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In-vivo Assessment of Extrahepatic Metabolism of Paeoniflorin in Rats: Relevance to Intestinal Floral Metabolism

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Abstract

The extraction ratios of paeoniflorin in gut wall (E_G), liver (E_H) and lung (E_L) were assessed by comparing AUCs after various routes of its administration to estimate the first-pass effects and the metabolism by intestinal flora.

Pulmonary extraction ratio of paeoniflorin was assessed by comparing AUCs calculated from venous and arterial plasma concentrations after its intravenous administration ($0.5\,mg\,kg^{-1}$). The mean pulmonary extraction ratio was estimated to be 0·06. The hepatic extraction ratio (E_H was assessed by comparing AUCs after intraportal and intravenous administrations (0·5 and $5\,mg\,kg^{-1}$). The plasma concentration profiles of paeoniflorin after intraportal administration were very close to those after intravenous administration, suggesting a negligible hepatic extraction ratio of paeoniflorin. The AUC value after intraperitoneal administration ($0.5\,mg\,kg^{-1}$) was greater than that after intraportal or intravenous administration. This finding suggests that paeoniflorin is not metabolized in the gut wall. The transference of paeoniflorin from the serosal side to the mucosal side was evaluated by the in-vitro everted sac method. The low intestinal permeability (19·4% at 60 min) was demonstrated by the comparison with phenobarbital (63·1% at 60 min).

We conclude that paeoniflorin is not metabolized by gut wall, liver and lung, its poor absorption from the intestine results in extremely low bioavailability and the unabsorbed fraction of paeoniflorin is degraded by the intestinal flora.

Paeoniflorin which is contained in paeony root (Japanese name: Shakuyaku) has several pharmacological actions, such as analgesic (Takagi & Harada 1969a; Sugishita et al 1984), muscle relaxant (Takagi & Harada 1969b) and anti-inflammatory effects (Takagi & Harada 1969c). Paeony root is prescribed in a great number of Kampo medicines (oriental herbal medicine) including Toki-syakuyaku-san or Syakuyaku-kanzo-to for the treatment of abdominal pain or syndromes such as stiffness of abdominal muscles. Its therapeutic effects are mostly explained by the pharmacological actions of paeoniflorin (Harada 1969). Previously (Takeda et al 1995), we have reported the absorption and excretion of paeoniflorin after its intravenous or oral administration in rats. Approximately 50% of the dose is excreted in urine after intravenous administration, little being excreted in bile, suggesting that paeoniflorin is metabolized in the body and the rest excreted mainly in urine. On the contrary, the bioavailability, calculated by the area under the plasma concentration-time curves (AUC), after oral administration is only 3–4%, and extremely low faecal excretion is observed. These findings indicate that the low bioavailability is induced by first-pass effects in the gut wall and liver; another possibility being degradation resulting from bacterial hydrolysis which has the opportunity to occur due to poor intestinal absorption, as occurs with glycyrrhizin which is poorly absorbed (Yamamura et al 1995). Hattori et al (1985) have reported the production of several metabolites after incubation of paeoniflorin with human faecal suspension. Furthermore, Kobayashi et al (1990) have reported that the inhibitory effect of paeoniflorin on the carbachol-induced contraction of rat isolated proximal colon was found only in-vivo. These results suggest that paeoniflorin may not be the active constituent in paeony root. Thus, it is very important to establish whether paeoniflorin is poorly absorbed or undergoes the first-pass metabolism extensively to elucidate its pharmacological effects.

In this paper, we compare the AUCs after various routes of administration of paeoniflorin with those after intravenous or oral administration, previously reported (Takeda et al 1995), and evaluated the extraction ratios in the gut wall (E_G), liver (E_H), and lung (E_L) to estimate the contribution of metabolism by intestinal flora.

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Materials and Methods

Materials

Paeoniflorin was supplied by the Technical Department in our Laboratories. β-Galactosidase was purchased from Boehringer Mannheim (Mannheim, Germany). Bovine serum albumin and 7-β-D-galactopyranosyloxy-4-methyl coumarin were purchased from Sigma (St. Louis, USA), physiological saline from Fusou Pharmaceutical Industry (Osaka, Japan), Freund's complete adjuvant from Difco and goat anti-rabbit IgG (Marcella 10) for enzyme-immunoassay (EIA) from Dainippon Pharmaceutical Industry (Osaka, Japan). All other reagents were of special or high performance liquid chromatography (HPLC) analytical grade obtained from Wako Pure Chemical Industry (Osaka, Japan).

Animals, treatment and sampling for evaluation of systemic availability

Male Sprague–Dawley rats (7–8 weeks old, Charles River, Japan) were used. Animals were fed standard laboratory chow with free access to water and fasted overnight before the experiment after accommodation for a week. Five to 7 rats were used in each study.

Paeoniflorin was dissolved in physiological saline and given intraportally (0·5 and 5 mg kg^{-1}) or intraperitoneally (0·5 mg kg^{-1}). The dose of 0·5 mg kg^{-1} was administered intravenously for the calculation of the pulmonary extraction ratio. A previous study (Takeda et al 1995), on intravenous and oral administration, was used as a reference for the estimation of the organ extraction ratio.

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 the concentration of 100 int. units mL^{-1} solution. In the case of intraportal administration, the polyethylene tubing was cannulated to the left femoral artery and portal vein via mesenteric vein to inject the drug solution. After recovery from ether anaesthesia, drugs were given through the venous cannula or by intraperitoneal injection. Arterial blood samples (100 μL) were taken at a fixed time after administration. For the calculation of pulmonary extraction ratio, blood samples (100 μL) were simultaneously taken through the arterial and venous cannula after intravenous administration of drug into the caudal vein. The rats were given the same volume of saline to replace the lost blood. Plasma samples were immediately separated by the centrifugation of the blood and stored at −20°C until analysis. Rats were allowed to have free access to water during the experiment.

Absorption by an in-vitro everted sac method

The procedure was followed as described by Barr & Riegelman (1970). The overnight-fasted rats (Sprague–Dawley, 370–408 g) were killed by decapitation and the jejunum, 30-cm length from Traitz ligament, was removed and everted with a stainless steel prove. The everted sacs, reduced to about 10 cm length, were filled with 1·0 mL of Krebs–Henseleit bicarbonate solution and treated with 95% O_2–5% CO_2 gas prior to experiment. These everted sacs were incubated in 50-mL glass tubes containing 30 mL of Krebs–Henseleit bicarbonate solution prepared with 100 μg mL^{-1} of paeoniflorin or 300 μg mL^{-1} of phenobarbital at 37°C for 60 min and 95% O_2–5% CO_2 gas supplied during experiment. The serosal and mucosal fluids were sampled at each time interval and used for drug analysis by HPLC. At the end of experiment, the jejunal tissue was homogenized with 4 vol. of buffer solution and centrifuged. The concentration of drug remaining in tissues was also determined by HPLC.

Determination of paeoniflorin

The determination of paeoniflorin in rat plasma was carried out by EIA as reported previously (Takeda et al 1995). The samples, serosal and mucosal fluids or upper layer of jejunal tissue homogenates, were determined by HPLC after filtration through a membrane filter (Millipore). The analysis was performed by an LC-6A pump, a C-R5A recorder, SPD-6A UV detector and a CTO-6A column oven, all from Shimadzu (Kyoto, Japan). The chromatographic determinations for paeoniflorin and phenobarbital were carried out by a column packed with a STR ODS-II (15 × 4·6 mm i.d., Shimazdu Techno Research Kyoto, Japan) or a GFF-S-5-80 (25 × 4·6 mm i.d., Pinkerton ISRP) at 40°C and at a flow rate of 1·2 or 0·6 mL min^{-1}. The mobile phase was acetonitrile-water (12:88, v/v) for paeoniflorin and acetonitrile–0·1 M phosphate buffer (20:80, v/v) for phenobarbital and the chromatograms were monitored at 232 or 254 nm wavelength, respectively.

Pharmacokinetic analysis

AUCs after various routes of administration were calculated by 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 parameters for systemic availability were determined as follows:

$$F = (AUC_{p.o.})/(AUC - i.v.) \quad (1)$$

$$F_L = (AUC_{arterial})/(AUC_{venous}) \quad (2)$$

$$F_H = (AUC_{h.p.v.})/(AUC_{i.v.}) \quad (3)$$

$$f_a F_G = (AUC_{p.o.})/(AUC_{h.p.v.}) \quad (4)$$

Where F is the oral bioavailability, f_a is the fraction of the absorbed dose into gut wall, and F_L, F_G and F_H are the fractions of the dose escaping metabolism by the lung, gut wall and liver, respectively. The subscripts, p.o., i.v. and h.p.v. refer to drug administration by the oral, intravenous, and hepatic portal venous routes, respectively, and 'arterial' and 'venous' indicate the sampling site of blood. Organ extraction ratio (E) was calculated by equation, $E = 1 - F$. Intraperitoneal route was used to estimate the contribution of microfloral metabolism. The data represented mean ± s.e.m.

Results

Pulmonary extraction ratio

The concentrations of paeoniflorin (0·5 mg kg^{-1}) in the venous and arterial plasma samples after its intravenous administration were determined (multiple sites of sampling method) and plasma levels at each sampling site are shown in Fig. 1. Plasma concentrations declined bi-exponentially after dosing, and their profiles in the artery and vein showed similar results. Since AUCs calculated by the arterial and venous plasma concentrations were 20·29 ± 0·99 μg min mL^{-1} and 22·00 ± 1·12 μg min mL^{-1}, respectively, the pulmonary extraction ratio ($E_L = 1 - F_L$) was estimated to be 0·06 ± 0·09 (n = 5).

Systemic availability after oral, intraportal and intraperitoneal administration

Plasma concentration profiles of paeoniflorin in rats after intraportal administration (0·5 and 5 mg kg^{-1}), and those after intravenous dosing for retrospective comparison, are shown in Fig. 2. Plasma concentrations declined bi-exponentially after both routes of administration and continued decreasing for 20 min. The elimination rates of paeoniflorin in plasma at terminal phase after intravenous dose at 5 mg kg^{-1} and 0·5 mg kg^{-1} were slightly faster or slower than those after intraportal administration, but the difference was small. Plasma

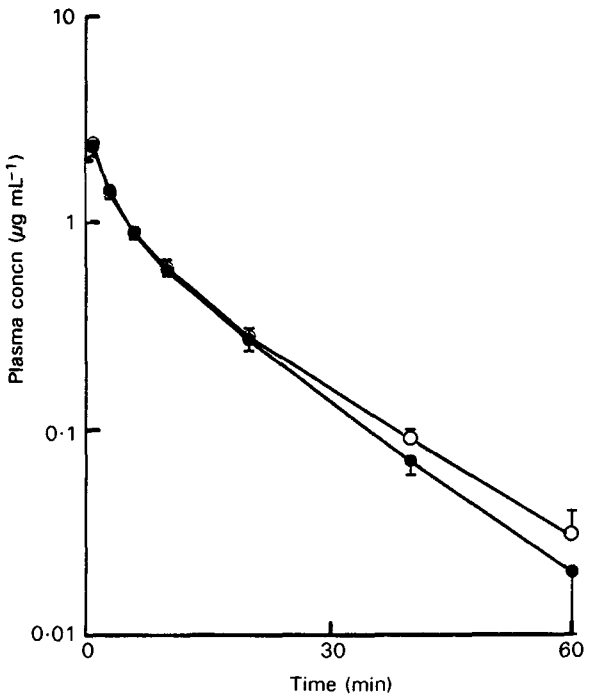

FIG. 1. Arterial (●) and venous (○) plasma concentration profiles of paeoniflorin after intravenous administration at a dose of 0·5 mg kg^{-1} in rats. The samples were simultaneously obtained at each time and determined by multiple sites of sampling method. Each value represents mean ± s.e.m. of 5 rats.

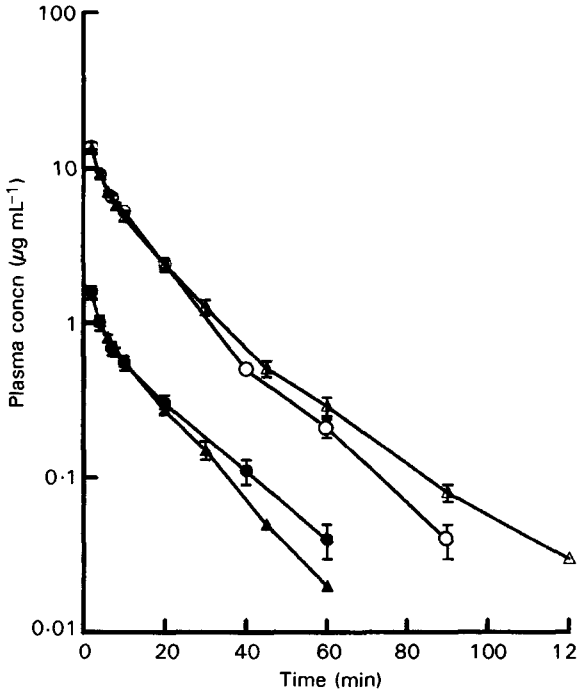

FIG. 2. Arterial plasma concentration profiles of paeoniflorin after intraportal administration at a dose of 0·5 (●) and 5 (○) mg kg^{-1} in rats, together with profiles after intravenous administration (▲, △) as previously reported (Takeda et al 1995). Each value represents mean ± s.e.m. of 6 rats.

concentration after intraperitoneal administration (0·5 mg kg^{-1}) in rats is shown in Fig. 3. Plasma paeoniflorin level reached maximum, 0·68 μg mL^{-1}, at 10 min after injection and thereafter decreased rapidly.

The systemic availabilities calculated by the AUC values after various routes of administration are shown in Table 1. The oral bioavailability was 0·03–0·04, as reported previously (Takeda et al 1995), whereas in this study, systemic availabilities (F_H) after intraportal doses were calculated to be 0·997 and 1·11, respectively, which were comparable to corresponding intravenous doses. The systemic availability after intraperitoneal administration was estimated to be 1·42, i.e. greater than that observed following intravenous administration.

Transference of paeoniflorin in in-vitro everted sacs

The transference was evaluated by calculating the ratio of serosal level to mucosal level. The ratio of paeoniflorin transferred was 3·5 and 19·4% at 15 and 60 min, respectively, while that of phenobarbital was 8·3 and 63·1%, respectively (Table 2). Phenobarbital was transferred at a level three times higher than paeoniflorin and accumulated in tissues, which suggested that paeoniflorin poorly penetrates intestinal mucosa.

Discussion

Attempts at the measurements of the relative contribution of the intestinal mucosa, liver and lung in first-pass metabolism have been made by comparing AUCs after various routes of

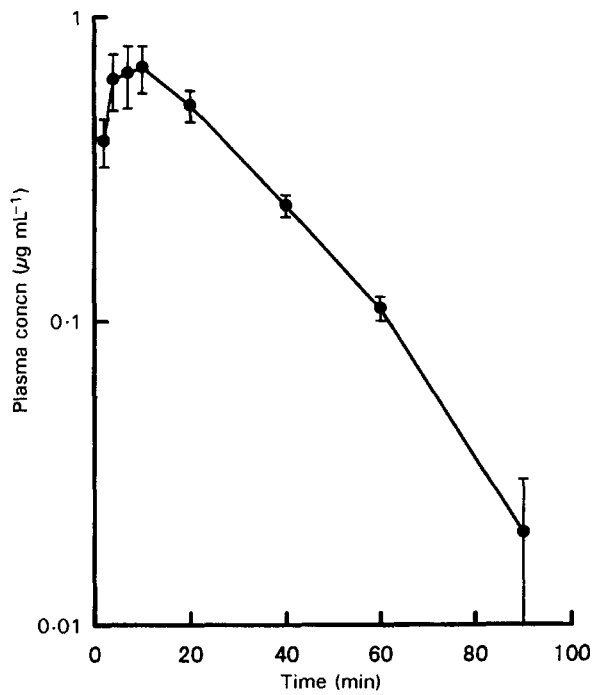

FIG. 3. Arterial plasma concentration profile of paeoniflorin after intraperitoneal administration at a dose of 0·5 mg kg^{-1} in rats. Each value represents mean ± s.e.m. of 6 rats.

Table 1. AUC values and systemic availabilities of paeoniflorin after administration by intravenous, oral, intraportal and intraperitoneal routes.

Route of administration	Dose ($mg\,kg^{-1}$)	AUC[a] ($\mu g\,min^{-1}\,mL^{-1}$)	Systemic availability[b]
Intravenous[c]	0·5	17·2 ± 0·9	1
	5	156·2 ± 8·2	1
Oral[c]	0·5	0·56 ± 0·10	0·033[1] 0·029[2]
	5	5·96 ± 1·24	0·038[1] 0·038[2]
Intraportal	0·5	19·1 ± 2·6	1·11[3]
	5	155·7 ± 7·6	0·997[3]
Intraperitoneal	0·5	24·5 ± 2·9	1·42[4]

[a]From time zero to infinity, mean ± s.e.m. of 5 to 7 rats. [b]Mean values were used for calculation. [1]$F = (AUC_{p.o.})/(AUC_{i.v.})$. [2]$f_aF_G = (AUC_{p.o.})/(AUC_{h.p.v.})$. [3]$F_H = (AUC_{h.p.v.})/(AUC_{i.v.})$. [4]$F_{ip} = (AUC_{i.p.})/(AUC_{i.v.})$. [c]Previous data (see Takeda et al 1995).

Table 2. Transferance of paeoniflorin and phenobarbital by in-vitro everted sacs of rat intestine.

	Time (min)	Mucosa ($\mu g\,mL^{-1}$)	Tissue (μg (g wet tissue)$^{-1}$)	T/M ratio (%)	Serosa ($\mu g\,mL^{-1}$)	S/M ratio (%)
Paeoniflorin	15	87·3 ± 8·5			3·0 ± 0·9	3·5 ± 1·1
	30	87·7 ± 10·2			7·3 ± 1·4	8·4 ± 1·5
	45	87·9 ± 6·3			12·5 ± 2·4	14·2 ± 2·2
	60	89·2 ± 4·7	15·6 ± 3·5	17·4 ± 3·1	17·3 ± 3·3	19·4 ± 3·7
Phenobarbital	15	280·3 ± 3·2			23·4 ± 7·2	8·3 ± 2·6
	30	282·1 ± 9·2			89·9 ± 15·9	32·1 ± 6·5
	45	273·8 ± 8·5			128·1 ± 12·6	47·1 ± 4·5
	60	272·4 ± 9·4	406·8 ± 118·6	148·4 ± 41·1	171·5 ± 26·2	63·1 ± 9·9

Each value represents mean ± s.e.m.(n = 5).

administration because of simplicity and convenience (Cassidy & Houston 1980; Hirai et al 1981; Minchin & Ilett 1982; Klippert et al 1983; Iwaki et al 1990). Two distinct methods, multiple sites of input and multiple sites of sampling, for assessing the extrahepatic metabolism have been demonstrated (Mistry & Houston 1985). In the present study, pulmonary extraction ratio was estimated by the multiple sites of sampling method, because it allowed determination of the specific organ extraction values, with minimum inter-individual variance of animals. The results obtained indicate that pulmonary extraction of paeoniflorin is negligible.

The hepatic extraction ratio ($E_H = 1 - F_H$) was assessed by comparing AUCs after intraportal and intravenous doses (Table 2). The initial disposition phase for both routes of administration was nearly the same and plasma concentration increased proportionally with increasing dose, which suggested no saturable hepatic tissue binding or another dose-related saturation including no saturable serum protein binding ranging from 1 to 10 $\mu g\,mL^{-1}$ (data not shown). Accordingly, we conclude that hepatic extraction is negligible. This is supported by the fact that paeoniflorin is not degraded when it is incubated with whole homogenates of rat liver (data not shown).

The AUC value after intraperitoneal administration was greater than that after intraportal or intravenous dosing, as shown in Table 2. Gillette & Pang (1977) have reported that intraperitoneally administered drug is absorbed through the gut wall or via the mesentery, and that there is the possibility that the drug is metabolized by intestinal mucosal enzyme in gut wall during absorption. Thus, the AUC value by the intraperitoneal route will be less than that by the portal route, if paeoniflorin is metabolized by intestinal mucosal enzyme after its intraperitoneal administration. From the finding that the AUC value after intraperitoneal administration is not smaller than that after intraportal administration, paeoniflorin seems not to be metabolized in gut wall. The reason for the greater AUC value after intraperitoneal administration is, perhaps, based on the inter-individual variance of animals by the separated experimental design or over-estimation of AUC value obtained by the extrapolation to infinity.

The above discussion leads to the suggestion that paeoniflorin is hardly metabolized in gut wall, liver and lung. As paeoniflorin is relatively stable in artificial gastric juice (data not shown), most of the orally administered dose reaches the small intestine without any degradation. Ishida et al (1990) have examined the intestinal absorption of paeoniflorin by the in-situ recirculating perfusion technique in rats and they observed the slower absorption rate constant (k_a value of 0·00177 min^{-1}; for comparison, k_a value of 0·0112 min^{-1} with salicylic acid reported by Karino et al 1982). The transference from the serosal side to the mucosal side was assessed by the in-vitro everted sac method in our study. The low intestinal permeability of paeoniflorin was obviously demonstrated as shown in Table 3 and this agreed well with the results of in-situ perfusion method by Ishida et al (1990). Metabolism in the intestine consists of an enzymatic process in gut wall and metabolism by bacterial flora. The estimated f_aF_G value was very close to the oral bioavailability (Table 2) and it was dramatically enhanced by intraperitoneal administration. Furthermore, Hattori et al (1985) have examined the metabolism of paeoniflorin by incubating with human intestinal bacteria in-

vitro and they found that paeoniflorin is transformed to several metabolites such as paeonimetaboline I, II and III. Shu et al (1987) have also investigated the structures of 7*S*- and 7*R*-paeonimetabolines I and II formed by *Bacteroides fragilis* and *Lactobacillus brevis;*. These findings suggest that the extrahepatic metabolism, i.e. the gut floral metabolism of paeoniflorin, is due to its poor absorption from the intestine. On the other hand, when paeoniflorin was intravenously administered, only half of the dose was recovered in the urine with slight excretion in bile (Takeda et al 1995). Thus, the organ in which paeoniflorin is metabolized still remains unclear and is expected to be clarified.

In conclusion, paeoniflorin is not metabolized by gut wall, liver and lung, its poor absorption from intestine results in extremely low oral bioavailability and the unabsorbed fraction of paeoniflorin is degraded by the intestinal flora.

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