J. Pharm. Pharmacol. 1995, 47: 1036–1040
Received October 31, 1994
Accepted April 4, 1995

Absorption and Excretion of Paeoniflorin in Rats

SHUICHI TAKEDA, TOMOKO ISONO, YOKO WAKUI, YUTAKA MATSUZAKI, HIROMI SASAKI, SAKAE AMAGAYA AND MASAO MARUNO

Drug Safety and Metabolism Department, Tsumura Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan

Abstract

The absorption and excretion of paeoniflorin after intravenous and oral administration was studied in rats to evaluate the significance of paeoniflorin in the pharmacological action of Paeony root.

The plasma concentration of paeoniflorin after intravenous administration at the doses of 0·5, 2·0 and 5·0 mg kg^{-1} rapidly decreased, simulated by a biexponential curve, with mean terminal half-lives of 11·0, 9·9 and 12· 6 min, respectively. The Vd_{ss} values were 0·332, 0·384 and 0·423 L kg^{-1} and the CL_{tot} values were 26·1, 31·2 and 30·3 mL min^{-1} kg^{-1} at each dose. When given orally at the same doses, the absolute bioavailability values (F) determined by the AUC were 0·032, 0·033 and 0·038, respectively. The cumulative urinary and faecal excretions of paeoniflorin at the dose of 5 mg kg^{-1} after intravenous administration were 50·5 and 0·22% of the dose within 72 h, and 1·0 and 0·08% of the dose after oral administration within 48 h, respectively. Cumulative biliary excretion after intravenous or oral administration at a dose of 0·5 mg kg^{-1} was 6·9 and 1·3% of the dose within 24 h, respectively. The total CL_R and CL_B value after intravenous dosing was less than the CL_{tot} value. These findings suggest that paeoniflorin is metabolized in other organs as well as in the liver.

We conclude that paeoniflorin absorbed is excreted mainly in urine, it has a low bioavailability and the metabolites may be involved in the pharmacological action of Paeony root.

Paeony root (*Paeoniae radix*) is one of the crude drugs composed of Kampo medicine (oriental herbal medicine) and contains paeoniflorin (Fig. 1) and albiflorin as active constituents (Nishizawa et al 1979). Paeoniflorin has several pharmacological effects including antiallergic (Yamahara et al 1982), anticonvulsive (Takagi & Harada 1969a), analgesic (Takagi & Harada 1969b), muscle relaxant (Takagi & Harada 1969a; Sugishita et al 1984) and anti-inflammatory (Takagi & Harada 1969c) actions and the therapeutic effects of Paeony root are explained by the pharmacological actions of paeoniflorin (Harada 1969). However, these pharmacological effects of paeoniflorin are mainly studied by the intraperitoneal route in mice and rats; although most Kampo medicines containing paeoniflorin are administered orally to patients in clinical use, there are few reports on the pharmacokinetics of paeoniflorin after oral dosing.

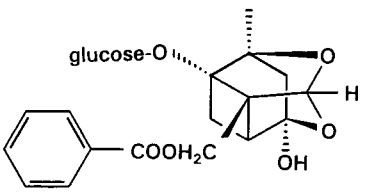

FIG. 1. Chemical structure of paeoniflorin.

In this paper we describe the absorption and excretion of paeoniflorin after single intravenous and oral administration in rats and evaluate the significance of paeoniflorin in the pharmacological action of Paeony root.

Correspondence: S. Takeda, Drug Safety and Metabolism Department, Tsumura Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan.

Materials and Methods

Materials

Paeoniflorin was supplied by the Technical Department in our laboratories. β-Galactosidase was purchased from Boehringer Mannheim GmbH. Bovine serum albumin (BSA) and 7-β-D-galactopyranosyloxy-4-methyl coumarin were from Sigma, physiological saline was from Fusou Pharmaceutical Industries (Osaka, Japan) and Freund's complete adjuvant was from Difco. Other reagents were of special or HPLC (high performance liquid chromatography) analytical grade obtained from Wako Pure Chemical Industries (Tokyo, Japan). Goat anti-rabbit IgG (Marcella 10) as the second antibody employed in the enzyme immunoassay (EIA) was purchased from Dainippon Pharmaceutical Industries (Osaka, Japan).

Animals

Male Sprague-Dawley rats (7–8 weeks old, Charles River Japan) were used. Animals were fasted overnight before the experiment with free access to water.

Paeoniflorin was dissolved in physiological saline for the intravenous dose and distilled water for the oral dose. Rats were given paeoniflorin intravenously or orally at the doses of 0·5, 2·0 and 5·0 mg kg^{-1} (n = 5–7). Paeoniflorin was also given intravenously or orally at a dose of 5 mg kg^{-1} (n = 5–7) for the urinary and faecal excretion studies and 0·5 mg kg^{-1} (n = 5–7) for the biliary excretion studies.

Under light anaesthesia with ether, the left femoral vein and artery were cannulated with SP 10 polyethylene tubing (Natsume Seisakusho Tokyo, Japan) which was filled with sodium heparin at a concentration of 100 int. units mL^{-1}. After recovery from ether anaesthesia, drugs were given to

the conscious rats by gastric intubation or through the venous cannula. Arterial blood samples (100 μL) were obtained at a fixed time after administration. Plasma samples were immediately separated by centrifugation of the blood and stored at −20°C. The rats were given the same volume of saline instead of the lost blood. The rats were kept in metabolic cages to collect the urine and faeces after intravenous or oral administration. Urine samples were stored at −20°C and faeces samples stored after grinding the freeze-dried faeces. The rats were also lightly anaesthetized with ether and cannulated into the common bile duct with SP 10 polyethylene tubing to collect the bile samples at a fixed time. After recovery from anaesthesia, paeoniflorin was intravenously or orally administered. The bile samples obtained were stored at −20°C until analysis.

Determination of paeoniflorin

The concentrations of paeoniflorin in plasma, urine, bile and faeces were determined according to the method of Kanaoka et al (1984) with minor modifications. Antiserum and labelled antigen were prepared in the same manner. For the preparation of antiserum to paeoniflorin, 6′-glutaryl paeoniflorin was coupled with bovine serum albumin (BSA) by the *N*-hydroxysuccinimide ester method to give hemiglutaryl paeoniflorin (immunogen). The immunogen was dissolved in saline and emulsified with complete Freund's adjuvant. The emulsion was injected into domestic albino female rabbits (Tokyo Jikken Dobutsu, 1·8–2·0 kg) subcutaneously at multiple sites on the back. After several booster injections, the blood was withdrawn and sera were separated by centrifugation. The antisera were stored at −80°C until use. For the preparation of labelled antigen, 6′-hemisuccinyl paeoniflorin was coupled with β-galactosidase by the *N*-hydroxysuccinimide ester method to give hemisuccinyl paeoniflorin-β-galactosidase (labelled antigen). The sample, antiserum and labelled antigen were diluted with buffer A (20 mM phosphate-buffered saline containing 0·1% BSA, 0·1% NaN_3, 1 mM $MgCl_2$, pH 7·0) at appropriate concentrations. The sample or standard solution containing paeoniflorin (100 μL) was added to 20 000-fold diluted anti-paeoniflorin antiserum (100 μL) and incubated at 4°C overnight. After addition of 40 000-fold diluted labelled antigen (50 μL), this mixture was stored at room temperature (21°C) for 2 h and then 10-fold diluted marcella 10 was added to the mixture. After further incubation for 1 h, 1 mL buffer A was added and the solution was centrifuged (2000 *g*) for 15 min at 4°C. The supernatant was removed and the immune precipitate was washed with buffer A followed by the recentrifugation. The resulting immune precipitate was incubated with $1·5 \times 10^{-4}$ M 7-β-D galactopyranosyloxy-4-methyl coumarin (100 μL) at 30°C for 30 min. Then, 2 mL 0·1 M glycine-NaOH buffer (pH 10·3) was added to the reaction mixture and the fluorescence intensity of 7-hydroxy-4-methylcoumarin formed was measured at 364 and 448 nm wavelength for excitation and emission, respectively.

Sample preparations and calibration curves

The plasma, urine and bile samples were diluted with buffer A. To the faeces, weighing 10 mg, was added 5 mL of buffer A and then centrifuged (18 000 rev min^{-1}, 15 min). The supernatant was diluted with buffer A for the assay. The calibration curve was prepared by drug-free biofluids with added standards of paeoniflorin and constructed with the linearized logit-log plot. The calibration curve ranged from 15·3 pg mL^{-1} to 7·81 ng mL^{-1}. The intra- and inter-day assay precisions (CV%) were 11·1 and 14·3% at 156 pg mL^{-1} and 3·9 and 1·9% at 1·56 ng mL^{-1}, respectively.

Pharmacokinetic analysis

The individual plasma concentration data after intravenous administration were fitted to the equation:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

by nonlinear least-squares regression analysis. The area under the plasma concentration curve from time zero to infinity (AUC), the plasma total body clearance (CL_{tot}), the apparent volume of the central compartment (V_c), the distribution volume at steady state (Vd_{dss}) and the elimination half-life of the α and β-phases ($t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$) were calculated by the following equations:

$$AUC = A/\alpha + B/\beta \quad (2)$$

$$CL_{tot} = \text{dose}/AUC \quad (3)$$

$$Vc = \text{dose}/(A + B) \quad (4)$$

$$Vd_{ss} = \text{dose} \cdot (A/\alpha^2 + B/\beta^2)/(A/\alpha + B/\beta)^2 \quad (5)$$

$$t_{\frac{1}{2}\alpha \text{ or } \beta} = 0·693/\alpha \text{ or } \beta \quad (6)$$

Pharmacokinetic analysis of the oral dosage form was carried out using model-independent methods. The peak plasma concentration (C_{max}) and the time to C_{max} (t_{max}) were determined from the individual profile by inspection. AUC

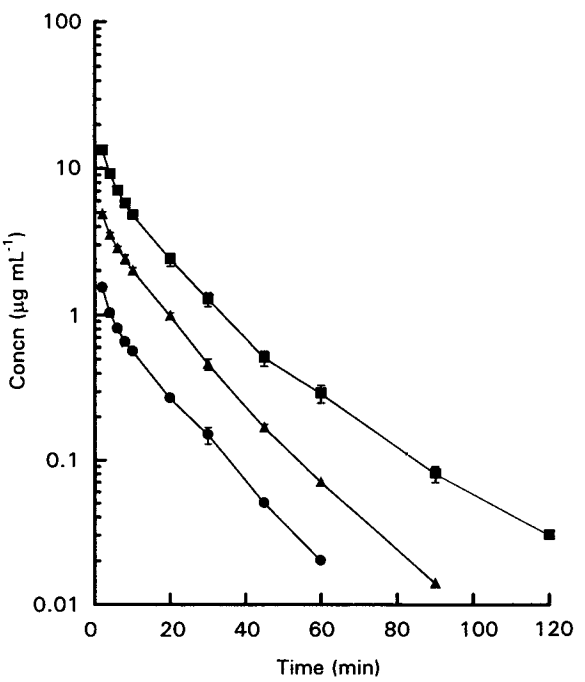

FIG. 2. Plasma concentration–time curves of paeoniflorin after intravenous administration of 0·5 (●), 2·0 (▲) and 5·0 (■) mg kg^{-1} to rats. Each value represents the mean ± s.e.m. of five to seven rats.

Table 1. Pharmacokinetic parameters of paeoniflorin after intravenous administration in rats.

Parameter	Dose (mg kg^{-1})		
	0·5 (n=6)	2 (n=5)	5 (n=7)
A (μg mL^{-1})	2·34±0·38	7·17±2.08	13·80±1·67
α (min^{-1})	0·64±0·13	0·85±0·23	0·38±0·05
B (μg mL^{-1})	1·00±0·09	4·04±0·42	7·27±0.79
β (min^{-1})	0·06±0·004	0·07±0·004	0·05±0·003
$t_{\frac{1}{2}\alpha}$ (min)	1·4±0·4	1·1±0·4	2·1±0·4
$t_{\frac{1}{2}\beta}$ (min)	11·0±0·7	9·9±0·6	12·6±0·9
V_c (L kg^{-1})	0·165±0·023	0·207±0·035	0·246±0·018
Vd_{ss} (L kg^{-1})	0·332±0·018	0·384±0·029	0·423±0·020
CL_{tot} (mL min^{-1} kg^{-1})	26·1±1·3	31·2±1·8	30·3±1·3
AUC (μg min mL^{-1})	19·5±1·1	64·9±3·4	167·1±8·3
AUC_{tr} (μg min mL^{-1})	17·2±0·9	59·2±2·9	156·2±8·2

Each value represents mean ± s.e.m.

Table 2. Pharmacokinetic parameters of paeoniflorin after oral administration in rats.

Dose (mg kg^{-1})	$AUC_{0-\infty}$ (ng min mL^{-1})	C_{max} (ng mL^{-1})	t_{max} (min)	$t_{\frac{1}{2}}$ (min)	F
0.5	555·9±95·7	9·8±2·1	8·8±0·7	49·7±10·0	0·032±0·006
2	1926·0±360·0	30·7±2·4	9·6±2·6	80·2±23·5	0·033±0·006
5	5963·4±1242·7	101·5±18·6	10·2±2·5	31·4±4·5	0·038±0·008

Each value represents mean ± s.e.m. of five rats.

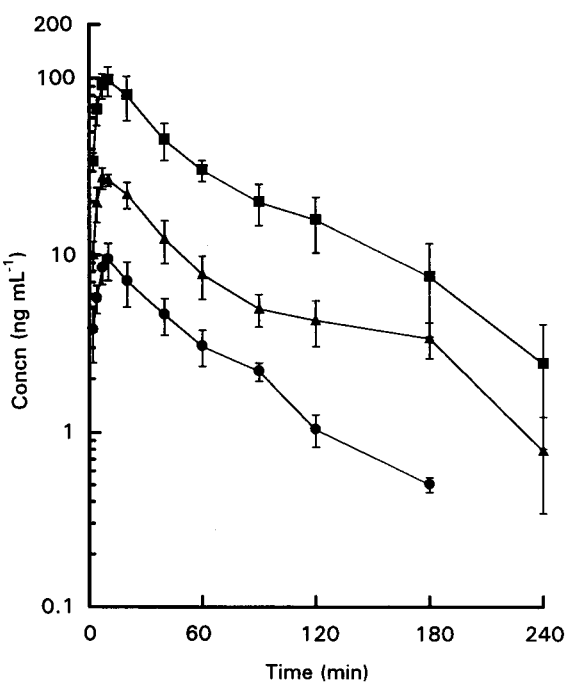

FIG. 3. Plasma concentration–time curves of paeoniflorin after oral administration of 0·5 (●), 2·0 (▲) and 5·0 (■) mg kg^{-1} to rats. Each value represents the mean ± s.e.m. of five rats.

after intravenous or oral dose was calculated by the trapezoidal rule and added to the value of plasma concentration at the detected last time divided by λ (the terminal elimination rate constant), which was calculated by the least squares method on a semi-logarithmic plot. The apparent half-life ($t_{\frac{1}{2}}$) was calculated as $\ln 2/\lambda$.

The systemic bioavailability (F) was calculated by the following equation:

$$F = AUC_{po}/AUC_{iv} \quad (7)$$

Pharmacokinetic parameters were represented as an estimated value ± s.e.m. Statistical analysis was performed by analysis of variance with the level of significance at 0·05.

Results

Fig. 2 shows the plasma concentration–time curves of paeoniflorin after intravenous administration at the doses of 0·5, 2·0 and 5·0 mg kg^{-1} to rats. The pharmacokinetic parameters were calculated and are listed in Table 1. The plasma disappearance of paeoniflorin in rats was described by the biexponential curves. Fig. 3 shows the plasma concentration–time curves of paeoniflorin after oral administration at doses of 0·5, 2·0 and 5·0 mg kg^{-1} to rats. Table 2 summarizes the pharmacokinetic parameters. After oral dosing, paeoniflorin was absorbed in a short time and reached a maximum within 10 min, followed by a biexponential decrease in each dose. There was a large variability in $t_{\frac{1}{2}}$ values at the terminal phase at each dose, because of a second small peak around 90 to 180 min after oral administration. The AUC values increased proportionally to the administered dose.

Table 3 shows the cumulative urinary and faecal excretions of paeoniflorin after intravenous or oral administration at a dose of 5 mg kg^{-1} to rats. Data are expressed as % of the administered dose.

Table 3. Cumulative urinary and faecal excretions of paeoniflorin after intravenous or oral administration at a dose of 5 mg kg^{-1} to rats.

Time (h)	Intravenous (n = 6)		Oral (n = 5)	
	Urine	Faeces	Urine	Faeces
	(% of dose)			
0–3	38·1 ± 3·4	—	0·5 ± 0·08	—
6	44·0 ± 3·3	—	0·8 ± 0·05	—
12	46·7 ± 2·9	—	0·9 ± 0·04	—
24	48·8 ± 2·5	0·20 ± 0·1	1·0 ± 0·08	0·07 ± 0·05
36	49·4 ± 2·4	0·22 ± 0·1	1·0 ± 0·10	0·08 ± 0·05
48	50·2 ± 2·2	0·22 ± 0·1	1·0 ± 0·11	0·08 ± 0·05
72	50·5 ± 2·1	—	—	—

—; not performed. Each value represents mean ± s.e.m.

Table 4. Cumulative biliary excretion of paeoniflorin after intravenous or oral administration at a dose of 0·5 mg kg^{-1} to rats.

Time (h)	Intravenous (n = 7)	(% of dose)	Oral (n = 5)
0–2	6·7 ± 0·8		0·9 ± 0·24
4	6·9 ± 0·8		1·2 ± 0·28
6	6·9 ± 0·8		1·3 ± 0·27
8	6·9 ± 0·8		1·3 ± 0·27
10	6·9 ± 0·8		1·3 ± 0·27
24	6·9 ± 0·8		1·3 ± 0·27

Each value represents mean ± s.e.m.

Table 4 shows the cumulative biliary excretion, expressed as % of the dose, of paeoniflorin after intravenous or oral administrations at a dose of 0·5 mg kg^{-1} to rats.

Discussion

Paeony root is one of the most important crude drugs in Kampo medicine, and contains paeoniflorin as an effective constituent (Nishizawa et al 1979). Xu et al (1990) have reported the low bioavailability of paeoniflorin after oral administration at a large dose of 250 mg kg^{-1} in rabbits. The dose-dependent pharmacokinetics of paeoniflorin after intravenous administration at the doses of 20, 40 and 100 mg kg^{-1} in rats has also been reported (Ishida et al 1990). In that report, Vd_{ss}, CL_{tot} and CL_R (renal clearance) were significantly decreased at the maximal dose, and this may be caused by the decreasing effect of paeoniflorin on renal plasma flow and saturable transport from plasma into liver. Kampo medicine is a decoction of several herbs and is often used for the treatment of chronic diseases in Japan and China. Kampo drugs containing Paeony root include a few percent of paeoniflorin at most. Furthermore, most Kampo drugs are administered orally to man and the plasma paeoniflorin concentration after oral dosing is considered to be very low. However, there is no report on the pharmacokinetics of paeoniflorin at the usual oral dose. Thus, we have established a highly sensitive method for determination of paeoniflorin by enzyme immunoassay (Kanaoka et al 1984) and calculated the bioavailability of paeoniflorin after usual oral dosing in rats to evaluate the significance of paeoniflorin in the pharmacological effects of Paeony root.

The EIA for paeoniflorin employed here had a high sensitivity with a detection limit of approximately 0·1 ng mL^{-1} and specificity because the anti-paeoniflorin antiserum hardly cross-reacted with other structure-related constituents, albiflorin and benzoylpaeoniflorin, as previously reported (Kanaoka et al 1984). The urinary concentration values of paeoniflorin after intravenous dosing determined by EIA were in good agreement with those by HPLC (data not shown). Accordingly, the results obtained in this report provide the pharmacokinetics of unchanged paeoniflorin.

The time course of plasma paeoniflorin concentration after intravenous administration was well simulated by the two-compartment model at each dose. Since constant pharmacokinetic parameters were observed in the range 0·5–5·0 mg kg^{-1}, the plasma-concentration profiles seemed to be linear. The concentration of paeoniflorin after intravenous dosing rapidly decreased because of a smaller elimination half-life. The Vd_{ss} value derived from A, α, B and β was very close to that reported previously (Ishida et al 1990). The pharmacokinetic analysis of the oral dosing was performed using model-independent methods because the plasma concentration data did not fit the compartment model. The plasma concentration of paeoniflorin after usual oral dosing was very low, with C_{max} values of 9·8, 30·7 and 101·5 ng mL^{-1} at 0·5, 2·0 and 5·0 mg kg^{-1}, respectively, when compared with that after intravenous dosing. After oral dosing, the t_{max} was observed within 10 min and the AUC values increased proportionally to the administered dose. Paeoniflorin was suggested to be rapidly absorbed from the gastrointestinal tract with linear pharmacokinetics at the usual dose. A large variability in $t_{\frac{1}{2}}$ values was observed due to the small peak around 60 to 180 min after dosing. This might be caused by the enterohepatic circulation. In fact 1·3% of paeoniflorin after oral dosing was excreted in bile, suggesting enterohepatic cycling may have occurred. The systemic bioavailability in rats after oral dosing was extremely low as reported in rabbits (Xu et al 1990). The bioavailability of a drug is generally defined by the absorbed fraction and first-pass effect. We therefore studied the excretion of paeoniflorin to confirm the low bioavailability.

Approximately 50% of the dose was excreted in urine after intravenous administration, little being excreted in the faeces and bile. This indicates that paeoniflorin absorbed is mostly excreted in the urine. CL_R and biliary clearance (CL_B) are calculated from the total excretory amounts in urine and bile divided by AUC. The total CL_R and CL_B values were lower than the CL_{tot} value. This suggested that paeoniflorin was metabolized in other organs, including liver, which had a major role in the drug elimination. These observations are in agreement with the previous report (Ishida et al 1990). After oral administration, the excretion of paeoniflorin in urine, faeces and bile was low. This coincided with the low F value from AUCs after intravenous and oral dosing. As described above, F is characterized by the fraction of absorption and first-pass effect. The low F value, about 3%, obtained here might be caused by poor absorption from the gastrointestinal tract, metabolism or decomposition in the intestine by bacterial microflora, or the first-pass metabolism by gut wall or liver.

Hattori et al (1985) have reported that paeoniflorin was metabolized to paeonimetaboline I by human intestinal microflora in-vitro. The anti-cholinergic actions of paeoniflorin in-vivo and in-vitro were compared in rats and its inhibitory effect on the carbachol-induced contractile responses of isolated rat proximal colon was found only in-vivo (Kobayashi et al 1990). These observations suggest that paeoniflorin is not the active constituent. Our findings of low bioavailability support this view and it seems difficult to ascribe the therapeutic effects of Paeony root to paeoniflorin. The first-pass metabolism and pharmacokinetics of paeoniflorin in germ-free rats to study the influence of bacterial microflora on the absorption are under investigation and will be reported.

In conclusion, paeoniflorin absorbed is excreted mainly in urine, has low bioavailability (3%), and the metabolites of paeoniflorin may be involved in the pharmacological action of Paeony root.

References

Harada, M. (1969) Pharmacological studies on herb paeony root. IV. Analysis of therapeutic effects of paeony-and licorice-containing frequent prescriptions in Chinese medicine and comparison with effects of experimental pharmacological tests. Yakugaku Zasshi 89: 899–908

Hattori, M., Yue-Zhong Shu, Shimizu, M., Hayashi, T., Morita, N., Kobashi, K., Guo-Jun Xu, Namba, T. (1985) Metabolism of paeoniflorin and related compounds by human intestinal bacteria. Chem. Pharm. Bull. 33: 3838–3846

Ishida, S., Sakiya, Y., Ichikawa, T. (1990) Dose-dependent pharmacokinetics of paeoniflorin in rats. Xenobiot. Metabol. Dispos. 5; 771–782

Kanaoka, M., Yano, S., Kato, H., Nakanishi, K., Yoshizaki, M. (1984) Studies on the enzyme immunoassay of bio-active constituents contained in oriental medicinal drugs. III. Enzyme immunoassay of paeoniflorin, a constituent of Chinese paeony root. Chem. Pharm. Bull. 32: 1461–1466

Kobayashi, M., Ueda, C., Aoki, S., Tajima, K., Tanaka, N., Yamahara, J. (1990) Anti-cholinergic action of paeony root and its active constituents. Yakugaku Zasshi 110: 964–968

Nishizawa, M., Yamagishi, T., Horikoshi, T., Homma, N. (1979) Chemical studies on Paeoniae Radix (part I); quantitative determination of glucosides in Paeoniae Radix. Shoyakugaku Zasshi 33: 65–71

Sugishita, E., Amagaya, S., Ogihara, Y. (1984) Studies on the combination of Glycyrrhizae Radix in shakuyakukanzoto. J. Pharmocobiodyn. 7: 427–435

Takagi, K., Harada, M. (1969a) Pharmacological studies on herb paeony root.: I. Central effects of paeoniflorin and combined effects with licorice component FM 100 Yakugaku Zasshi 89: 879–886

Takagi, K., Harada, M. (1969b) Pharmacological studies on herb paeony root. II. Anti-inflammatory effect, inhibitory effect on gastric juice secretion, preventive effect on stress ulcer, antidiuretic effect of paeoniflorin and combined effects with licorice component FM 100. Yakugaku Zasshi 89: 887–892

Takagi, K., Harada, M. (1969c) Pharmacological studies on herb paeony root. III. Effects of paeoniflorin on circulatory and respiratory systems and isolated organs. Yakugaku Zasshi 89: 893–898

Xu, S., Chen, C., Chen, G. (1990) The pharmacokinetics of paeoniflorin. Eur. J. Pharmacol. 183: 2390

Yamahara, J., Yamada, T., Kimura, H., Sawada, T., Fujimura, H. (1982) Biologically active principles of crude drugs: anti-allergic principles of "shoseiryu-to": I. Effect on delayed-type allergy reaction. Yakugaku Zasshi 102: 881–886