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Pharmacokinetic study of paeoniflorin in mice after oral administration of Paeoniae radix extract

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Abstract

Quantification of paeoniflorin, the principal bioactive component of Paeoniae radix, in mice plasma following oral administration of Paeoniae radix extract was achieved by using a simple and rapid high-performance liquid chromatographic method. The calibration curve for paeoniflorin was linear ($r^2 = 0.998$) over the concentration range 10–200 ng/ml. The coefficients of variation of intra- and inter-day assays were 15.04, 7.31, 6.14, 6.55, 6.63% and 12.71, 6.07, 3.61, 5.51, 4.52% at concentrations of 10, 60, 100, 160, 200 ng/ml, respectively. The recoveries of paeoniflorin from mice plasma were found to be 74.49, 76.83, 80.38 and 80.56% for concentrations of 30, 80, 120 and 160 ng/ml, respectively. The plasma concentration–time curves were fitted with mean terminal half-lives ($t_{1/2}$) of 94.16 min.

Keywords: Paeoniflorin

1. Introduction

Paeoniae alba radix (White Paeony Root; Baishao), the dried peeled root of *Paeonia lactiflora* Pall. (family Paeoniaceae), is one of the Chinese traditional tonic crude drugs. It has been used as a spasmolytic and pain-relieving agent and has long been used to regulate the menstrual flow, for the treatment of menstrual disorders and to relieve abdominal spasmodic pain and muscle stiffness. Paeoniflorin (structure shown in Fig. 1), a water-soluble substance isolated from the root of *P. lactiflora* in 1963 [1], is one of the bioactive components in Paeoniae radix and has been reported to exhibit anticoagulant [2], neuromuscular blocking [3–10], cognition-enhancing [11–15], immunoregulating [16] and antihyperglycemic effects [17]. Because knowledge of the pharmacokinetic processes can help us to explain and predict a variety of events related to the efficacy and toxicity of herbal prepara-

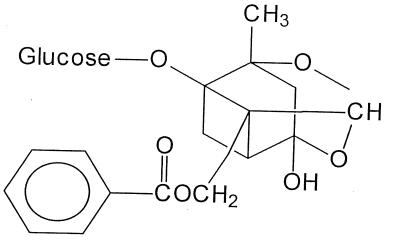

Fig. 1. Structure of paeoniflorin.

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tions, it is important to investigate the pharmacokinetics of paeoniflorin for further evaluation of its clinical applications.

Although paeoniflorin has been demonstrated to exhibit neuromuscular blocking and cognition-enhancing effects, there are no appropriate pharmacokinetic data to support an ideal dosing regimen for these clinical applications at this stage. From a pharmacokinetic point of view, to determine a rational dosing interval (e.g., tid or qid regimen), the half-life ($t_{1/2}$) of paeoniflorin should be considered first. In addition the time to reach maximum concentration (t_{max}) is required for the estimation of onset of pharmacological response. Therefore, the pharmacokinetics of paeoniflorin are useful for providing an ideal dosing regimen and enhancing the safety and efficacy of paeoniflorin in clinical applications.

Because most Chinese medicines are administered orally as extract powders, decoctions or extract granules in clinical use, the pharmacokinetics after administration of purified paeoniflorin reported in recent years [18–20] could not used as a suitable reference in clinical application. To obtain the available pharmacokinetic data of paeoniflorin, a simple and rapid high-performance liquid chromatographic (HPLC) method was developed to determinate paeoniflorin in mice plasma after oral administration of Paeoniae radix extract. The present study describes the absorption and excretion of paeoniflorin; the related pharmacokinetic profiles of paeoniflorin are evaluated and discussed.

2. Experimental

2.1. Crude drugs

Paeoniae Alba Radix, the dried roots of *Paeonia lactiflora* Pall. of Paeoniaceae, was purchased from Sun-yun Herbal Shop (Taipei, Taiwan). The herb materials were extracted twice by refluxing with boiling water (1:10, v/v) for 1 h, and the solution obtained was concentrated and then made into freeze-dried powder extracts. A 24.6-g amount of Paeoniae radix extracts was obtained from 200 g raw material (the yield was 12.3%). The freeze-dried extracts were stored at 4°C until use.

2.2. Chemicals and reagents

The reference standard of paeoniflorin was purchased from Nacalai Tesque (Kyoto, Japan). The internal standard, pentoxifylline, was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and ether (HPLC grade) were obtained from Merck (Darmstadt, Germany). Triple-deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.3. Animals

Male ICR mice (20–30 g) were obtained from the Laboratory Animal Center at the National Taiwan University (Taipei, Taiwan). Animals were kept in an environmentally controlled breeding room (temperature: 24±1°C, humidity: 60±5%, 12 h dark–light cycle) for 1 week before the start of the experiments. They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments.

2.4. HPLC system

The HPLC system consisted of a pump (solvent delivery system 400, Applied Biosystems), an autosampler (715 Ultra Wisp, Waters), an UV detector (UV-975 intelligent UV–Vis detector, Jasco), and an integrator (HP 3396A, Hewlett-Packard). A Cosmosil 5 C_{18}-AR reversed-phase column, 250×4.6 mm, 5 μm (Nacalai, Japan) was used. The mobile phase was water–acetonitrile (84:16, v/v), filtered through a 0.45-μm Millipore filter and degassed prior to use. The flow-rate was 1 ml/min. Detection was performed at a wavelength of 231 nm under a constant temperature (22±1°C).

2.5. Content of paeoniflorin in extract of Paeoniae radix

Experimental mice were orally administered with Paeoniae radix extracts (at a dose containing 10 mg/kg paeoniflorin) in this study. To calculate the administered dose, the content of paeoniflorin in the Paeoniae radix extract had to be quantitatively analyzed first. The freeze-dried extract of Paeoniae radix was mixed with distilled water and diluted to 50 μg/ml. The mixture was centrifuged at 1500 *g* for 15 min at 8–10°C and the supernatant solution

was taken. After the addition of fixed amount of internal standard, 20 μl of this solution were injected onto the HPLC system (described above) for analysis. The content of paeoniflorin in the freeze-dried extract of Paeoniae radix was determined (13.0%) from the peak height ratios by using the equation for linear regression obtained from the calibration curve.

2.6. Drug administration and blood sampling

Aqueous solutions of Paeoniae radix extracts were orally administered to mice at a dose containing 10 mg/kg paeoniflorin. Under anesthesia with ether in a glass chamber, blood samples (0.8–1 ml) were collected from each mouse by cardiac puncture according to the specific schedule (at times of 5, 10, 15, 20, 40, 60, 120, 180 and 240 min after dosing). Data from these samples were used to construct pharmacokinetic profiles by plotting drug concentration vs. time.

2.7. Preparation of plasma samples

Each collected blood sample was immediately transferred to a heparinized microcentrifuge tube and centrifuged at 1500 *g* for 15 min at 8–10°C. The resulting plasma (0.4 ml) was then mixed with 0.8 ml acetonitrile and 80 μl internal standard (pentoxifylline 5 μg/ml). The denatured protein precipitate was separated by centrifugation at 1500 *g* for 15 min at 8–10°C. The supernatant was mixed with 4 ml ether by ultrasonic vortex for 2 min. Then the mixture was centrifuged at 1500 *g* for 15 min at 8–10°C. The ether layer was discarded to get rid of the non-polar interfering impurities. The water layer was evaporated to dryness below 40°C in vacuo and then dissolved in 0.1 ml of mobile phase. A 20-μl volume of this sample solution was injected onto HPLC for analysis. The same sample handling process was used for recovery and precision determinations in plasma.

2.8. Calibration curve

A calibration curve was constructed based on the analysis of various concentrations (10, 30, 60, 80, 100, 120, 160 and 200 ng/ml) of paeoniflorin spiked in mice plasma by HPLC. The concentrations of paeoniflorin in plasma were determined from the peak height ratios by using the equation for linear regression obtained from the calibration curve.

2.9. Recovery

Plasma samples were spiked with four different concentrations (30, 80, 120 and 160 ng/ml) of paeoniflorin. After preparation of plasma sample, fixed amounts of internal standard (pentoxifylline) were added to plasma for normalization. The resulting peak height ratios (corresponding ratios of paeoniflorin: internal standard) were compared with those standards carried in distilled water to provide the recovery values.

2.10. Precision

Precision over the entire working dose range was determined by triplicate analyses of plasma samples ($n=3$) spiked with five different concentrations (10, 60, 100, 160 and 200 ng/ml) of paeoniflorin. To determine intra-day variance, the assays were carried out on the same samples at different times during the day. Inter-day variance was determined by assaying the spiked samples over 3 consecutive days. Coefficients of variation (C.V.s) were calculated from these values.

2.11. Pharmacokinetic analysis

All data were subsequently processed by the computer program WINNONLIN (SCI, Lexington, USA). The non-compartmental pharmacokinetic parameters of half-life ($t_{1/2}$), mean residence time (MRT), area under the plasma concentration–time curve (AUC), clearance/bioavailability (Cl/F) and volume of distribution/bioavailability (V_d/F) were calculated based on the moment theory.

3. Results

3.1. HPLC chromatograms

Under the condition described above, the HPLC chromatograms of blank plasma, plasma spiked with paeoniflorin (100 ng/ml) and the plasma obtained 20 min after oral administration of Paeoniae radix

extract (at a dose containing 10 mg/kg paeoniflorin) are shown in Fig. 2. The retention times of paeoniflorin and pentoxifylline (internal standard) were 9.44 and 16.39 min, respectively. No interfering peaks were observed within the time frame in which paeoniflorin and pentoxifylline were detected.

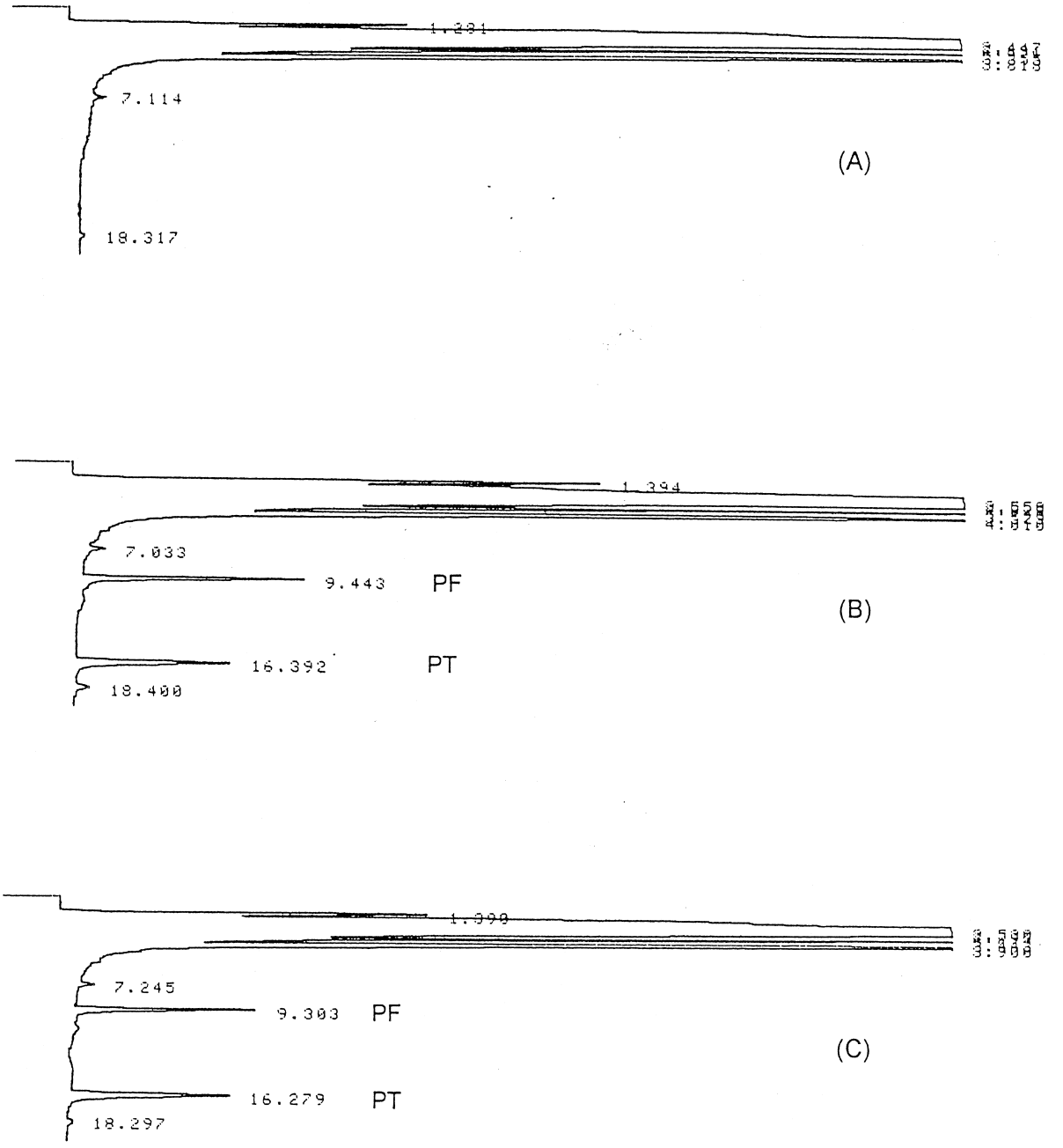

Fig. 2. Chromatograms of paeoniflorin in mice plasma: (A) blank plasms; (B) blank plasma spiked with paeoniflorin (100 ng/ml) and internal standard (pentoxifylline); (C) plasma sample obtained 20 min after oral administration of Paeoniae radix extract. PF, paeoniflorin; PT, pentoxifylline.

3.2. Calibration curves

Paeoniflorin was dissolved in distilled water and diluted to give standard solutions (10, 30, 60, 80, 100, 120, 160 and 200 ng/ml) for the calibration curve of the drug in plasma. The calibration curve for paeoniflorin was linear ($r^2 = 0.998$) over the concentration range of 10–200 ng/ml. With the least-squares method, a regression equation of $y = 0.01309x + 0.10000$ (x: peak height ratio of paeoniflorin to pentoxifylline, y: concentration of paeoniflorin in plasma) was obtained.

3.3. Recovery tests and reproducibility

The recoveries of paeoniflorin from mice plasma were 74.49, 76.83, 80.38 and 80.56% for the concentrations of 30, 80, 120 and 160 ng/ml, respectively (Table 1). The reproducibility of the method was defined by examining both intra- and inter-day variance. The C.V. values of intra-day assay were 15.04, 7.31, 6.14, 6.55 and 6.63% at concentrations of 10, 60, 100, 160 and 200 ng/ml, respectively (Table 2). The C.V. values of inter-day assay were 12.71, 6.07, 3.61, 5.51, and 4.52% at concentrations of 10, 60, 100, 160, and 200 ng/ml, respectively (Table 3). These validation results indicated that the method is suitable for the present study.

3.4. Determination of paeoniflorin in plasma

The plasma concentration versus time profile of paeoniflorin in mice is presented in Fig. 3. After oral administration of aqueous solution of Paeoniae radix extract (at dose containing 10 mg/kg paeoniflorin), the plasma level of paeoniflorin declined with a half-life of 94.16 min. The concentration was lower than quantitative limit (10 ng/ml) after 4 h.

Table 2
Validation of the intra-day assay[a]

Spiked conc. (ng/ml)	Measured conc. (ng/ml)	Accuracy (%)	C.V. (%)
10	9.80±1.47	98.03	15.04
60	60.13±4.40	100.22	7.31
100	98.38±6.04	98.38	6.14
160	151.51±9.92	94.69	6.55
200	191.37±12.68	95.69	6.63

[a] Each value represents the mean±SD, ($n = 3$).

Table 3
Validation of the intra-day assay[a]

Spiked conc. (ng/ml)	Measured conc. (ng/ml)	Accuracy (%)	C.V. (%)
10	11.35±1.44	113.53	12.71
60	58.58±3.56	97.64	6.07
100	94.11±3.40	94.11	3.61
160	146.02±8.05	91.26	5.51
200	185.33±8.39	92.66	4.52

[a] Each value represents the mean±SD, ($n = 3$).

3.5. Kinetic analysis

As calculated from the plasma concentrations of paeoniflorin following oral administration of Paeoniae radix extracts, the pharmacokinetic parameters of paeoniflorin are listed in Table 4.

4. Discussion

Nowadays, scientific results are required to support the clinical efficacy of tradition Chinese medicines (TCMs). In addition to the study of

Table 1
Recovery of the paeoniflorin assay[a]

Spiked conc. (ng/ml)	Peak height ratio		Recovery (%)	C.V. (%)
	Untreated	Treated		
30	0.5733±0.0302	0.4258±0.0278	74.49±7.57	10.16
80	1.4611±0.0411	1.1210±0.0497	76.83±5.60	7.30
120	2.2176±0.0175	1.7819±0.1028	80.38±5.29	6.58
160	2.9033±0.0422	2.3373±0.1454	80.56±6.06	7.53

[a] Each value represents the mean±SD, ($n = 3$).

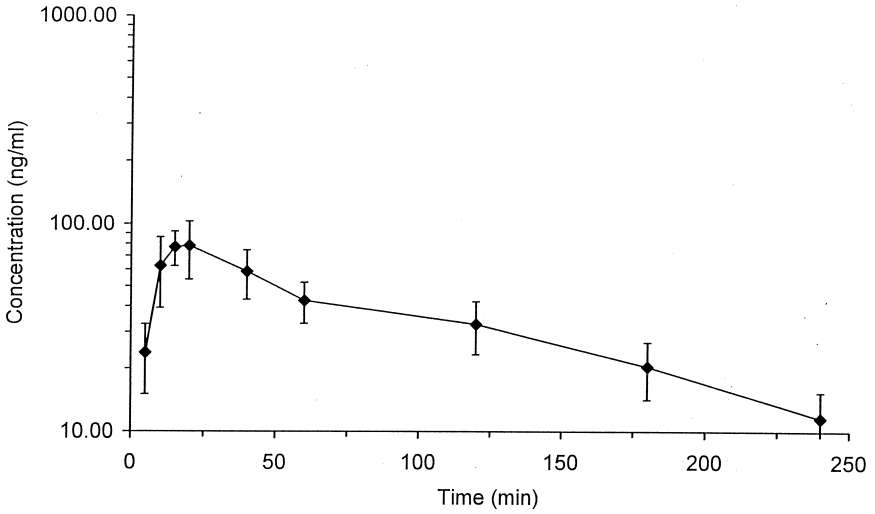

Fig. 3. Plasma concentration–time curve of paeoniflorin in mice after oral administration of Paeoniae radix extracts (at a dose containing 10 mg/kg paeoniflorin). Each point and bar represent the mean±SD ($n=5$).

pharmacological mechanisms, the pharmacokinetic study of TCMs is an important and useful approach [21,22]. Through the results derived from the pharmacokinetic studies of TCMs, their conditions of absorption, distribution, metabolism and excretion in the human body are elucidated, and reasonable dosing regimens can be suggested. From a pharmacokinetic point of view, to determine a rational dosing interval (e.g., tid or qid regimen), the $t_{1/2}$ and t_{max} values need to be established for estimation of the onset of pharmacological response. Because there are no appropriate pharmacokinetic data to support an ideal dosing regimen of paeoniflorin at this stage, the pharmacokinetics of paeoniflorin is useful in providing the dosing information and thus enhancing the safety and efficacy of paeoniflorin in clinical applications.

Table 4
Pharmacokinetic parameters of paeoniflorin in mice plasma ($n=5$) after oral administration of extracts of Paeoniae radix (at a dose containing 10 mg/kg paeoniflorin)

Parameter	Estimate (mean±SD)
t_{max} (min)	14.00±4.18
C_{max} (ng/ml)	86.34±18.67
AUC_{0-t} [a] (ng·min/ml)	8126±2004.62
$AUC_{0-\infty}$ (ng·min/ml)	9746.10±2554.62
$t_{1/2}$ (min)	94.16±12.35
CL/F (ml/min·kg)	1113.35±420.63
V_d/F [b] (l/kg)	147.44±40.78
MRT[c] (min)	135.64±18.51

[a] AUC, area under the curve.
[b] V_d, volume of distribution.
[c] MRT, mean residence time.

Pharmacokinetic studies of paeoniflorin, the major component of Paeoniae radix, are limited to its absorption and excretion in animal studies after administration of purified paeoniflorin (parenteral or oral) [18–20]. However, since TCMs are orally administered in the form of a crude extract in clinical practice, these related results are not enough to reveal the clinical efficacy of paeoniflorin. Therefore, this research was designed to administer aqueous Paeoniae radix extracts to animal subjects, and to determine the plasma profiles and pharmacokinetic parameters of paeoniflorin.

HPLC and EIA are the two major methods that have been used for the quantification of paeoniflorin. The sensitivity of EIA is higher (≈0.1 ng/ml), however the process is cumbersome. Compared with EIA, HPLC is a simpler analytical method. So HPLC was chosen for this study to quantitatively analyze the concentrations of paeoniflorin. The accuracy and precision of the calibration curve, except the C.V. value of the low concentration point (10 ng/ml) which was 10.06%, were <10%. C.V.s of intra- and

inter-day assay were also <10%, except at 10 ng/ml. Judging from these results, the HPLC method has good reproducibility, accuracy and precision, and could be applied for the quantitative assay of paeoniflorin in blood samples. Recoveries for this assay were around 74.49–80.56%. Although the relatively low recovery was due to the loss of paeoniflorin during sample handling process, the protein binding capacity of paeoniflorin was also an important factor which contributed to the loss of paeoniflorin.

In the present study, paeoniflorin plasma levels were assayed after oral administration of aqueous Paeoniae radix extracts (at a dose containing 10 mg/kg paeoniflorin). The pharmacokinetic parameters of paeoniflorin derived from the plasma profiles are listed in Table 4. The bioavailability could not be obtained since the crude extract is not suitable for i.v. injection; therefore, clearance and volume of distribution were indicated by CL/F and V_d/F, respectively. To compare with the previous results from oral administration of purified paeoniflorin [19], our results indicated the longer $t_{1/2}$ and later t_{max}, implying a smoother absorption and excretion of paeoniflorin after oral administration of Paeoniae radix extracts.

In case of oral administration of Paeoniae radix extracts, the much lower paeoniflorin concentrations in mice plasma reflected a higher V_d/F value. Although this might imply that paeoniflorin has a higher binding activity to organs and a lower blood distribution, the low bioavailability was also a possible cause of the low paeoniflorin concentration in plasma. Since the bioavailability of paeoniflorin is low (about 3%) [19], if we can find an ideal vehicle or use combination treatment to increase absorption of paeoniflorin, a great benefit for clinical efficacy of paeoniflorin will be obtained.

In conclusion, the pharmacokinetic studies of TCMs could be a very important reference for TCM researches in clinical trials and applications. In the present study, aqueous solution of Paeoniae radix extract was oral administered to evaluate the pharmacokinetic parameters of paeoniflorin. The features of paeoniflorin's pharmacokinetics could be applied as a reference for evaluating its clinical efficacy. Since the bioavailability for oral administration of paeoniflorin is low, how to efficiently increase its bioavailability, enhance its concentration in blood and improve the clinical efficacy will be our goals of future projects. In addition, most of the TCMs are prescribed by Chinese medicine prescriptions, and are used to engender synergistic effects. Therefore, the effect of Chinese medicine prescriptions on the pharmacokinetics of TCMs will also be an important topic for further studies.

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