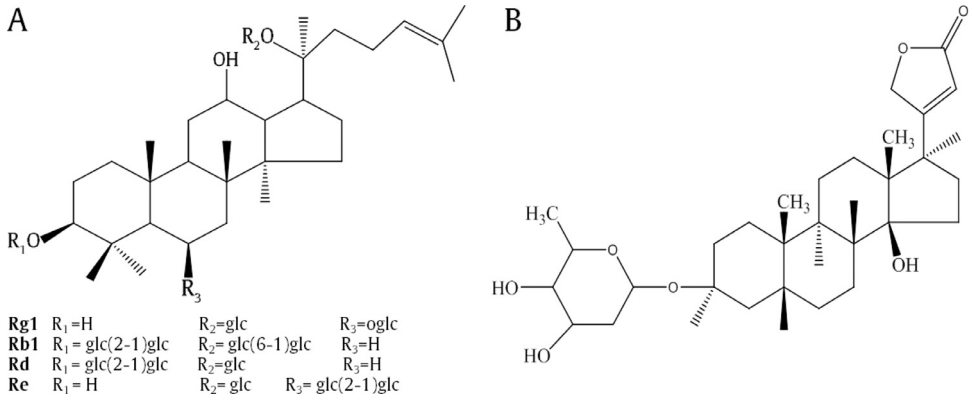

Fig. 1. Chemical structures of Rg1, Rb1, Re, and Rd (A) and internal standard digoxin (B).

the other group was orally administered with PR. The four ginsenosides were given to the rats in each group by dosages of 18 mg/kg, 25 mg/kg, 65 mg/kg, and 7.0 mg/kg, respectively. Blood samples (0.3 mL) were collected via eye puncturing at 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 36 h, before accepting immediate centrifugation at 15000 r/min for 5 min. Plasma (0.15 mL) was spiked with acetonitrile (0.3 mL) and IS (20 μL) by vortex mixing for 30 min. The mixture was centrifugation at 15 000 r/min for 5 min to separate the precipitated protein. The supernatant was injected into LC-MS/MS. The plasma concentration of four ginsenosides was expressed in the form of mean ± standard deviation. All data were processed by non-compartmental analysis using WinNonlin 6.1.

2.7. Validation of LC-MS/MS assay

The four ginsenosides and digoxin were accurately weighed and dissolved to a final concentration of 1000.0 μg/L for Rg1, 1000.0 μg/L for Re, 2000.0 μg/L for Rb1, 2000.0 μg/L for Rd, and 1000.0 μg/L for digoxin, respectively. Then, final standard solutions were diluted with blank plasma, resulting in five working solutions, including Rg1 (1.0, 5.0, 50.0, 100.0, 500.0 μg/L), Re (1.0, 5.0, 50.0, 100.0, 500.0 μg/L), Rb1 (5.0, 50.0, 500.0, 1000.0, 2000.0 μg/L), and Rd (5.0, 50.0, 500.0, 1000.0, 2000.0 μg/L). In the same way, quality control (QC) samples were prepared with blood plasma for Rg1 (1.0, 50.0, 500.0 μg/L), Re (1.0, 50.0, 500.0 μg/L), Rb1 (5.0, 500.0, 2000.0 μg/L), and Rd (5.0, 500.0, 2000.0 μg/L), respectively.

The precision and accuracy of the method were investigated by analyzing six replicates of QC samples in the same analytical method. Through comparing the response rate obtained for extracted QC samples with that obtained for spiked plasma extracts, the extraction recovery of the QC sample could be evaluated. The storage stability of plasma samples was measured according to the ginsenoside concentrations of five replicate QC plasma samples stored at −80 °C for 30 d. Freeze-thaw stability from −80 °C to room temperature was measured. The post-preparation stability was determined by assaying the extracted QC samples stored in the auto-sampler (5 °C) for 24 h.

2.8. Pharmacokinetic analysis

After oral administration of PG and PR, the concentrations of the four ginsenosides in rat plasma were measured by UPLC-MS/MS. The WinNonlin software was used to calculate the following PK parameters: C_{max}, peak plasma concentration; T_{max}, time at C_{max}; $AUC_{0-12\ h}$, area under the curve when blood concentration is plotted against time from 0 to 12 h; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; MRT, mean residence time; CL/F, clearance rate; and $t_{1/2}$, elimination half-life.

3. Results

3.1. Validation of UPLC-MS/MS method

The selectivity of the analytical method was evaluated (Fig. 2). The correlation coefficient of determination (r) was over 0.995, indicating a good linearity within the selected range (Table 1). The assay accuracy was 90.2%–108.8%, and the precision (relative standard deviation) was less than 9.9%, indicating a high level of reproducibility (Table 2). The extraction recoveries of the four ginsenosides were within the range from 75.2% to 91.5% (Table 3). According to the stability values in Table 4, all analytes remained stable after storing at ambient temperature for 4 weeks, followed by three freeze-thaw cycles for three consecutive days, and stored at −80 °C for 4 weeks or at 5 °C in the auto-sample pool for 24 h.

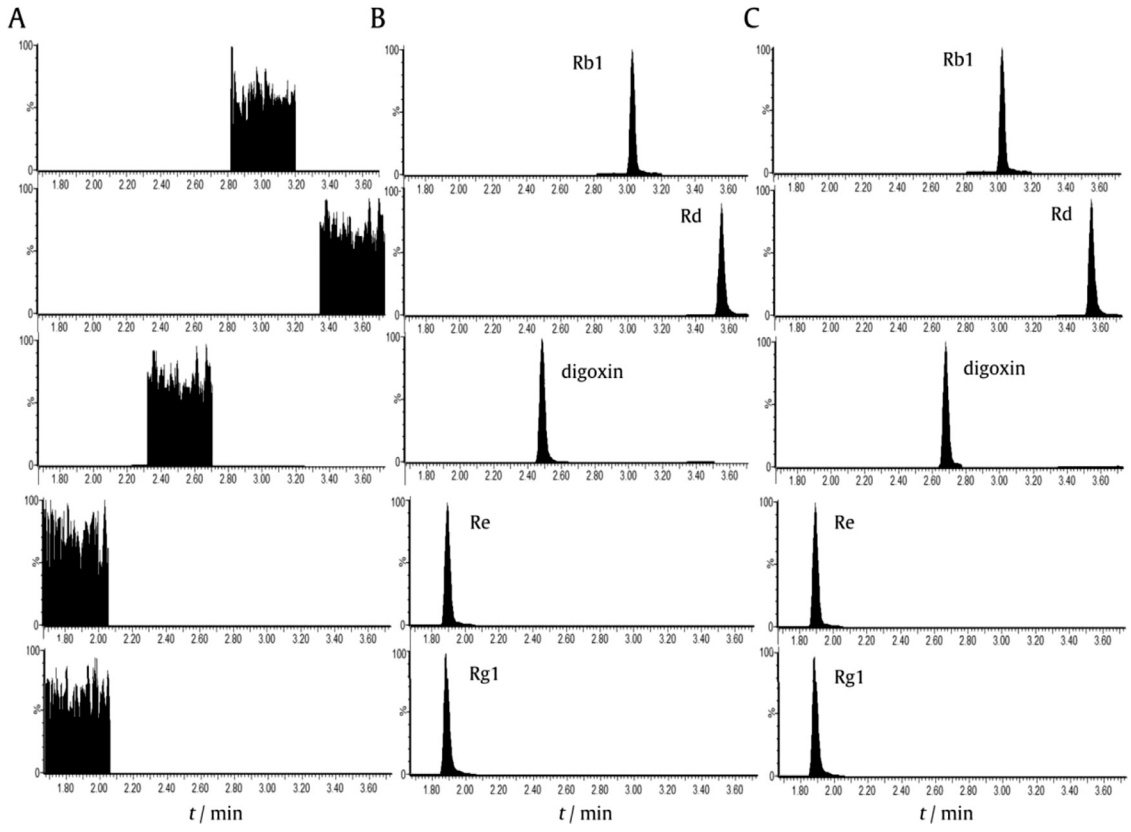

Fig. 2. Typical MRM chromatograms of blank plasma (A), blank plasma spiked with standard compounds Rg1, Re, Rb1 and Rd and digoxin (IS) (B), and plasma samples 15 min following oral administration of PR (C).

Table 1
Regression data and LLOQ of analytes determined ($n = 6$).

Ginsenosides in plasma	Linear regression equations	r^2	Linear range/($\mu g \cdot L^{-1}$)	LLOQ/($\mu g \cdot L^{-1}$)
Rg1	$y = 0.142x + 0.04$	0.999	1.0–500.0	1.0
Re	$y = 0.217x + 0.12$	0.995	1.0–500.0	1.0
Rb1	$y = 0.395x + 0.03$	0.998	5.0–2000.0	5.0
Rd	$y = 0.413x + 0.11$	0.999	5.0–2000.0	5.0

Table 2
Accuracy and precision of analytes in blank plasma.

Ginsenosides	Spiked/($\mu g \cdot L^{-1}$)	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
		Accuracy/%	Precision RSD/%	Accuracy/%	Precision RSD /%
Rg1	10.0	103.1	9.5	105.7	9.1
	50.0	92.3	9.8	105.6	8.5
	100.0	96.9	5.3	92.7	5.4
Re	10.0	95.1	4.5	93.1	8.2
	50.0	90.2	9.5	107.4	9.2
	100.0	108.8	4.0	96.2	8.8
Rb1	100.0	103.2	7.1	91.1	7.4
	500.0	93.5	9.7	91.5	6.3
	2000.0	93.3	8.9	91.5	4.3
Rd	100.0	94.5	4.2	90.1	6.6
	500.0	94.3	7.3	101.3	9.9
	2000.0	97.7	8.4	108.8	7.5

Table 3
Extraction recovery of analytes in rat plasma ($n = 3$).

Ginsenosides	Spiked/($\mu g \cdot mL^{-1}$)	Extraction recovery/%	RSD/%
Rg1	10.0	77.1	8.4
	50.0	82.2	6.3
	100.0	84.4	7.8
Re	10.0	75.2	8.6
	50.0	87.4	10.6
	100.0	85.2	8.8
Rb1	100.0	77.2	10.7
	500.0	85.5	6.7
	2000.0	91.5	9.8
Rd	100.0	89.2	5.3
	500.0	82.5	8.2
	2000.0	80.8	8.6

3.2. Forced swimming test

Fig. 3 showed the effect of *Raphani Semen* on the results of FST. It indicated that PR group had shorter forced swimming time than PG group ($P < 0.01$). The average running duration before exhaustion was (420 ± 52) s for PR group, which was 19.2% shorter than that of PG group, (520 ± 45) s. In addition, the forced swimming time of PG group was longer than PR and RS group.

3.3. Analysis of BLA, SUN and GLU

As shown in Fig. 4, it can be seen that after FST, BLA level (13.4 mmol/L) of PR group was higher than that of PG (11.2 mmol/L) group ($P < 0.05$); SUN level of PR (12.4 mmol/L) group was higher than that of PG and lower than that of RS group (10.2 mmol/L) ($P < 0.01$); GLU level of PR group was 14.2 mg/g, which was higher than that of RS group (16.3 mg/g) ($P < 0.01$) and lower than that of PG group.

Table 4
Stability of analytes under different conditions (mean ± SD).

Ginsenosides	Spiked/($\mu g \cdot mL^{-1}$)	Accuracy/%			
		Short-term stability	Long-term stability	Freeze-thaw stability	Post-preparative stability
Rg1	10.0	101.0 ± 5.4	104.7 ± 8.1	103.1 ± 3.5	91.4 ± 4.6
	50.0	95.2 ± 7.1	103.1 ± 9.3	105.0 ± 8.4	105.1 ± 6.1
	100.0	93.1 ± 8.4	94.6 ± 5.4	97.2 ± 4.1	96.5 ± 7.5
Re	10.0	94.3 ± 5.5	94.6 ± 7.8	98.2 ± 8.1	95.4 ± 4.7
	50.0	94.5 ± 6.5	107.6 ± 6.7	104.3 ± 6.1	102.5 ± 7.1
	100.0	95.4 ± 7.0	94.3 ± 4.5	95.2 ± 8.2	94.5 ± 6.0
Rb1	100.0	103.5 ± 9.4	96.7 ± 7.8	95.2 ± 6.6	97.2 ± 6.6
	500.0	91.4 ± 4.5	97.7 ± 8.1	99.5 ± 7.3	92.7 ± 4.0
	2000.0	98.6 ± 3.2	93.2 ± 4.4	97.3 ± 7.1	97.1 ± 6.2
Rd	100.0	92.3 ± 4.1	98.6 ± 7.3	96.5 ± 7.7	103.7 ± 7.5
	500.0	95.6 ± 5.1	105.3 ± 8.6	105.7 ± 6.3	97.2 ± 6.5
	2000.0	107.3 ± 6.1	103.5 ± 8.3	106.3 ± 8.6	97.2 ± 8.5

3.4. Pharmacokinetic study

After oral administration of PG and PR, the pharmacokinetics of the four ginsenosides in rat plasma was investigated using the validated UPLC-MS/MS method. The mean plasma concentration

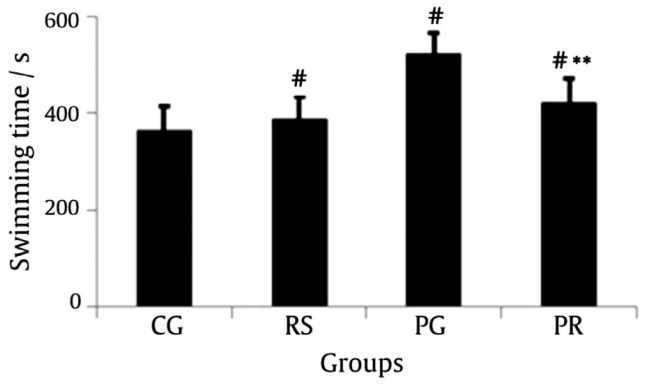

Fig. 3. Effect of *Raphani Semen* on forced swimming time in mice. $^{**}P < 0.01$ *vs* PG; $^{\#}P < 0.05$ *vs* CG.

curves of Rg1, Re, Rb1, and Rd versus time were shown in Fig. 5. The estimated pharmacokinetic parameters were shown in Table 5. Most pharmacokinetic parameters were significantly different between PG group and PR group ($P < 0.05$), suggesting the occurrence of herb-herb interaction.

In the PG group, the C_{max} of Rg1, Re, Rb1, and Rd was 45.0, 49.3, 1139.2, and 606.8 μg/L, respectively. In the PR group, C_{max} of Rg1, Re, Rb1, and Rd reached 38.9 ($P < 0.05$), 40.2, 283.3 ($P < 0.01$), and 83.3 ($P < 0.05$) μg/L, respectively. Thus, the presence of *Raphani Semen* in the *P. ginseng* extract was associated with the reduction in C_{max} by 15.7%, 22.6%, 302.1%, and 627.5% for Rg1, Re, Rb1, and Rd, respectively. Compared to the values reported for the PG group, the AUC values of Rg1 (190.9 μg·h/L), Re (767.9 μg·h/L), Rb1 (7992.9 μg·h/L, $P < 0.01$), and Rd (2277.1 μg·h/L, $P < 0.05$) decreased by 24.9%, 27.3%, 155.3%, and 518.5% in the PR group, respectively. The CL/F and $T_{1/2}$ of the four ginsenosides in the PR group were decreased and the MRT was increased significantly compared to the corresponding values in the PG group.

4. Discussion and conclusion

Both synergistic and antagonistic effects between TCM's components may occur as a result of herb-herb interactions. Researches on herb-herb interactions are conductive to clarifying the synergistic and antagonistic interactions between TCM herbs (Wang, 2012). Indeed, such assay will aid to modernize clinical applications of TCM.

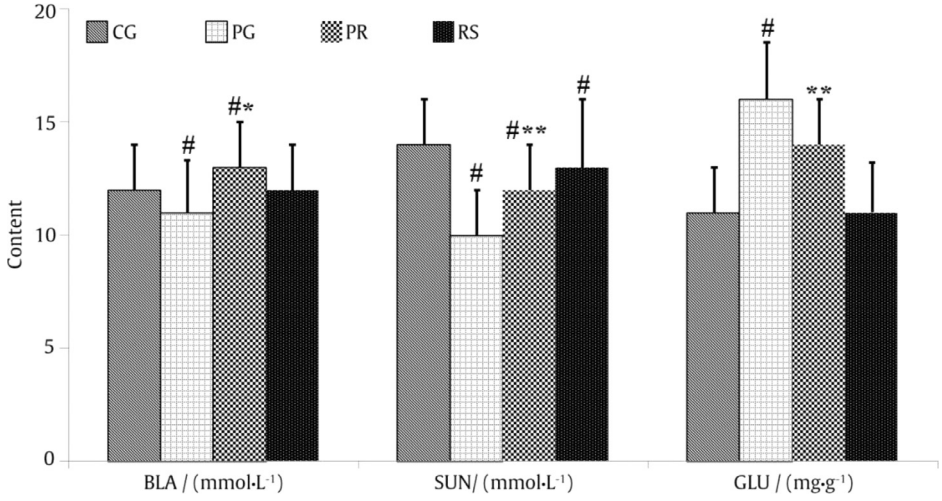

Fig. 4. Effect of *Raphani Semen* on blood lactate (BLA), serum urea nitrogen (SUN) and hepatic glycogen (GLU) in mice. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* PG; $^{\#}P < 0.05$ *vs* CG.

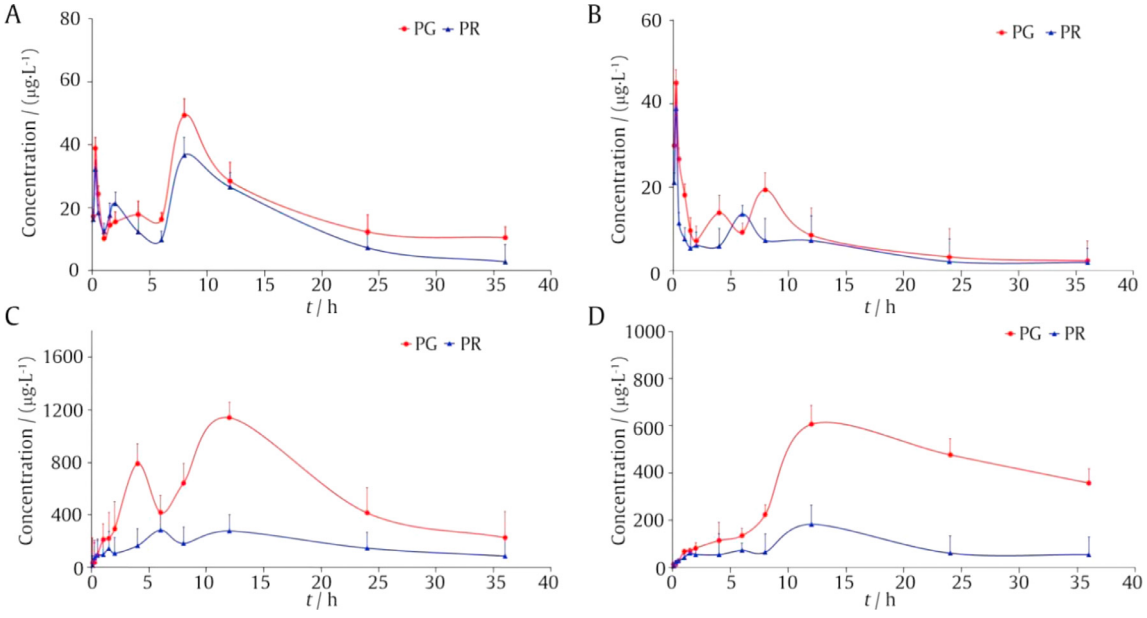

Fig. 5. Mean plasma concentration-time profiles of ginsenosides Rg1 (A), Re (B), Rb1 (C) and Rd (D) following oral administration of PG and PR to rats.

Table 5
Pharmacokinetic parameters of ginsenosides Rg1, Re, Rb1 and Rd in rat plasma following oral administration of PG and PR (mean ± SD, $n = 5$).

Ginsenosides	Groups	$C_{max}/(\mu g \cdot L^{-1})$	T_{max}/h	$t_{1/2}/h$	$CL/F/(L \cdot h \cdot kg^{-1})$	$AUC/(\mu g \cdot h \cdot L^{-1})$	MRT/h
Re	PG	49.3 ± 12.3	8.5 ± 1.2	25.7 ± 7.8	10.5 ± 4.5	977.7 ± 123.5	16.6 ± 4.5
	PR	40.2 ± 8.9	6.5 ± 1.3*	13.1 ± 2.6*	15.6 ± 5.6*	767.9 ± 145.3*	12.4 ± 5.6*
Rg1	PG	45.0 ± 12.3	0.25 ± 0.02	12.8 ± 4.6	9.8 ± 3.2	238.6 ± 78.6	18.5 ± 7.5
	PR	38.9 ± 7.6*	0.25 ± 0.03	10.1 ± 2.6	7.8 ± 1.8	190.9 ± 89.6*	13.4 ± 5.6
Rb1	PG	1139.2 ± 236.5	12.0 ± 2.3	16.1 ± 5.6	21.5 ± 7.8	20,410.2 ± 458.6	24.5 ± 7.6
	PR	283.3 ± 45.3**	6.0 ± 1.3	10.2 ± 4.5*	18.9 ± 8.9*	7992.3 ± 389.6**	19.9 ± 5.6*
Rd	PG	606.8 ± 98.6	12.0 ± 4.2	13.2 ± 4.6	19.6 ± 8.9	14,084.2 ± 453.6	19.9 ± 4.5
	PR	83.8 ± 22.3*	12.0 ± 3.6	9.5 ± 2.3	17.5 ± 9.6	2277.1 ± 387.9*	17.7 ± 6.3*

The results of this study showed that *Raphani Semen* had a significant effect on the anti-fatigue properties of *P. ginseng*, which indicated that combination administration of *P. ginseng* and *Raphani Semen* can enhance the accumulation of BLA and increase significantly level of SUN and lead to a higher production of SUN compared with administration of *P. ginseng* alone. A research has confirmed that a prolonged physical activity can consume stored hepatic glycogen (Jung, Han, Kwon, Lee, & Kim, 2007). Our study indicated that the combination administration of *P. ginseng* and *Raphani Semen* can decrease the level of GLU than the administration of *P. ginseng* alone. All results showed that *Raphani Semen* can reduce the anti-fatigue activity of *P. ginseng* when mixing with *Raphani Semen*.

Our results showed that there were significant differences in several pharmacokinetic parameters (AUC, MRT) among the four ginsenosides after oral administration of PG and PR. Compared with *P. ginseng, Raphani Semen* could decrease the bioavailability of the four ginsenosides in rat plasma. The mechanism leading to the differences in pharmacokinetic behaviors of the four ginsenosides between *P. ginseng* extracts and *P. ginseng* combined with *Raphani Semen* is not clear. A previous study investigated differences in the PK parameters of paeoniflorin between rats administered with pure paeoniflorin and *Moutan Cortex* extract or Shuang-Dan extract (Wu et al., 2009). Moreover, some ginsenosides are the substrates of p-glycoprotein (P-gp) which is a drug efflux pump (Yang, Wang, & Niu, 2012). Some studies have shown that one herb can increase or reduce the absorption of the other by inhibiting or activating intestinal P-gp. For example, *Hypericum perforatum* can reduce plasma concentration of digoxin, benzodiazepine, theophylline, and warfarin by activating the intestinal P-gp (Tan, 2009). It is possible that compounds in *Raphani Semen* activate the intestinal P-gp and reduce the bioavailability of the four ginsenosides.

In conclusion, co-administration of *P. ginseng* and *Raphani Semen* made a significant effect on anti-fatigue activities and pharmacokinetics of the four ginsenosides in rats. The result showed that the combined administration of *Raphani Semen* and *P. ginseng* could decrease the anti-fatigue activity of *P. ginseng* and reduce blood exposure of the four ginsenosides in rats. This study for the first time evaluates the effect of combined application of *Raphani Semen* and *P. ginseng* on anti-fatigue activity and pharmacokinetics of the four ginsenosides in rats, which provides a reference for investigating the herb-herb interaction of *Raphani Semen* and *P. ginseng*.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

This research was supported by Central Public-interest Scientific Institution Basal Research Fund (No. 1610342017017).

References

Ang-Lee, M. K., Moss, J., & Yuan, C. S. (2001). Herbal medicines and perioperative care. *Journal of the American Medical Association, 286*(2), 20–28.
Attele, A. S., Wu, J. A., & Yuan, C. S. (1999). Ginseng pharmacology: Multiple constituents and multiple actions. *Biochemical Pharmacology, 58*, 1685–1693.
Chen, L. C., Chou, M. H., Lin, M. F., & Yang, L. L. (2002). Pharmacokinetics of paeoniflorin after oral administration of Shao-yao Gan-chao Tang in mice. *Japanese Journal Pharmacology, 88*(3), 250–255.
Choi, P. J., Park, J. Y., Kim, T. J., Park, S. H., Kim, H. K., & Kang, K. S. (2015). Improved anticancer effect of ginseng extract by microwave-assisted processing through the generation of ginsenosides Rg3, Rg5 and Rk1. *Journal of Functional Foods, 14*, 613–622.
Dai, F. G., Luo, R., Wang, Y. G., & Xia, C. Y. (2005). Compatible effects of euphorbia and glycyrrhiza on CYP2E1 expression and activity in rat liver. *Acta Academiae Medicinae Mlitaris Tertiae, 27*, 742–744.
Liao, Q. F., Yang, W., Jia, Y., Chen, X. H., Gao, Q. T., & Bi, K. S. (2005). LC–MS determination and pharmacokinetic studies of ursolic acid in rat plasma after administration of the traditional Chinese medicine preparation Lu-Ying extract. *Yakugaku Zasshi, 125*(6), 509–515.
Jung, K. A., Han, D., Kwon, E. K., Lee, C. H., & Kim, Y. E. (2007). Anti-fatigue effect of *Rubbus coreanus* Miquel extract in mice. *Journal of Medicinal Food, 10*, 689–693.
Kim, K. H., Kim, C. S., Park, Y. J., Moon, E., Choi, S. U., Lee, J. H., et al. (2015). Anti-inflammatory and antitumor phenylpropanoid sucrosides from the seeds of *Raphanus sativus*. *American Journal of Chinese Medicine, 25*(1), 96–99.
Li, W., Gu, C., Zhang, H., Awang, D. V., Fitzloff, J. F., Fong, H. H., & Breemen Van, R. B. (2000). Use of high-performance liquid chromatography-tandem mass spectrometry to distinguish *Panax Ginseng* C. A. Meyer (Asian ginseng) and *Panax quinquefolius* L. (North American ginseng). *Journal of Radioanalytical and Nuclear Chemistry, 72*(3), 5417–5422.
Lars, P. C., Martin, J., & Ulla, K. (2006). Simultaneous determination of ginsenosides and polyacetylenes in American ginseng root (*Panax quinquefolium* L.) by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 54*(24), 8995–9003.
Mook-Jung, I., Hong, H. S., Boo, J. H., Lee, K. H., Yun, S. H., Cheong, M. Y., et al. (2001). Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. *Journal of Neuroscience Research, 63*(6), 509–515.
Sham, T. T., Yuen, A. C. Y., Ng, Y. F., Chan, C. O., Mok, D. K. W., & Chan, S. W. (2013). A review of the phytochemistry and pharmacological activities of *Raphani semen*. *Evidence-Based Complementary and Alternative Medicine, 2013*, 1–16.
Saito, H., Yoshida, Y., & Takagi, K. (1974). Effect of *Panax ginseng* root on exhaustive exercise in mice. *Japanese Journal of Pharmacology, 24*(1), 119–127.
Tan, W., Yu, K. Q., Liu, Y. Y., Ouyang, M. Z., Yan, M. H., Luo, R., et al. (2012). Antifatigue activity of polysaccharides extract from *Radix Rehmanniae Praeparata*. *International Journal of Biological Macromolecules, 50*(1), 59–62.
Tan, R. (2009). P-glycoprotein interaction with Chinese traditional medicine and its chemical composition. *Chinese Hospital Pharmcy Journal, 29*, 2028–2029.
Wang, C. H., Wang, R., Cheng, X. M., He, Y. Q., Wang, Z. T., & Wu, C. (2008). Comparative pharmacokinetic study of paeoniflorin after oral administration of decoction of *Radix Paeoniae Rubra* and *Radix Paeoniae Alba* in rats. *Journal of Ethnopharmacology, 117*(3), 467–472.
Wang, J. H. (2012). Traditional Chinese medicine and the positive correlation with homeostatic evolution of human being: Based on medical perspective. *Chinese Journal of Integrative Medicine, 18*(8), 629–634.
Wu, H., Zhu, Z. Y., Zhang, G. Q., Zhao, L., Zhang, H., Zhu, D. L., et al. (2009). Comparative pharmacokinetic study of paeoniflorin after oral administration of pure paeoniflorin, extract of *Cortex Moutan* and Shuang-Dan prescription to rats. *Journal of Ethnopharmacology, 125*(3), 444–449.
Yamaguchi, Y., Higashi, M., & Kobayashi, H. (1996). Effects of ginsenosides on impaired performance caused by scopolamine in rats. *European Journal of Pharmacology, 312*(2), 149–151.
Yang, Z., Wang, J. R., & Niu, T. (2012). Inhibition of P-glycoprotein leads to improved oral bioavailability of compound K, an anti-cancer metabolite of red ginseng extract produced by gut microflora. *Drug Metabolism and Disposition, 40*(6), 1538–1544.
Zhang, X., Wang, L. N., & Song, F. R. (2007). Study on the variation of chemical constituents during combination of ginseng with *Trogopteroum Feces* and *Semen Raphani* by high performance liquid chromatography-mass spectrometry. *Chinese Journal of Analytical Chemistry, 35*, 559–563.