Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Alterations in the Pharmacokinetics of Paeoniflorin and Albiflorin in a Collagen-Induced Arthritis Rat Model

Ya-xin Han[1,2], Mu-xin Gong[1], Jin-e Peng[3], Yong-song Xu[4], Feng Qiu[1], Kai-li Xie[1], Xue-rong Dong[1], Bin-bin Wang[1], Xiao-xin Chai[1], Zhe Ma[1], Si-hui Wang[1], Nan Nan[1]

[1]Department of Pharmaceutics of TCM, Beijing Key Lab of TCM Collateral Disease Theory Research, School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, [2]Division of Chemical Metrology and Analytical Science, National Institute of Metrology, Beijing 100029, [3]Department of Pharmacy, Beijing Ditan Hospital Capital Medical University, Beijing 100015, China, [4]Beijing Key Laboratory of Diabetes Research and Care, Center for Endocrine Metabolism and Immune Diseases, Luhe Hospital Capital Medical University, Beijing 101149, PR China

Submitted: 22-Nov-2021 Revised: 25-Mar-2022 Accepted: 27-Apr-2022 Published: 19-Sep-2022

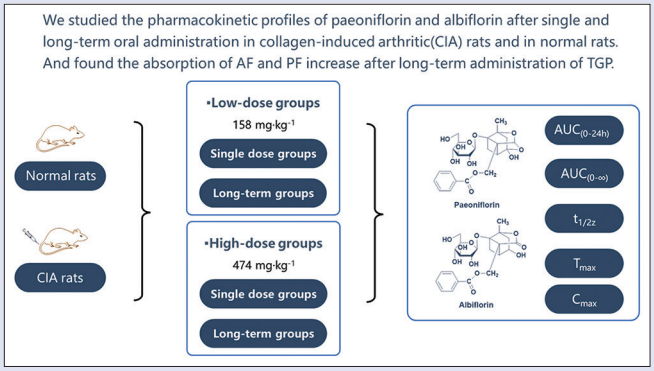
ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune disease that seriously affects the patient's quality of life. Total glucosides of peony (TGP) have long been used to treat RA in China despite the need for long-term administration. The main active ingredients in TGP are paeoniflorin (PF) and albiflorin (AF). This study aimed to clarify the effective mechanism of TGP only after long-term administration, and the pharmacokinetics of PF and AF following single and chronic oral TGP administration in collagen-induced arthritic (CIA) and normal rats. **Materials and Methods:** Comparative pharmacokinetic studies of TGP were conducted in male Sprague Dawley rats. Plasma concentrations of PF and AF were determined with ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). DAS software was used to estimate pharmacokinetic parameters. **Results:** Bimodal phenomenon was observed. After a single TGP dose, the absorption of AF and PF were both lower in CIA rats compared to control rats. Compared with single-dose groups, the absorption of AF and PF in CIA rats increased after long-term administration of TGP. No significant differences were seen between the groups after chronic administration in low-dose groups in normal rats. **Conclusion:** We found that after long-term administration of TGP, the absorption of PF and AF are promoted in CIA rats. These results, combined with the existing literature, may help substantiate the TGP-related changes in gut microbiota.

Key words: Albiflorin, paeoniflorin, pharmacokinetics, rheumatoid arthritis, total glucosides of peony

SUMMARY

- In this work, we evaluated the pharmacokinetic characteristics of PF and AF after single and long-term oral administration of TGP in CIA rats and normal rats first. We found that the absorption of AF and PF was lower in CIA rats after a single dose of TGP administration compared to control rats, whereas there were no significant differences between the two groups after chronic administration. Oral administration of TGP has been shown to reverse the changed intestinal flora of CIA rats to that of normal rats. We propose the hypothesis that intestinal flora may affect TGP efficacy by affecting AF and PF metabolism.



Abbreviations used: AF: Albiflorin; AI: Arthritis index; BP: Benzoyl-paeoniflorin; CIA: Collagen-induced arthritis; GP: Galloyl-paeoniflorin; IS: Internal standard; LHC: Long-term high-dose CIA; LHN: Long-term high-dose normal; LLC: Long-term low-dose CIA; LLN: Long-term low-dose normal; LLOQ: Lower limit of quantification; OP: Oxypaeoniflorin; PF: Paeoniflorin; RA: Rheumatoid arthritis; RSD: Relative standard deviation; SHC: Single high-dose CIA; SHN: Single high-dose normal; SLC: Single low-dose CIA; SLN: Single low-dose normal; TGP: Total glucosides of peony.

Correspondence:

Prof. Mu-xin Gong,
Department of Pharmaceutics of TCM, Beijing Key Lab of TCM Collateral Disease Theory Research, School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, PR China.
E-mail: gongmuxin@ccmu.edu.cn
DOI: 10.4103/pm.pm_539_21

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Total glucosides of peony (TGP), the effective fraction of the traditional Chinese medicine Radix *Paeoniae* Alba, is reported to exhibit analgesic, anti-inflammatory, and hepatoprotective properties.[1,2] Commercially available as Bai Shao Zong Gan Jiao Nang (Ningbo Lihua Pharmaceutical Co., Ltd), TGP capsules have been approved by the National Medical Products Administration (https://www.nmpa.gov.cn/) since 1998 for the treatment of autoimmune diseases, including rheumatoid arthritis (RA), which is a common ailment in China.

Paeoniflorin (PF),[1] and albiflorin (AF),[3] are monoterpene glycosides and the two most abundant compounds in TGP, comprising 52.5% and 13.5%, respectively. These components have the most potent therapeutic effects both *in vitro* and *in vivo*: both are xanthine oxidase inhibitors that can potentially prevent hyperuricemia and gout.[4] PF has shown

Cite this article as: Han Y, Gong M, Peng J, Xu Y, Qiu F, Xie K, *et al*. Alterations in the pharmacokinetics of paeoniflorin and albiflorin in a collagen-induced arthritis rat model. Phcog Mag 2022;18:571-8.

anti-inflammatory and immune regulatory properties,[5] and is an active compound of radix *paeoniae* rubra with significant antithrombotic properties.[6] On the other hand, AF is known to possess a significant antidepressant-like effect.[7] Additionally, AF and PF can be degraded easily by intestinal flora.[8,9]

RA is characterized by chronic synovial joint inflammation,[10] and TGP is widely used in the treatment of RA because of its relatively fewer side effects. However, the drug must be taken over a long period to achieve a clinically significant effect. A previous study had reported that the pharmacokinetics of TGP are affected by the patient's pathologic state.[11] However, most pharmacokinetic studies regarding TGP have single-dose administration in normal animals rather than long-term administration in model animals. Additionally, dysbiosis of the gut microbiota may increase the risk of RA,[12] since the components of many bacteria can stimulate the secretion of rheumatoid factor antibodies and the intestinal flora of RA patients is known to change.[13] A previous study reported that dysbiosis of the gut flora in collagen-induced arthritic (CIA) rats were partly resolved after four weeks of TGP administration.[14] To further elucidate this, we carried out a four-week experiment to determine the pharmacokinetic profiles of PF and AF after long-term oral administration of TGP in a CIA rat model and normal rats. Additionally, the pharmacokinetic parameters of AF and PF after single-dose administration were studied. We also studied the metabolism of AF and PF in feces by intestinal flora under different TGP administration conditions to clarify its clinical role.

MATERIALS AND METHODS

Reagents and chemicals

TGP (lot no: 181008) was purchased from Liwah Pharmaceutical (Ningbo, China). Standard PF (PS161215-02), AF (PS010200), and the Internal Standard (IS) geniposide (PS0064-0025MG) were obtained from Chengdu PUSH Bio-Technology Co. (Chengdu, China). The structures of PF, AF, and IS are shown in Figure 1. Bovine type II collagen (lot no.: 190078), Complete Freund's adjuvant (batch no.: 190021), and Incomplete Freund's adjuvant (batch no.: 190001) were obtained from Chondrex (Redmond, WA, USA). Chromatographic-grade methanol, acetonitrile, and formic acid (A117-50) were acquired from Thermo Fisher Scientific (Waltham, MA, USA). Double-distilled water as well as all other solvents, reagents, and chemicals were analytical-grade and commercially available.

Instrument and chromatography conditions

The ultra-high performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UPLC-ESI-MS) system consisted of a UPLC system (Agilent Technologies, Palo Alto, CA, USA) comprising a model-1290 G4220A pump, model-1290 Infinity Autosampler, and a linear ion-trap quadrupole liquid chromatography-tandem mass spectrometry equipped with an ESI source (QTRAP 6500; AB Sciex, Framingham, MA, USA). The Analyst v1.6.2 software (AB Sciex) was used to control the system, and acquire and analyze data. Separation was carried out on a Poroshell 120 EC-C_{18} column (2.1 × 100 mm, 2.7 µm, Agilent Technologies). The mobile phase contained 0.1% (v/v) formic acid, both in water (A) and methanol (B). The solvent gradients were 0.00–1.00 min with 10% B, 1.00–2.00 min with 10%–43% B, 2.00–3.00 min with 43%–50% B, 3.00–6.00 min with 50%–100% B, 6.00–7.00 min with 100% B, and 7.01–8.00 min with 10% B. The flow rate was set at 0.3 mL/min. The injection volume was 1 µL.

The mass spectrometer was operated in negative mode. Quantification was carried out by multiple reaction monitoring of the precursor ion to produce the ion ratio. The parameters were set as follows: *m/z* 525→449 for PF, *m/z* 525→479 for AF, and *m/z* 433→225 for IS. The optimized parameters used were: ion spray voltage at −3000 V; temperature at 120°C; ion source gas-1 (N_2) at 55 psi; ion source gas-2 (N_2) at 55 psi, curtain gas (N_2) at 40 psi; collision cell entrance potential at −10 V; and collision cell exit potential at −13 V. The collision energies of PF, AF, and IS were −35, −17, and −19 eV, respectively, and the declustering potentials were −140, −60, and −36 V, respectively. The PF, AF, and IS fragments are shown in Figures 2–4.

Animals

Animal grouping

Healthy male Sprague Dawley rats (mean weight: 260 ± 20 g) were purchased from the Animal Center of Capital Medical University, Beijing, China and were housed in a room with controlled temperature (23 ± 2°C), humidity (57 ± 3%), and illumination (12:12 hr light/dark cycle). Before carrying out the experiments, the rats were fed standard chow for one week and allowed to adapt to the laboratory conditions. Animal welfare was respected and the experiments were performed under strict supervision. The animal experimental protocol was approved by the Animal Ethics Committee of Capital Medical University (Approval no.: AEEI-2014-128).

Sixty rats were randomly divided into the following 10 groups (n = 6 each): normal control, CIA control, single low-dose normal (SLN), single high-dose normal (SHN), single low-dose CIA (SLC), single high-dose CIA (SHC), long-term low-dose normal (LLN), long-term high-dose normal (LHN), long-term low-dose CIA (LLC), and long-term high-dose CIA (LHC).

Establishing the CIA model

Collagen-related arthritis was induced in rats, and the arthritis index (AI) was calculated as described in a previous study.[14] The degree of arthritis

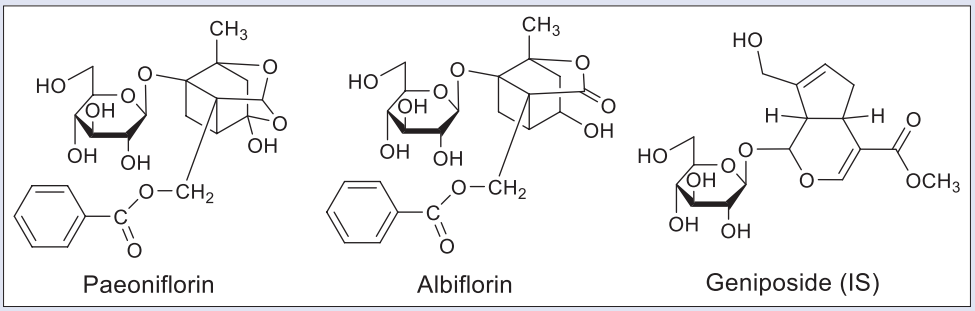


Figure 1: Chemical structures of PF, AF, and IS

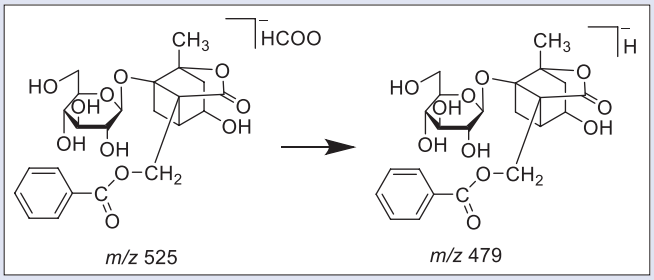


Figure 2: The fragmentation process of AF

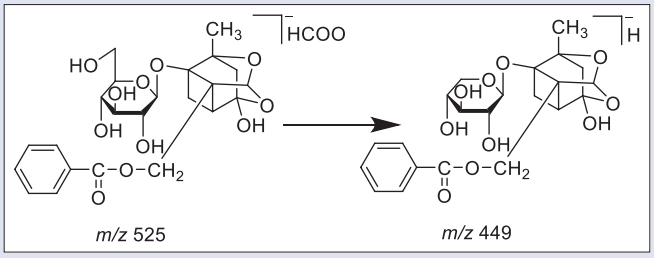


Figure 3: The fragmentation process of PF

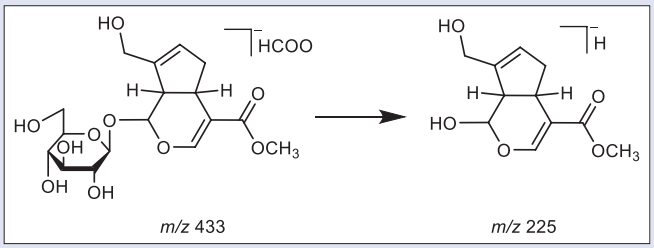


Figure 4: The fragmentation process of IS

was scored and the total AI was calculated as the sum of scores for all four limbs.

Pharmacokinetic study

Drug administration and blood sampling

The animals were kept fasting overnight (for 12 hr) before the experiments, but had free access to water. The low dose (clinical equivalent: an equivalent dose between human and animal) of TGP was considered 158 mg/kg, and the high dose (three times the clinical equivalent dose) was set at 474 mg/kg in a volume of 5 mL/kg.

For the single-dose administration group, 100 μL of blood was taken from the caudal vein at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hr after oral administration of TGP, It was collected in pre-heparinized tubes and then centrifuged for 15 min (5,000 rpm, 4°C). The supernatant was collected and immediately frozen at − 80°C. The rats also received 100 μL saline at each time point. For the chronic administration group, TGP was given three times a day with an interval of 8 hr consecutively for 28 days. Before TGP administration at 08:00 a.m. on days 26, 27, and 28, and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after administration of TGP on day 28, 100 μL of blood was collected in pre-heparinized tubes and processed as in the single-dose group. Thereafter, the rats were euthanized with ethyl carbamate (1.0 g/kg, *i.p.*).

Preparation of stock solutions, calibration solutions, and quality control samples

The IS stock solution was prepared at 0.25 mg/L in methanol/acetonitrile (1:1, v/v), whereas the PF and AF stock solutions were prepared in methanol. A series of working solutions were prepared by diluting each stock solution to final concentrations of 0.050, 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 8.00, 10.0, 20.0, 30.0, 40.0, and 50.0 mg/L. Quality control samples were prepared by spiking different concentrations of PF and AF into blank plasma.

Sample preparation

A 50-μL volume of rat plasma sample was transferred to an empty 1.5 mL Eppendorf tube, and 100 μL of methanol/acetonitrile (1:1, v/v) containing 125 μg/L IS and 5 μL methanol were added to it. After subjecting to vortex for 1 min, the sample was centrifuged for 10 min (14,000 rpm, 4°C), and the supernatant was stored at 4°C until analysis.

Method validation

Specificity

Specificity was assessed by comparing the peaks corresponding to blank plasma. The blank plasma was spiked with PF and AF of the lower limit of quantification (LLOQ) and IS, and the *in vivo* plasma sample was collected 0.5 hr after oral administration of TGP to eliminate the effects of interference from endogenous compounds.

Linearity

The calibration samples were prepared by combining 5 μL of the working solution, 50 μL of the rat plasma sample, and 100 μL of the methanol/acetonitrile (1:1, v/v) containing 125 μg/L of IS, followed by pre-treatment. The resultant plasma contained 5.0–5000 μg/L of PF and AF.

The linearity of each calibration curve was determined by plotting the peak area ratio (y) of the analyzed sample to the IS against the theoretical concentration (x) of the analyzed sample, with 1/x as the weighting factor.

LLOQ was defined as the lowest concentration on the standard curve that could be quantified with an accuracy of ±15% and a relative standard deviation (RSD) less than ±15% based on the analysis of five replicate samples.

Precision and accuracy

Accuracy was determined by analyzing the replicate samples containing known concentrations of the target compound; the actual values were within ±15% of the nominal value. The precision of intra- and inter-day measurements was evaluated using three concentrations of five replicate samples with an RSD of ±15%. The accuracy and precision of the LLOQ were <20%.

Recovery and matrix effect

Recovery was evaluated at three concentrations (5, 100, and 5,000 μg/L) by comparing the concentrations of the target compound in spiked samples with those in blank plasma and post-extraction samples. The matrix effect was determined by comparing the peak responses of samples when the extracted matrix was spiked with standard and near-standard solutions.

Stability

Short-term stability, long-term stability, and stability after freeze–thawing were assessed using five replicate samples stored for 24 hr at room temperature, stored at −80°C for three months, and after

three freeze–thaw cycles, respectively. The samples were considered stable when the accuracy bias was within ±15% of the theoretical concentration.

Data processing and analysis

The pharmacokinetic parameters of PF and AF were analyzed for each rat using DAS v2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The differences in parameter values between the two groups were assessed by three-way analysis of variance (ANOVA) using SPSS v. 21.0 (SPSS Inc, Chicago, IL, USA). The differences were considered significant if $P < 0.05$.

RESULTS

Validation of the methodology

Typical chromatograms of a blank rat plasma (A), plasma spiked with IS, LLOQ of PF, and AF (B), and plasma samples obtained 0.5 hr after oral administration of TGP (C) are shown in Figure 5. No endogenous plasma components showed peaks interfering with those corresponding to AF (2.8 min), PF (3.3 min), and IS (3.0 min).

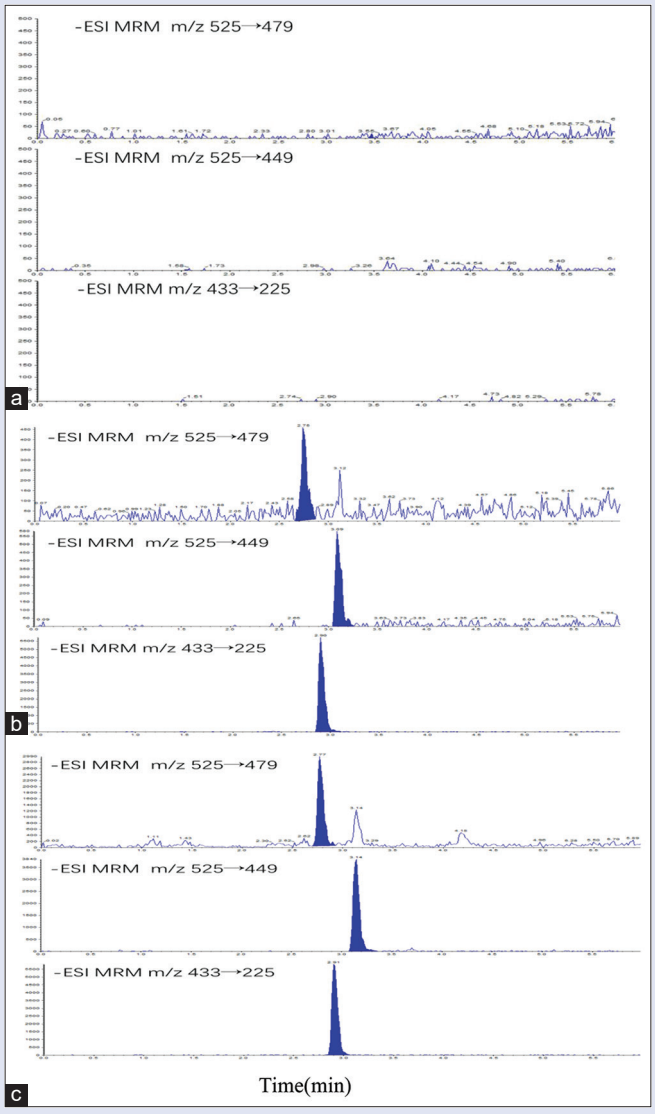


Figure 5: Representative chromatograms of AF (*m/z* 525→479), PF (*m/z* 525→449), and IS (m/z 433→225) in rat plasma. (a) blank plasma; (b) blank plasma spiked with internal standard (IS), and LLOQ of PF and AF; (c) plasma sample collected at 0.5 hr after oral administration

Also, no significant matrix effects were observed for AF, PF, or IS.

The regression equations and correlation coefficients of AF and PF were obtained as y = 0.00130x + 0.0651 (r = 0.9994) and y = 0.00291x + 0.2380 (r = 0.9995), respectively. The calibration curves were linear in the range of 5–5000 μg/L for both AF and PF; the LLOQ for both AF and PF was 5 μg/L. The percentage recovery of the extracted compounds lay between 85.80% and 118%.

AF and PF were stable under all conditions including short- and long-term storage and freeze–thawing. The relative error (%) and RSD (%) values were all below 15%.

The recovery and matrix effects of AF and PF are summarized. That the precision and accuracy of AF and PF were within acceptable limits. Depict the stability of AF and PF.

Pharmacokinetic analysis

There were no significant differences in body weight or AI scores between CIA groups. The pharmacokinetic parameters for the single-dose and chronic treatment groups are shown in Tables 1 and 2, respectively, and the mean plasma concentrations of PF and AF over time are shown in Figures 6 and 7, respectively.

Pharmacokinetics of AF and PF after single-dose TGP administration

At the same dose, the SLC group showed lower areas under the receiver operating characteristic curve (AUC) ($AUC_{[0-24h]}$ and $AUC_{[0-\infty]}$), shorter half-life period ($t_{1/2z}$) and peak time (T_{max}), and lower maximum concentration (C_{max}) than the SLN control group. The differences in $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$ between the two groups were statistically significant ($P < 0.01$). Meanwhile, the SHC group had lower $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, shorter $t_{1/2z}$, lower C_{max}, and longer T_{max} than the SHN control group. However, the differences were not statistically significant ($P > 0.05$).

Likewise, regarding the pharmacokinetic parameters of PF after single-dose administration of TGP, at the same dose, the SLC group had lower $AUC_{(0-24h)}$, $AUC_{(0-\infty)}$, and C_{max}, but a longer $t_{1/2z}$ than the SLN group. Also, the differences between the groups with respect to the $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$ were statistically significant ($P < 0.01$). Further, all pharmacokinetic parameters for the SHC group were lower than the SHN rats, but none showed a significant difference ($P > 0.05$).

Pharmacokinetics of AF and PF after chronic TGP administration

After chronic administration of TGP at the same dose, the LLC group had a lower $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, shorter T_{max}, lower C_{max}, and higher $t_{1/2z}$ than the LLN group. However, there were no significant differences ($P > 0.05$) for any of the parameters related to AF. On the other hand, compared to the LHN group, LHC rats had a higher $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, longer $t_{1/2z}$, higher C_{max}, and shorter T_{max}, with no differences between the groups regarding any of the parameters ($P > 0.05$).

For the pharmacokinetics parameters of PF, at the same dose of TGP, the LLC group had a lower $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, shorter $t_{1/2z}$, lower C_{max}, and longer T_{max} than the LLN group, but none of the differences were significant ($P > 0.05$). The LHC group had a higher $AUC_{(0-24h)}$ and C_{max}, lower $AUC_{(0-\infty)}$, and shorter $t_{1/2z}$ and T_{max} compared to the LHN group, with no significant differences ($P > 0.05$).

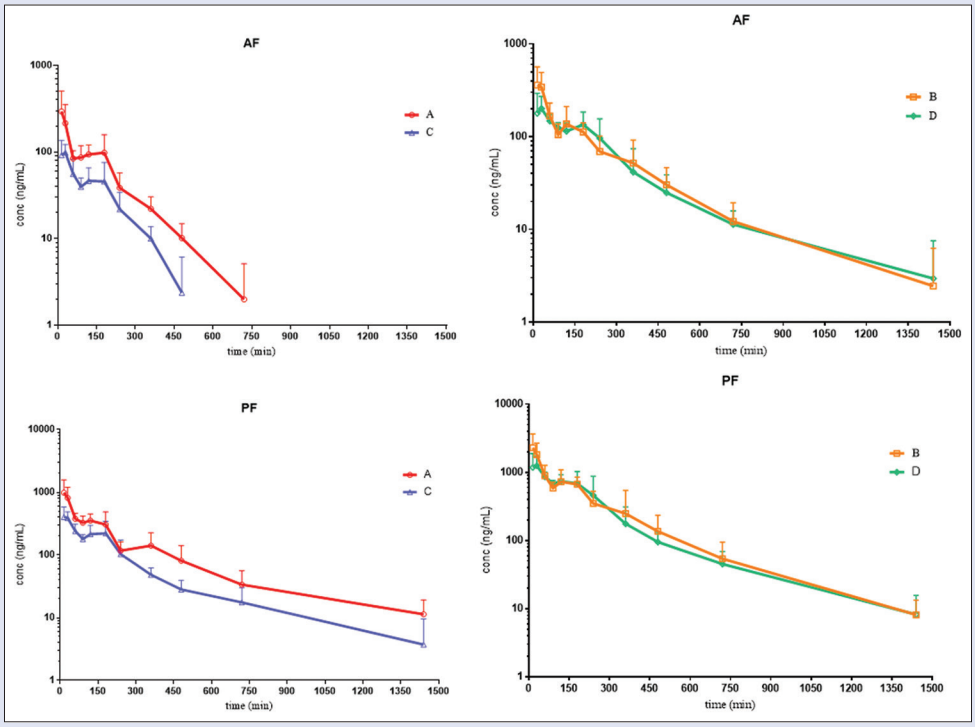


Figure 6: Concentration–time curve of AF and PF after single-dose administration of TGP in the CIA model and control rats (mean ± standard deviation, $n = 6$). A, normal rats administered a low dose of TGP; B, normal rats administered a high dose; C, CIA rats administered a low dose; D, CIA rats administered a high dose

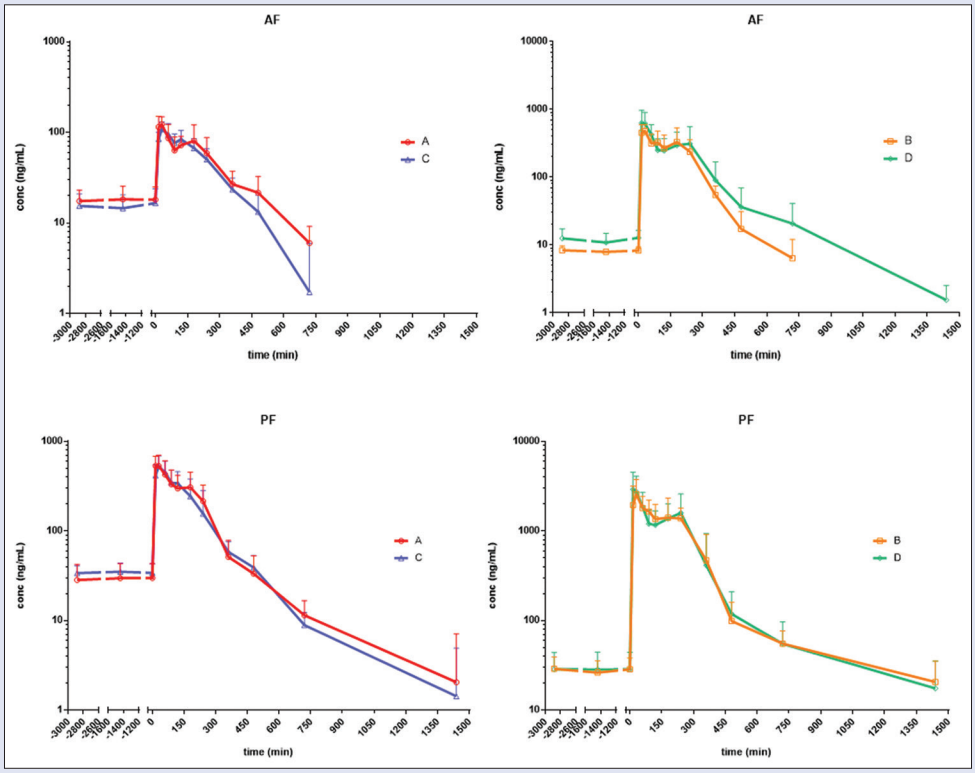


Figure 7: Concentration–time curve of AF and PF after long-term administration of TGP in the CIA model and control rats (mean ± standard deviation, $n = 6$). A, normal rats administered a low dose of TGP; B, normal rats administered a high dose of TGP; C, CIA rats administered a low dose of TGP; D, CIA rats administered a high dose of TGP

Table 1: Pharmacokinetic parameters of AF in CIA and control rats following administration of TGP ($\bar{X} \pm S$, $n = 6$)

Group	Parameters		Low-dose normal rats	High-dose normal rats	Low-dose CIA rats	High-dose CIA rats
Single-dose administration	$AUC_{(0-24h)}$	(hr*pg/mL)	529.78±170.07	933.89±226.95$^{\Delta\Delta}$	237.34±71.54**	845.04±291.41$^{\Delta\Delta}$
	$AUC_{(0-\infty)}$	(hr*pg/mL)	564.41±176.20	1007.08±220.01$^{\Delta\Delta}$	265.92±59.30**	895.22±265.90$^{\Delta\Delta}$
	$t_{1/2z}$	h	3.28±3.19	4.39±2.14	2.19±0.95	3.84±1.97
	T_{max}	h	0.63±0.52	0.67±0.67	0.42±0.13	0.88±1.05
	C_{max}	μg/L	304.00±213.81	397.33±186.23	110.22±32.94	212.83±96.48$^{\Delta}$
Long-term administration	$AUC_{(0-24h)}$	(hr*pg/mL)	501.96±150.74	1664.18±598.10$^{\Delta\Delta\blacktriangle}$	421.20±76.10$^{\blacktriangle\blacktriangle}$	2068.75±1175.33$^{\Delta\blacktriangle}$
	$AUC_{(0-\infty)}$	(hr*pg/mL)	528.71±152.57	1692.10±584.95$^{\Delta\Delta\blacktriangle}$	495.07±47.83$^{\blacktriangle\blacktriangle}$	2138.62±1175.86$^{\Delta\blacktriangle}$
	$t_{1/2z}$	h	2.50±0.50	1.45±0.78$^{\Delta\blacktriangle}$	3.13±1.38	3.22±2.06
	T_{max}	h	0.83±1.07	1.21±1.03	0.79±0.64	0.42±0.13
	C_{max}	μg/L	137.50±25.88	560.33±93.70$^{\Delta\Delta}$	120.25±20.73	678.50±338.01$^{\Delta\Delta\blacktriangle}$

AF, albiflorin; AUC, area under the receiver operating characteristic curve; CIA, collagen-induced arthritis; C_{max}, maximum concentration; $t_{1/2}$, half-life; TGP, total glucosides of white peony; T_{max}, maximum time. *$P < 0.05$, **$P < 0.01$ vs control group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ vs low-dose group; $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ vs single-dose group

Table 2: Pharmacokinetic parameters of PF in CIA and control rats following administration of TGP ($\bar{X} \pm S$, $n = 6$)

Group	Parameters		Low-dose normal rats	High-dose normal rats	Low-dose CIA rats	High-dose CIA rats
Single-dose administration	$AUC_{(0-24h)}$	(hr*pg/mL)	2499.83±516.80	5118.94±1767.04$^{\Delta}$	1288.24±193.04**	4413.52±1635.88$^{\Delta\Delta}$
	$AUC_{(0-\infty)}$	(hr*pg/mL)	2597.19±555.80	5377.16±2004.55$^{\Delta}$	1458.80±321.07**	4633.86±1634.75$^{\Delta\Delta}$
	$t_{1/2z}$	h	4.68±2.06	5.30±5.18	7.20±5.80	4.44±1.98
	T_{max}	h	0.42±0.13	0.63±0.69	0.42±0.13	0.46±0.10
	C_{max}	μg/L	1048.00±606.61	2411.67±1352.62	460.33±138.65	1321.33±610.80$^{\Delta}$
Long-term administration	$AUC_{(0-24h)}$	(hr*pg/mL)	1828.55±583.82	9500.39±2782.20$^{\Delta\Delta\blacktriangle\blacktriangle}$	1716.62±578.69	9630.09±5364.40$^{\Delta}$
	$AUC_{(0-\infty)}$	(hr*pg/mL)	1879.32±614.77	9820.08±2849.11$^{\Delta\Delta\blacktriangle}$	1748.74±596.26	9723.70±5390.35$^{\Delta}$
	$t_{1/2z}$	h	3.26±3.69	8.19±6.44	2.76±1.48	3.70±2.04
	T_{max}	h	0.46±0.29	1.25±0.99	0.67±0.26	0.33±0.13$^{\Delta}$
	C_{max}	μg/L	577.00±146.20	2737.92±1120.85$^{\Delta\Delta}$	570.33±169.36	3051.67±1602.68$^{\Delta\blacktriangle}$

AUC, area under the receiver operating characteristic curve; CIA, collagen-induced arthritis; C_{max}, maximum concentration; PF, paeoniflorin; $t_{1/2}$, half-life; TGP, total glucosides of white peony; T_{max}, maximum time. *$P < 0.05$, **$P < 0.01$ vs control group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ vs low-dose group; $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$ vs single-dose group

Comparison of pharmacokinetic parameters after single-dose versus chronic administration

Chronic administration of TGP resulted in a lower $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, shorter $t_{1/2z}$, lower C_{max}, and longer T_{max} for AF in the LLN group compared to the SLN group, but the differences were not statistically significant ($P > 0.05$). The LHN group had a higher $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, longer T_{max}, higher C_{max}, and shorter $t_{1/2z}$ than the SHN group; significant differences ($P < 0.05$) between the two groups were observed for $AUC_{(0-24h)}$, $AUC_{(0-\infty)}$, and $t_{1/2z}$. In CIA rats receiving low doses of TGP, the pharmacokinetic parameters had higher values after chronic administration versus single-dose administration (LLC vs SLC), with $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$ values differing significantly between the two groups. A higher $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, higher C_{max}, and shorter $t_{1/2z}$ and T_{max} were observed for the LHC group compared to the SHC group, with significant differences ($P < 0.05$) between the groups for $AUC_{(0-24h)}$, $AUC_{(0-\infty)}$, and C_{max}.

For PF, the LLN group had lower $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, shorter $t_{1/2z}$, lower C_{max}, and longer T_{max} than the SLN group, but the differences were not significant in each case ($P > 0.05$). In normal control rats treated with high doses of TGP, all parameters had higher values after chronic administration as compared to single-dose administration (LHN vs SHN), with significant differences ($P < 0.05$) for $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$. For low-dose CIA rats, chronic administration resulted in higher $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, longer T_{max}, higher C_{max}, and shorter $t_{1/2z}$ compared to single-dose administration (LLC vs SLC), with no significant differences ($P > 0.05$). A higher $AUC_{(0-24h)}$ and $AUC_{(0-\infty)}$, higher C_{max}, and shorter $t_{1/2z}$ and T_{max} were recorded in the LHC group compared to the SHC group, with non-significant differences ($P > 0.05$) except for C_{max} ($P < 0.05$).

Mean plasma concentration-time profiles

In both normal and CIA rats, double absorption peaks were observed for AF and PF after both single-dose and chronic administration. After a single dose of TGP, AF showed a second peak in the SLN, SLC, and SHC rats after about 3 hr, whereas the SHN group reached the second peak at 2 hr. Likewise, the second peak in PF concentration was observed at 2 hr in the SLN, SHN, and SHC groups and 3 hr in the SLC group. As can be seen in Figure 6, in all groups, the first peak in concentration was higher than the second.

As can be seen in Figure 7, after long-term TGP administration, AF showed a second peak in concentration in the LLN, LHN, and LHC groups at 3 hr and in the LLC group at 2 hr. The second peak in PF concentration in LLN and LHN rats occurred after about 3 hr, and there was no bimodal phenomenon in the LLC group. The LHC group showed a second peak in PF concentration at 4 hr.

DISCUSSION

Establishment of the LC-MS method

AF and PF are isomers with relative molecular masses of 480 with fragments of *m/z* 525 → 479 (parent ion $[M + HCOO]^-$ and product-ion $[M-H]^-$) obtained during scanning. However, under the experimental conditions, the retention time was more than 0.5 min apart, i.e., the ion pairs of AF and PF did not interfere with each other.

We evaluated geniposide,[15] and puerarin,[16] as potential IS and observed that both were pure chemically stable substances that were not present in the test sample. However, as the puerarin peak overlapped with that of the test sample under our experimental conditions, geniposide was selected as the IS. Accordingly, the specificity results indicated that the chromatographic peak of IS showed no interference from impurities.

In this study, both positive and negative ion modes were investigated to establish the LC-MS method. We observed that irrespective of the working parameters' values of the mass spectrometer in the positive ion scan mode, the response of AF, PF, oxypaeoniflorin (OP), and other reference materials were inferior to that of the negative ion mode; thus the negative ion scan mode was selected for further analysis. OP, benzoyl-paeoniflorin (BP), and galloyl-paeoniflorin (GP) are also included in TGP.[17,18] However, these components were involved in the present study. In quality control samples containing 2.00 μg/L of OP and BP, significant peaks were observed (S/N > 10). We, therefore, concluded that these compounds were not detected due to their poor absorption. As GP was scarcely detectable by triple quadrupole MS with a minimum LOQ as high as 800 μg/L in our study, we did not perform quantification of GP in plasma.

Bimodal phenomenon

A previous study also reported a bimodal phenomenon after a single-dose oral gavage of the Radix *Paeoniae* Alba extract in rats.[19] Factors like enterohepatic and general circulation, rate of gastric emptying, absorption, and formulation of compounds may all contribute to this bimodal pattern.[20] Enterohepatic circulation causes drugs to be reabsorbed in the intestine after being discharged into the intestinal tract through bile and returned to the liver via the portal vein, yielding an initial peak in concentration followed by a second lower peak. In one study, PF was detected in bile after both intravenous and oral administration of TGP, supporting the occurrence of enterohepatic cycling.[8] Likewise, irregular or delayed gastric emptying may also result in a bimodal distribution of concentration peaks.[21] TGP has good solubility in water, and the absorption of AF and PF may be affected by the gastric emptying rate. PF can be absorbed along with the whole intestinal segments.[22] Incubating the jejunum and ileum with different concentrations of PF showed that the absorption followed first-order kinetics.[23] Furthermore, at a high concentration (120 mg/L), absorption was higher in the ileum than in the jejunum. However, the shape of the peak was not consistent with a pharmacokinetic curve, suggesting that the bimodal phenomenon was caused not by irregular gastric emptying, but by another factor.

AF, PF, OP, BP, and GP, all share a similar pinane structure. PF can be converted into paeonimetaboline-I.[24] AF was detected following *in vitro* incubation of PF with anaerobic intestinal flora from rats.[25] Accordingly, the presence of compounds with similar parent nucleus interconversion may explain the bimodal phenomenon.

We observed that there were individual differences in pharmacokinetic parameters after TGP administration. Although there was no bimodal pattern in the average blood concentration–time curve for PF in CIA rats treated chronically with a low dose of TGP, it was observed in some animals in the group. Analyzing the bimodal phenomenon provides reference values for the safety and rational clinical use of drugs.

Pharmacokinetics of TGP in normal and CIA rats after single-dose administration

After a single dose of TGP, the blood concentrations of AF and PF showed the same trend, i.e., the two compounds were absorbed and quickly eliminated in all rats. Furthermore, the degree of absorption of AF and PF at a low (clinically equivalent) dose of TGP was reduced in CIA rats compared to normal rats, while absorption at a high dose was initially low, which increased slowly. Absorption was greater at the high dose than the low dose in both normal and CIA rats.

PF is a P-glycoprotein (P-gp) substrate,[26] and its absorption through passive diffusion follows first-order kinetics.[27] The poor absorption and low bioavailability of PF may be due to limited transport, degradation, or metabolism in the intestinal lumen,[28] although a previous study showed that enzymes in the intestinal tract could not hydrolyze PF.[29] Moreover, PF is not metabolized in the lungs, liver, or gut wall but is degraded only by intestinal flora.[8] The efflux due to P-gp and/or degradation by intestinal flora, which showed an altered profile in CIA rats, could have resulted in lower absorption of PF in these animals compared to normal controls at low doses of TGP. P-gp is expressed in the absorptive cells of jejunal villi,[30] and P-gp levels are elevated in RA patients.[31] When administered at low doses, the PF concentration in rats is largely affected by P-gp efflux. A previous study reported that the degradation rate of PF was higher upon incubation with intestinal flora from CIA rats as compared to normal rats. RA is known to disrupt the normal metabolic activity of gut microbiota.[14] This explains why the absorption of the low-dose TGP in the CIA rats was lower than that of the normal rats. However, for high doses, the efflux effect of P-gp in the rats was saturated, while the effect on absorption was relatively small. Intestinal flora has a limited ability to degrade high concentrations of a drug. This may have resulted in no significant absorption of single high doses of TGP in the normal and CIA groups.

On the other hand, the absorption of AF can be affected by P-gp.[32] It can be metabolized by human intestinal bacteria to obtain paeonilactone A and B.[9] Similar to our PF results, a previous study showed that AF was degraded at a higher rate by the intestinal flora of CIA rats compared to normal rats, suggesting that AF was poorly absorbed in the former following low-dose administration of TGP (The data has not been published yet). However, because of the limited ability of intestinal flora to degrade high concentrations of AF, there was no difference between normal and CIA rats in terms of AF absorption after administration of a high TGP dose.

To clarify its clinical characteristics, the metabolic rate of AF and PF by intestinal flora in feces under different administration conditions of TGP was also studied. The results of the incubation experiment indicated that the intestinal flora in CIA rats metabolized TGP faster, and because the colonization of the intestinal flora takes a long time, the single dose of TGP had a limited effect on the intestinal flora. This may explain why the absorption by model rats was worse than that by normal rats after a single-dose administration.

Pharmacokinetics of TGP in normal and CIA rats after chronic administration

We observed that after long-term administration, there were no differences in the absorption of PF and AF between normal and CIA rats, regardless of the dose. This suggests that TGP absorption is similar in CIA and normal rats after long-term administration. Clinical effects can be observed early in RA patients, about one month after they start taking TGP,[33] since long-term administration of TGP reverses most of the changes in the gut microbiota profile that may have occurred in RA. While PF is slowly metabolized in human liver microsomes,[34] AF is stable in both rat and human liver microsomes.[35] Additionally, PF and AF are excreted in their original form in feces.[36] Thus, the degradation of AF and PF by intestinal flora is the main factor influencing their absorption.

A previous study found that PF has a cumulative effect when multiple doses are administered and absorption is accelerated,[37] which is consistent with our results. A previous study found that long-term administration of TGP altered fecal flora and cecal microbiota profiles in both normal and CIA rats in a dose-dependent manner.[14] Thus, the changes in the intestinal flora possibly contribute to accelerating AF metabolism in normal rats and increased T_{max} in CIA rats after administration of a high dose of TGP.

Lastly, after long-term administration in the normal rats, the metabolic rate of PF in high-dose rats was significantly higher than that of low-dose rats. As for normal rats, it is not possible to explain the pharmacokinetic parameters only by the results of microbial metabolism. Considering

that PF is a substrate of P-gp, a high intestinal metabolic rate may reduce the intestinal concentration of PF after high-dose administration and reduce its outflow, thereby promoting its absorption. In addition, it also shows that the TGP regulation in the intestinal flora of normal rats is different from that of model rats.

CONCLUSION

In this study, we established a highly reproducible UPLC-ESI-MS/MS method for the quantification of compounds in rat plasma. We found that the absorption of AF and PF differed between single-dose and chronic TGP administration, and the metabolic rate of AF and PF were significantly slower after long-term TGP intervention. We propose that the latter reduces the degradation of PF and AF by gut microbiota and promotes their absorption; therefore, long-term TGP administration is required for it to be clinically effective. However, it is also possible that these compounds exert direct effects on gut microbiota or regulate intestinal mucosal immunity. Nonetheless, these results provide a reference for clarifying the effect of CIA on TGP's intestinal metabolism and may be useful for establishing clinical guidelines for the use of TGP in the treatment of RA.

Financial support and sponsorship

This study was financially supported by the National Natural Science Foundation of China (Grant no. 81773860).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. He DY, Dai SM. Anti-inflammatory and immunomodulatory effects of Paeonia lactiflora Pall, a traditional Chinese herbal medicine. Front Pharmacol 2011;2:10.
2. Yu XR, Cao CF, Chen YZ. Application progress of TGP in autoimmune disease. Acta Chin Med Pharmacol 2019;47:127-30.
3. Gao LN, Zhang Y, Cui YL, Akinyi OM. Comparison of paeoniflorin and albiflorin on human CYP3A4 and CYP2D6. Evid Based Complement Alternat Med 2015;2015:470219.
4. Wang J, Shi D, Zheng M, Ma B, Cui J, Liu C, *et al.* Screening, separation, and evaluation of xanthine oxidase inhibitors from Paeonia lactiflora using chromatography combined with a multi-mode microplate reader. J Sep Sci 2017;40:4160-7.
5. Zhang L, Wei W. Anti-inflammatory and immunoregulatory effects of paeoniflorin and total glucosides of paeony. Pharmacol Ther 2020;207:107452.
6. Xie P, Cui L, Shan Y, Kang WY. Antithrombotic effect and mechanism of Radix *Paeoniae* rubra. BioMed Res Int 2017;2017:9475074.
7. Wang YL, Wang JX, Hu XX, Chen L, Qiu ZK, Zhao N, *et al.* Antidepressant-like effects of albiflorin extracted from Radix *paeoniae* Alba J Ethnopharmacol 2016;179:9-15.
8. Takeda S, Isono T, Wakui Y, Mizuhara Y, Amagaya S, Maruno M, *et al. in-vivo* assessment of extrahepatic metabolism of paeoniflorin in rats: Relevance to intestinal floral metabolism. J Pharm Pharmacol 1997;49:35-9.
9. Hattori M. Metabolism of albiflorin by human intestinal bacteria. J Med Pharm Soc Wakan-Yaku 1985;2:398-404.
10. Bae SC, Lee YH. Alcohol intake and risk of rheumatoid arthritis: A Mendelian randomization study. Z Rheumatol 2019;78:791-6.
11. He X, Xing D, Ding Y, Li Y, Xu L, Du L. Effects of cerebral ischemia-reperfusion on pharmacokinetic fate of paeoniflorin after intravenous administration of *Paeoniae* Radix extract in rats. J Ethnopharmacol 2004;94:339-44.
12. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, *et al.* Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. eLife 2013;2:e01202. doi: 10.7554/eLife.01202.
13. Chen J, Wright K, Davis JM, Jeraldo P, Marietta EV, Murray J, *et al.* An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. Genome Med 2016;8:43.
14. Peng J, Lu X, Xie K, Xu Y, He R, Guo L, *et al.* Dynamic alterations in the gut microbiota of collagen-induced arthritis rats following the prolonged administration of total glucosides of paeony. Front Cell Infect Microbiol 2019;9:204.
15. Wang Q, Yang HY, Liu WN, Li XN, Liu RX, Bi KS, *et al.* Determination of albiflorin in rat plasma by LC-MS/MS and its pharmacokinetics. Chin Pharm J 2008;43:1170-3.
16. Xu W, Zhao Y, Qin Y, Ge B, Gong W, Wu Y, *et al.* Enhancement of exposure and reduction of elimination for paeoniflorin or albiflorin via co-administration with total peony glucosides and hypoxic pharmacokinetics comparison. Molecules 2016;21:874.
17. Liu J, Chen L, Fan CR, Li H, Huang MQ, Xiang Q, *et al.* Qualitative and quantitative analysis of major constituents of *Paeoniae* Radix Alba and *Paeoniae* Radix rubra by HPLC-DAD-Q-TOF-MS/MS. Zhongguo Zhong Yao Za Zhi 2015;40:1762-70.
18. Ye X, Xia L, Ren H, Deng Y, Yang M, Zhang J, *et al.* Research progress on processing history evolution, chemical constituents and pharmacological action of Paeoniae Radix Alba. Chin Tradit Herbal Drugs 2020;51:1951-69. doi: 10.7501/j.issn.0253-2670.2020.07.032.
19. Feng C, Liu M, Shi X, Yang W, Kong D, Duan K, *et al.* Pharmacokinetic properties of paeoniflorin, albiflorin and oxypaeoniflorin after oral gavage of extracts of Radix *Paeoniae* rubra and Radix *Paeoniae* Alba in rats. J Ethnopharmacol 2010;130:407-13.
20. Chen X, Ma YM, Zhong J. Research progress in double peak phenomenon of concentration-time curve. Chin J New Drugs Clin Rem 2012;31:432-7.
21. Takeda S, Isono T, Wakui Y, Matsuzaki Y, Sasaki H, Amagaya S, *et al.* Absorption and excretion of paeoniflorin in rats. J Pharm Pharmacol 1995;47:1036-40.
22. Yang XG, Zhang LB, Pan WS, Zhang JY. Studies on absorption kinetics of paeoniflorin in rat's intestines. Chin Pharm J 2006;41:854-7.
23. Shui WB, He Q, Ge ZW, Cheng YY. Studies on absorption of PF in rat small intestines by HPLC-MS. Chin Pharm J 2007;42:1098-101.
24. Shu YZ, Hattori M, Akao T, Kobashi K, Kagei K, Fukuyama K, *et al.* Metabolism of paeoniflorin and related compounds by human intestinal bacteria. II. Structures of 7S- and 7R-paeonimetabolines I and II formed by Bacteroides fragilis and Lactobacillus brevis. Chem Pharm Bull (Tokyo) 1987;35:3726-33.
25. Ke ZC, Yang N, Hou XF, Wang AD, Feng L, Jia XB. Metabolism of paeoniflorin by rat intestinal flora *in vitro*. Zhongguo Zhong Yao Za Zhi 2016;41:3839-45.
26. Dong Y, Zhang Y, Yang Q, Li Y, Zhu X. Absorption of extractive Radix *Paeoniae* Alba in rat everted gut sacs and its interaction with P-glycoprotein. Zhongguo Zhong Yao Za Zhi 2009;34:884-8.
27. Wang C, Wei W, Yang S, Nie X. Studies on absorption kinetics of paeoniflorin microemulsion in rat's intestines. Chin Pharmacol Bull 2009;25:181-5.
28. Chan K, Liu ZQ, Jiang ZH, Zhou H, Wong YF, Xu HX, *et al.* The effects of sinomenine on intestinal absorption of paeoniflorin by the everted rat gut sac model. J Ethnopharmacol 2006;103:425-32.
29. Yan C. Multi-channel analysis and comparison on absorption difference between paeoniflorin and Paeonia lactiflora extracts. China J Chin Mater Med 2012;37:2012-16.
30. Cong X, Fan J. Effects of P-glycoprotein on multidrug resistance in colon cancer. Chin J Biochem Mol Biol 2019;35:19-26.
31. Tsujimura S, Adachi T, Saito K, Kawabe A, Tanaka Y. Relevance of P-glycoprotein on CXCR4+B cells to organ manifestation in highly active rheumatoid arthritis. Mod Rheumatol 2018;28:276-86.
32. Hou J, Hu JC, Li YT, Liu T, Huang Y, Wang AM. Absorption characteristics of 5 components of Xinshao extract in Caco-2 cells. Zhongguo Zhong Yao Za Zhi 2016;41:2137-43.
33. Wang C, Wei W. Research progress of pharmacokinetic study of paeoniflorin. Chin Pharmacol Bull 2014;30:1646-50.
34. Tan Y, Shen GL, Zhuang XM, Yuan M, Li Y, Gao Y. Metabolic characteristics of paeoniflorin *in vitro*. J Int Pharm Res 2013;40:625-33.
35. Zhao ZX. Preclinical pharmacokinetic study of albiflorin [masters degree thesis]. China: University of Jinan; 2015.
36. Fei F, Yang H, Peng Y, Wang P, Wang S, Zhao Y, *et al.* Sensitive analysis and pharmacokinetic study of the isomers paeoniflorin and albiflorin after oral administration of total glucosides of white paeony capsule in rats. J Chromatogr B Analyt Technol Biomed Life Sci 2016;1022:30-7.
37. Wang HR, Wang HT, Liu LJ, Zhao SH, Zhang LT. Pharmacokinetics of Tongxinluo supermicro powder in rats. Lishizhen Med Mater Med Res 2014;25:1328-31.