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Influence of co-administered antibiotics on the pharmacokinetic fate in rats of paeoniflorin and its active metabolite paeonimetabolin-I from Shaoyao-Gancao-tang

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Abstract

The effects of orally co-administered antibiotics on the pharmacokinetics of paeoniflorin (PF) and paeonimetabolin-I (PM-I), a bioactive metabolite derived from PF by intestinal bacteria, from the traditional Chinese formulation, Shaoyao-Gancao-tang (SGT), were investigated in rats to clarify the effect of administering SGT together with some synthetic drugs. Co-administration of the antibiotics amoxicillin and metronidazole (AMPC-MET) significantly increased the area under the plasma concentration versus time curve (AUC) of PF, whereas it markedly decreased that of PM-I, to 2.6% of the normal AUC by administration of a single dose, and less than 1% by a 3-day pretreatment. Similar effects were observed using the combination of ofloxacin with SGT. The PF-metabolizing activity of intestinal bacteria was reduced to 16% and 33% of normal levels by treatment with AMPC-MET and ofloxacin, respectively, which caused alterations of that degree in the extent of absorption of PF and PM-I, but did not affect their rate of absorption or elimination. The present study suggests that it may not be appropriate to use SGT simultaneously with antibiotics such as AMPC-MET or ofloxacin, and also reveals the important role of intestinal bacteria in the pharmacokinetics of the active components of this traditional Chinese formulation.

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Introduction

In the Japanese medical care system, traditional Chinese formulations (called Kampo-medicines in Japan) are often used together with synthetic drugs. It has been reported that concomitant use of antibiotics and Kampo-medicines occurred in 7% of the cases of Kampo-prescriptions in Japan (Ishihara et al 2002). To ensure the safety and clinical efficacy of such simultaneous use of drugs, it is important to determine the drug–drug interactions during combination therapy. Some studies on the influence of traditional Chinese formulations on the pharmacokinetics of synthetic drugs have been reported (Hasegawa et al 1995; Nishimura et al 1998). In the course of our biopharmaceutical studies on traditional Chinese formulations, we previously examined the influence of the co-administration of antibiotics on the pharmacokinetics of glycyrrhetic acid, an active metabolite of glycyrrhizin from a traditional Chinese formulation, Shaoyao-Gancao-tang (SGT; Shakuyaku-Kanzo-To in Japanese) (He et al 2001).

SGT, composed of Shaoyao (Paeoniae Radix) and Gancao (Glycyrrhizae Radix), is widely prescribed for the treatment of abdominal pain (Katsura 1995), and sometimes used in combination with two antibiotics, amoxicillin (AMPC) and metronidazole (MET), to eradicate *Helicobacter pylori* for the treatment of peptic ulcer. SGT is also used for relieving colic pain in urolithiasis and cholelithiasis (Yamaguchi et al 1982), and sometimes used together with ofloxacin, an antibiotic popularly prescribed for therapy of urinary infection.

Paeoniflorin (PF) is one of the important glucosidic ingredients of SGT (Sugishita et al 1984). It is known that orally taken PF is biotransformed into a bioactive metabolite, paeonimetabolin I (PM-I) by intestinal bacteria and then absorbed into the blood (Meselhy & Hattori 2000 and references therein). Hence, the analgesic and

antispasmodic effects of SGT are owing to PM-I rather than PF itself (Abdel-Hafez et al 1999).

In this study, we investigated the influence of the co-administration in rats of two antibiotics, AMPC-MET and ofloxacin, on the pharmacokinetics of PF and PM-I from SGT. The mechanism of the pharmacokinetic alterations was elucidated by examining the PF-metabolizing activity of the intestinal bacteria in rat faeces, using our newly developed high-performance liquid chromatography (HPLC) assay (He et al 2002).

Materials and Methods

Materials

The freeze-dried extract of SGT (4.00 ± 0.06 g, a common daily dose for adult humans, containing PF at 39.7 ± 1.4 mg (g extract)$^{-1}$) used in the animal experiments was prepared as previously reported (He et al 2001). The voucher specimens of Shaoyao (Paeoniae Radix produced in Japan) and Gancao (Glycyrrhizae Radix imported from China; Dongbei-Gancao in Chinese) are deposited in the Department of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. To ensure the homogeneity of the formulation and to prepare batches of constant formulation, the HPLC profile of SGT was analysed as previously reported (He et al 2001).

PF and normal rabbit serum were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). AMPC, ofloxacin, β-galactosidase (from *Escherichia coli*) and goat anti-rabbit IgG were purchased from Sigma Chemical Co. (St Louis, MO, USA). MET was purchased from Aldrich Chem. Co., and phenylmercaptan (thiophenol) and 4-methylumbelliferyl β-D-galactoside from Nacalai Tesque, Inc. (Kyoto, Japan). All of the other chemicals and solvents used were of analytical and/or HPLC grade.

Animals and blood sampling

Male Wistar rats (8 weeks old, approx. 250 g) were purchased from Japan SLC Inc., (Hamamatsu, Japan) and maintained on a 12-h light–dark cycle at 21–24 °C in the Laboratory for Animal Experiments, Toyama Medical and Pharmaceutical University. Blood samples (approx. 5 mL for each) were collected (destructive sampling, $n = 6$ at each time point) from the inferior vena cava of different rats anaesthetized with ether at 5, 10, 20 min, 1, 2, 4, 6, 9, 12 and 24 h after the last drug administration. The samples were immediately centrifuged at 1100 *g* for 10 min and the plasma was stored at −20 °C until analysis. The rats were given free access to water and standard laboratory chow before experiments.

All animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Single simultaneous administration of antibiotics with SGT (single regimen)

Rats were fasted overnight with free access to water before treatment. Antibiotics at 10 times the usual single dose given to adult humans as AMPC-MET (83.3 mg kg^{-1} AMPC, 41.7 mg kg^{-1} MET) and ofloxacin (50 mg kg^{-1}) were each orally administered together with SGT (645 mg kg^{-1}, equivalent to 25 mg kg^{-1} PF, 10 times the daily single dose given to adult humans) at the same time. Control groups were given tap water together with SGT.

Three-day pre-administration of antibiotics before SGT (multiple regimen)

Antibiotics (at the same dose as used in the single regimen) were given twice a day for 3 days before SGT administration. On the fourth day, the antibiotics were administered together with SGT as in the single regimen.

Determination of PF and PM-I in plasma

The plasma PF (Kanaoka et al 1984) and PM-I (Hattori et al 1996) concentrations were determined by using the respective enzyme immunoassays (EIAs) as previously reported. Briefly, β-galactosidase-labelled antigen was reacted with antiserum in the presence of plasma samples. The separation of bound and free fractions was performed by the double antibody method using a goat antiserum to rabbit IgG. 4-Methylumbelliferyl β-D-galactoside was used as the substrate for the fluorometric assay of β-galactosidase activity. The respective antisera for PF and PM-I for the EIAs were generously provided by Professor M. Hattori, Institute of Natural Medicine of Toyama Medical and Pharmaceutical University.

Determination of PF-metabolizing activity of intestinal bacteria in rat faeces

The PF-metabolizing activity of intestinal bacteria in rat faeces was determined using our previously developed HPLC method (He et al 2002). This method is based on the assumption that the rate of biotransforming PF into PM-I by the intestinal bacteria is equivalent to that of biotransforming PF into 8-phenylthio-paeonimetabolin I (PT-PM-I; Figure 1). Briefly, fresh rat faeces (0.5 g) collected after a 3-day pre-administration of the antibiotics was suspended in Na-phosphate buffer (pH 7.2, 2.5 mL). The faecal suspension was incubated with PF (2.0 mM) in the presence of phenylmercaptan (5.0 mM) at 37 °C for 20 min. The reaction mixture was extracted with MeOH and the product PT-PM-I in the extract was detected by HPLC at 255 nm. The detection limit and recovery rate were 0.05 μg mL^{-1} and 94%, respectively. The HPLC system comprised a Jasco DG-980-50 three-line degasser, a Jasco PU-980 pump, a Jasco LG-1580-02 ternary

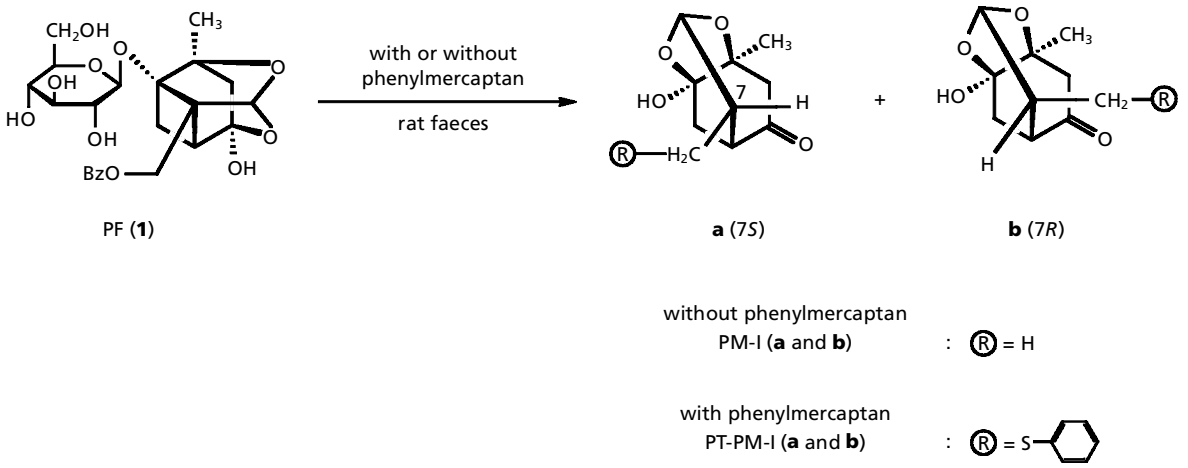

Figure 1 Biotransformation of paeoniflorin (PF) into paeonimetabolin-I (PM-I) and phenylthio-PM-I (PT-PM-I) by intestinal bacteria in rat faeces without or with phenylmercaptan. The rate of metabolism of PF into PM-I in rat faeces was estimated from the rate of formation of PT-PM-I from PF. The PT-PM-I formed in the reaction mixture of the faecal suspension with PF (2.0 mM) and phenylmercaptan (5.0 mM) was extracted with MeOH and measured using HPLC at 255 nm.

gradient unit, a Jasco MD-910 multiwavelength detector (Tokyo), a Rheodyne 7725 injector fitted with a 100-μL loop (USA) with a Jasco-Borwin chromatography data treatment system. The column was a YMC-Pack ODS-A-303 column (250 × 4.6 mm i.d., S-5 μm; YMC, Inc., USA) with a Develosil packed guard column (ODS-MG-5; Nomura Chemical Co., Ltd, Japan) housed in a Tosoh CO-8020 oven (Tokyo) set at 40 °C. The gradient mobile phase was H_2O/CH_3CN from 60:40 to 0:100 in 15 min, with a flow rate of 1.0 mL min^{-1}. The rate of metabolism of PF into PM-I was estimated by measuring the rate of formation of PT-PM-I from PF.

Data and statistical analysis

The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were determined directly from the actual drug concentrations in the plasma. The elimination rate constant (K) was estimated by linear regression analysis on the terminal portion of the semi-logarithmic plot of plasma concentration versus time. The absorption rate constant (K_a) was determined by the method of residuals (Gibaldi & Perrier 1982). The half-lives of elimination ($t_{\frac{1}{2}K}$) and absorption ($t_{\frac{1}{2}Ka}$) were calculated from $\ln 2/t_{\frac{1}{2}K}$ and $\ln 2/t_{\frac{1}{2}Ka}$, respectively. The time delay between administration and the start of absorption (t_{lag}) was estimated by the method of residuals (Rowland & Tozer 1995) or by the technique of Csizmadia & Endrenyi (1998). The resampling method (Mager & Goller 1998) was applied for the estimation of the mean values of the above pharmacokinetic parameters and their respective standard errors. The area under the mean concentration versus time curve from 0 to 24 h ($AUC_{0-24\,h}$) was calculated by using the trapezoidal rule. The variance of $AUC_{0-24\,h}$ was estimated by Bailer's method (Bailer 1988). $AUC_{0-24\,h}$ was evaluated statistically by the Bailer–Satterthwaite t-test (Nedelman et al 1995). Other pharmacokinetic parameters were statistically evaluated by the usual unpaired two-tailed Student's t-test. Differences were considered statistically significant at $P < 0.05$.

Results

Plasma concentration profile of PF and PM-I

Figure 2 shows the mean plasma concentration–time data of PF and PM-I, when SGT was given orally alone or with the single dose of AMPC-MET (single regimen). Significant decreases in the plasma PM-I concentration during the time interval 2–12 h, as well as delayed occurrence of the peak concentration, were observed in the group given SGT together with AMPC-MET. A significant increase in the plasma PF concentration during the time interval 10 min–12 h was observed.

Figure 3 shows similar but greater alterations in plasma concentration–time data of PF and PM-I, when SGT was given in the absence or presence of a single dose of AMPC-MET in rats with or without the 3-day antibiotic pretreatment (multiple regimen).

Figure 4 shows the mean plasma concentration–time data of PF and PM-I after SGT was administered with a single dose of ofloxacin (single regimen). The results were similar to those observed with the co-administration of SGT with AMPC-MET.

Pharmacokinetic parameters for PF and PM-I

Table 1 shows the pharmacokinetic parameters for PF and PM-I in six experiments. In the single regimen of AMPC-MET, in which the antibiotic was administered

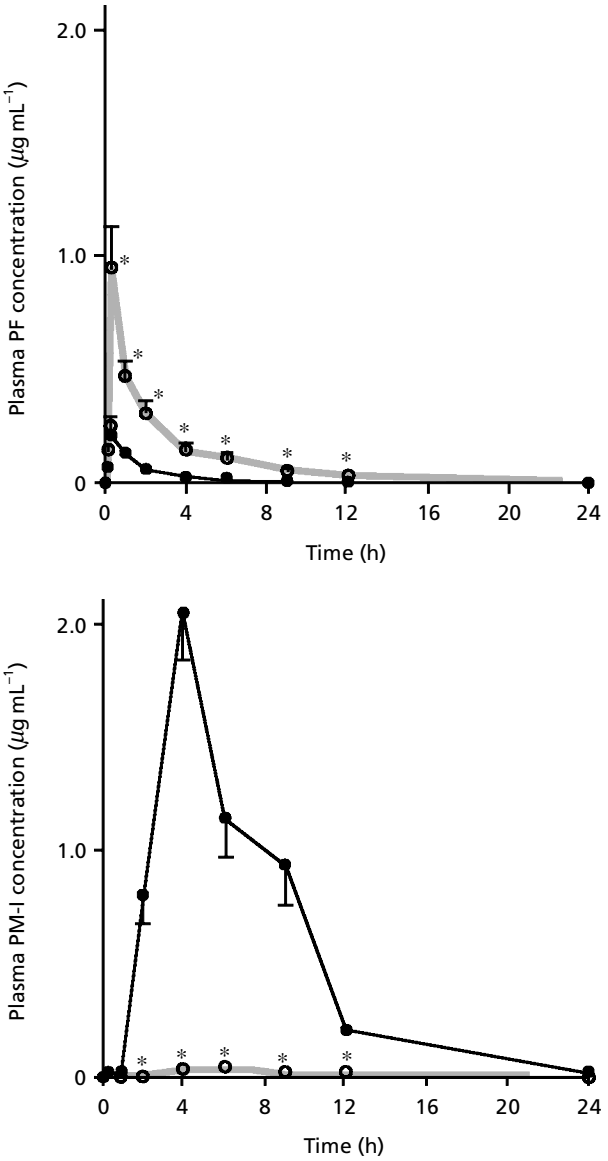

Figure 2 Plasma concentration–time curves of paeoniflorin (PF) and paeonimetabolin-I (PM-I) after single administration of amoxicillin and metronidazole (AMPC-MET) together with Shaoyao-Gancao-tang (SGT) (single regimen). Each point represents the mean ± s.e. (n = 6) determined by enzyme immunoassay. Spectrofluorometry was performed using a Shimadzu RF-550 spectrofluorometric detector (Kyoto, Japan). ●, SGT without AMPC-MET (control); ○, SGT with AMPC-MET. *$P < 0.01$ vs control.

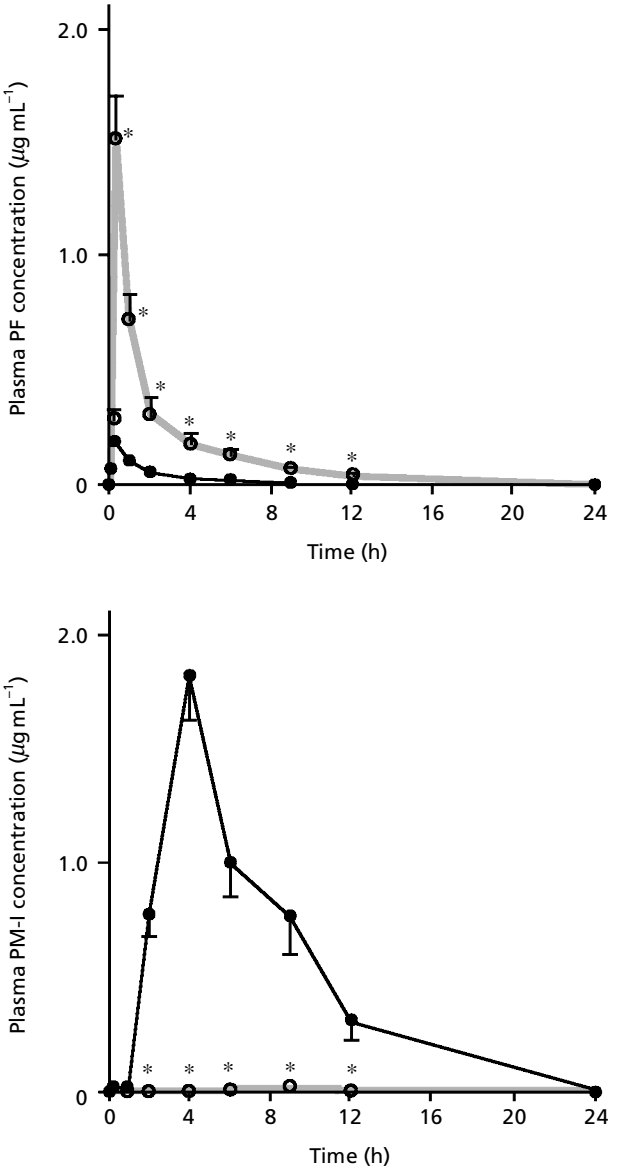

Figure 3 Plasma concentration–time curves of paeoniflorin (PF) and paeonimetabolin-I (PM-I) after amoxicillin and metronidazole (AMPC-MET) was pre-administered for 3 days and co-administered with Shaoyao-Gancao-tang (SGT) on the fourth day (multiple regimen). Each point represents the mean ± s.e. (n = 6) determined by enzyme immunoassay. ●, SGT without AMPC-MET (control); ○, SGT with AMPC-MET. *$P < 0.01$ vs control.

concomitantly with SGT at a single dose, the C_{max} and $AUC_{0-24\,h}$ of PM-I from SGT were significantly decreased to 2.0% and 2.6%, respectively, by the combination as compared with the respective values in rats treated with SGT alone (control group). The values of t_{max} and t_{lag} were found to be significantly prolonged. The other pharmacokinetic parameters, such as K_a and K, as well as their respective half-lives, were not affected. Conversely, both C_{max} and $AUC_{0-24\,h}$ of PF were significantly increased (by approx. 4–5-fold) in the AMPC-MET co-administered group compared with the control group; the other pharmacokinetic parameters were not significantly altered.

In the multiple regimen of AMPC-MET, in which the antibiotics were pre-administered for 3 days before administration in combination with SGT, a similar but more intense effect was observed. Both the C_{max} and $AUC_{0-24\,h}$ of PM-I were reduced by approximately 99%. The t_{max} and t_{lag} values were significantly prolonged to a greater extent than in the single regimen ($P < 0.05$). More significant increases in the C_{max} (1.52 vs 0.19 $\mu g\,mL^{-1}$) and

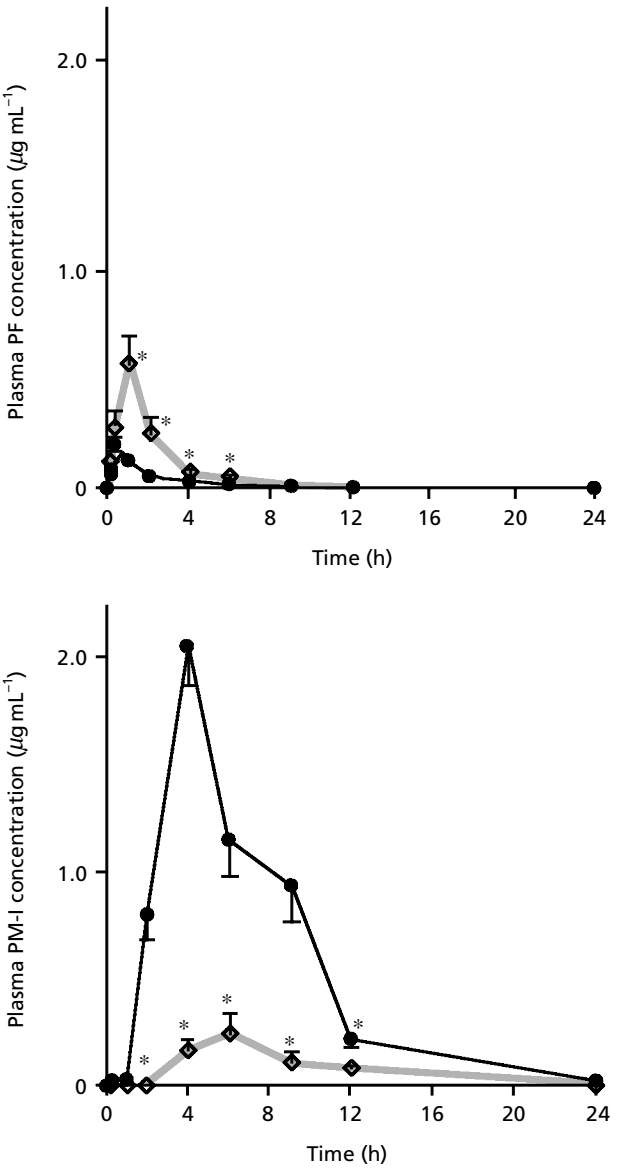

Figure 4 Plasma concentration–time curves of paeoniflorin (PF) and paeonimetabolin-I (PM-I) after single administration of ofloxacin together with Shaoyao-Gancao-tang (SGT) (single regimen). Each point represents the mean ± s.e. (n = 6) determined by enzyme immunoassay. ●, SGT without ofloxacin (control); ◇, SGT with ofloxacin. *$P < 0.01$ vs control.

$AUC_{0-24\,h}$ (2.97 vs 0.41 μg h mL^{-1}) of PF were caused by administration of the antibiotics with the 3-day pre-administration than without; the other pharmacokinetic parameters were not affected.

Similarly, the co-administration of ofloxacin significantly decreased the plasma PM-I concentration, whereas it significantly increased the plasma PF concentration. The influence on C_{max} and AUC of PM-I by the combination of SGT with ofloxacin was significantly weaker than that caused by combining SGT with AMPC-MET.

Correlation between AUC (or t_{lag}) and PF-metabolizing activity

To clarify the mechanism of the alterations of AUC and t_{lag} induced by the co-administered antibiotics, the correlations between AUC (or t_{lag}) and the PF-metabolizing activity of the intestinal bacteria in faeces were investigated in rats pretreated with AMPC-MET or ofloxacin for 3 days.

The PF-metabolizing activity was significantly decreased to 16% and 33% by treatment with AMPC-MET and ofloxacin, respectively (see Figure 5 for values). As shown in Figure 5, the PF-metabolizing activity was negatively correlated with the AUC of PF as well as t_{lag} of PM-I, but was positively correlated with the AUC of PM-I. Greater PF-metabolizing activity resulted in a larger AUC and shorter t_{lag} for PM-I, but a smaller AUC for PF, and *vice versa*.

Discussion

SGT is one of the most commonly used traditional Chinese formulation and is widely prescribed for the treatment of abdominal pain (Katsura 1995) and colic pain in urolithiasis and cholelithiasis (Yamaguchi et al 1982). During the course of our biopharmaceutical studies of drug–drug interactions between synthetic drugs and traditional Chinese formulations, we previously found that orally co-administered AMPC-MET significantly decreased the AUC of glycyrrhetic acid, an active metabolite of glycyrrhizin from orally administered SGT in rats. This reduction of AUC was caused by the decrease of glycyrrhizin-hydrolysing activity of intestinal bacteria after antibiotic treatment (He et al 2001). Since AMPC-MET is commonly used to eradicate *H. pylori* from ulcers, SGT is sometimes used together with AMPC-MET to relieve abdominal pain induced by *H. pylori* infections.

PF is a pharmacologically important constituent of Shaoyao and also of SGT, which is also transformed by intestinal bacteria in the gut into a bioactive metabolite, PM-I (Meselhy & Hattori 2000 and references therein). Some pharmacokinetic studies on SGT have been reported (Bando et al 2000; Chen et al 2002), however there is no report concerning the influence of co-administered antibiotics on the pharmacokinetic fate of PF in SGT. Thus, in the present study, we focused our attention on the pharmacokinetics of PF and its bioactive metabolite PM-I, when SGT was concurrently administered with the antibiotics AMPC-MET and ofloxacin. SGT and some other formulations containing Shaoyao such as Si-Wu-tang (Shimotsu-To in Japanese) are also sometimes used together with ofloxacin, as well as Zhuling-tang (Chorei-To in Japanese), to relieve pain in the treatment of urinary infections.

PF is reported to have anticholinergic activity after oral administration, but has no effect on the isolated rat proximal colon in-vitro (Kobayashi et al 1990 and references therein). Since intraperitoneal injection of PM-I is

Table 1 Pharmacokinetic parameters of paeoniflorin and paeonimetabolin-I after co-administration of antibiotics with Shaoyao-Gancao-tang (SGT).

	Parameter	SGT alone (control)	SGT with amoxicillin and metronidazole	SGT with ofloxacin
Single regimen				
Paeonimetabolin-I	K_a (h^{-1})	2.77 ± 0.17	2.98 ± 0.20	2.22 ± 0.33
	$t_{\frac{1}{2}Ka}$ (h)	0.25 ± 0.02	0.23 ± 0.02	0.31 ± 0.05
	K (h^{-1})	0.25 ± 0.04	0.23 ± 0.03	0.23 ± 0.04
	$t_{\frac{1}{2}K}$ (h)	2.79 ± 0.46	3.05 ± 0.40	3.04 ± 0.54
	t_{lag} (h)	1.89 ± 0.10	3.78 ± 0.35^{ad}	3.71 ± 0.29^{ad}
	t_{max} (h)	3.15 ± 0.40	5.37 ± 0.35^{ad}	5.21 ± 0.44^{a}
	C_{max} ($\mu g\ mL^{-1}$)	2.05 ± 0.19	0.04 ± 0.01^{abd}	0.25 ± 0.06^{ad}
	(C_{max} %)	(100)	(2.0 ± 0.5)	(12.2 ± 2.9)
	$AUC_{0-24\,h}$ ($\mu g\ h\ mL^{-1}$)	12.65 ± 0.91	0.33 ± 0.07^{ace}	1.96 ± 0.27^{ae}
	($AUC_{0-24\,h}$ %)	(100)	(2.6 ± 0.3)	(15.5 ± 1.1)
Paeoniflorin	K_a (h^{-1})	2.14 ± 0.30	2.77 ± 0.47	2.01 ± 0.35
	$t_{\frac{1}{2}Ka}$ (h)	0.32 ± 0.04	0.25 ± 0.04	0.34 ± 0.04
	K (h^{-1})	0.32 ± 0.03	0.25 ± 0.04	0.33 ± 0.05
	$t_{\frac{1}{2}K}$ (h)	2.17 ± 0.20	2.77 ± 0.46	2.10 ± 0.42
	t_{lag} (h)	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.02
	t_{max} (h)	0.34 ± 0.05	0.41 ± 0.10	0.59 ± 0.17
	C_{max} ($\mu g\ mL^{-1}$)	0.21 ± 0.03	0.95 ± 0.12^{abd}	0.59 ± 0.08^{ad}
	(C_{max} %)	(100)	(452.4 ± 7.6)	(281.0 ± 2.1)
	$AUC_{0-24\,h}$ ($\mu g\ h\ mL^{-1}$)	0.43 ± 0.04	2.30 ± 0.19^{acd}	1.42 ± 0.16^{ae}
	($AUC_{0-24\,h}$ %)	(100)	(534.9 ± 5.6)	(330.2 ± 6.5)
Multiple regimen				
Paeonimetabolin-I	t_{lag} (h)	1.85 ± 0.12	5.91 ± 0.64^{a}	5.48 ± 0.57^{a}
	t_{max} (h)	3.09 ± 0.43	7.29 ± 0.76^{a}	6.94 ± 0.78^{a}
	C_{max} ($\mu g\ mL^{-1}$)	1.83 ± 0.25	0.01 ± 0.01^{ab}	0.1 ± 0.03^{a}
	(C_{max} %)	(100)	(0.6 ± 0.2)	(5.5 ± 0.8)
	$AUC_{0-24\,h}$ ($\mu g\ h\ mL^{-1}$)	12.03 ± 0.86	0.09 ± 0.02^{ac}	0.97 ± 0.14^{a}
	($AUC_{0-24\,h}$ %)	(100)	(0.8 ± 0.1)	(8.1 ± 0.5)
Paeoniflorin	t_{max} (h)	0.32 ± 0.05	0.39 ± 0.08	0.62 ± 0.17
	C_{max} ($\mu g\ mL^{-1}$)	0.19 ± 0.02	1.52 ± 0.22^{a}	1.28 ± 0.21^{a}
	(C_{max}%)	(100)	(801.0 ± 31.9)	(673.7 ± 40.1)
	$AUC_{0-24\,h}$ ($\mu g\ h\ mL^{-1}$)	0.41 ± 0.03	2.97 ± 0.23^{a}	2.50 ± 0.25^{a}
	($AUC_{0-24\,h}$ %)	(100)	(724.4 ± 13.1)	(609.8 ± 16.4)

Each value represents the mean ± s.e. (s.e. of AUC was evaluated by Bailer's method, the others were evaluated by the resampling method, six rats per time point). [a] $P < 0.01$, significantly different compared with the control; [b] $P < 0.05$ and [c] $P < 0.01$ vs with ofloxacin; [d] $P < 0.05$ and [e] $P < 0.01$ vs multiple regimen.

reported to have anticonvulsant and muscle relaxation effects in mice (Abdel-Hafez et al 1999), the analgesic and antispasmodic effects of orally administered SGT are attributed to PM-I derived by biotransformation from PF, rather than PF itself. Thus, the extent of absorption of PM-I into the blood determines the efficacy of SGT.

When SGT was orally administered in the absence of antibiotics in rats, PF was absorbed rapidly and reached a peak concentration at about 20 min, and was then slowly eliminated (Figure 2). These results are in good agreement with the data obtained from the oral administration of SGT in mice (Chen et al 2002) and of pure PF in rats (Takeda et al 1995). The pharmacokinetic parameters obtained are summarized in Table 1. Both the C_{max} and AUC of PF were markedly lower than those of PM-I ($P < 0.001$). Moreover, a long t_{lag} was observed for PM-I, but not for PF ($P < 0.001$). This long t_{lag} of PM-I may be owing to the long time required for biotransformation of PF into PM-I by intestinal bacteria such as *Lactobacilli* and *Bacteroides* species (Shu et al 1987). The pharmacokinetic differences between PF and PM-I observed in the present study after the oral administration of SGT agree well with those observed in a study of pure PF (Heikal et al 1997), in which it was found that orally administered PF is poorly absorbed in its original form, whereas its metabolite PM-I is readily absorbed.

As shown in Table 1, even just a single concomitant administration of AMPC-MET with SGT greatly decreased the C_{max} and AUC of PM-I, whereas it significantly increased those of PF. The t_{max} of PM-I was markedly delayed, perhaps because of the extremely prolonged t_{lag}. The co-administered antibiotics did not affect the other pharmacokinetic parameters examined, such as K_a,

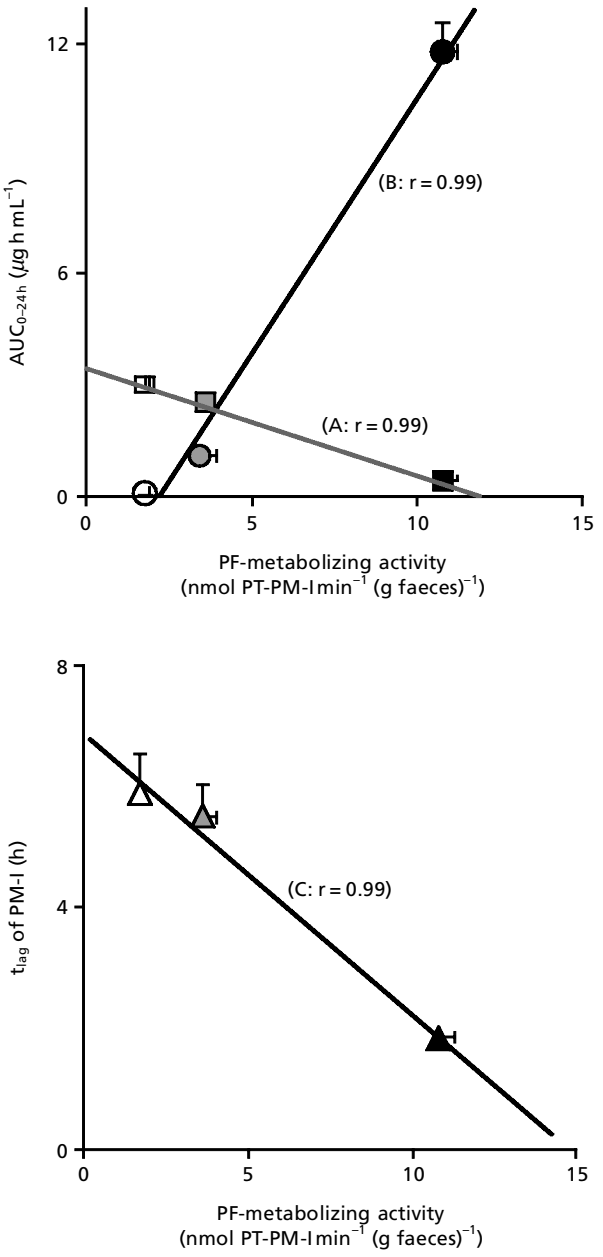

Figure 5 Correlation between AUC of paeoniflorin (PF) as well as paeonimetabolin-I (PM-I) or t_{lag} of PM-I and PF-metabolizing activity in faeces of rats pretreated with antibiotics for 3 days. Each point represents the mean ± s.e. of the AUC (s.e. evaluated by Bailer's method) or t_{lag} of PF-metabolizing activity. PF-metabolizing activity (rate of biotransformation from PF to PT-PM-I) was measured using HPLC, resulting in values of 10.75 ± 0.50, 1.71 ± 0.14 and 3.60 ± 0.44 nmol PT-PM-I min^{-1} (g faeces)$^{-1}$ (n = 60) for the control group, the amoxicillin and metronidazole (AMPC-MET)-treated group and the ofloxacin-treated group, respectively. A. Correlation between AUC of PF and PF-metabolizing activity (y = −0.29x + 3.49); ■, control group; □, AMPC-MET-treated group; ■, ofloxacin-treated group. B. Correlation between AUC of PM-I and PF-metabolizing activity (y = 1.35x − 2.90); ●, control group; ○, AMPC-MET-treated group; ●, ofloxacin-treated group. C. Correlation between t_{lag} of PM-I and PF-metabolizing activity (y = −0.47x + 6.90); ▲, control group; Δ, AMPC-MET-treated group; ▲, ofloxacin-treated group.

K and the half-lives, indicating that there was no effect of the antibiotics on the rates of absorption and elimination of either PF or PM-I, and thus that the reasons for those pharmacokinetic changes were not related to the processes of absorption or elimination. One single concomitant administration after a 3-day pre-administration of AMPC-MET (multiple regimen) showed a similar but much stronger effect ($P < 0.05$ vs single regimen). Since the antibiotics are regularly used for one or more weeks (Soll 1996), such reductions of the AUC and prolongation of the absorption lag time for PM-I may become much more marked in clinical practice.

These effects were further confirmed by a similar study of the influence of the combination of ofloxacin, another antibiotic predominantly used for the treatment of urinary tract infections (Drew & Gallis 1988), with SGT. In comparison with AMPC-MET, ofloxacin showed a similar but much weaker effect on C_{max} and AUC of PM-I ($P < 0.05$ and $P < 0.001$, respectively). These results are compatible with the evidence that the interference by ofloxacin on the PF-metabolizing activity (3.60 ± 0.44 nmol PT-PM-I min^{-1} (g faeces)$^{-1}$) was significantly weaker than that by AMPC-MET (1.71 ± 0.14 nmol PT-PM-I min^{-1} (g faeces)$^{-1}$). The reasons for this disparity between AMPC-MET and ofloxacin may lie in the difference in their antibacterial activity. The minimum inhibitory concentration of ofloxacin against 90% of *Bacteroides fragilis*, a strain having potent activity transforming PF into PM-I (Shu et al 1987), is 6.3 μg mL^{-1} (Drew & Gallis 1988), whereas that of MET is less than 0.39 μg mL^{-1} (Oguri & Kosakai 1979). Moreover, the minimum inhibitory concentration of AMPC against many anaerobic bacteria is less than 0.13 μg mL^{-1} (Aldridge et al 1983), lower than that of ofloxacin (Drew & Gallis 1988).

To clarify the mechanism of the alteration of the AUC for PF and PM-I, as well as the changes in t_{lag} for PM-I by the co-administered antibiotics, the correlations between the PF-metabolizing activity of intestinal bacteria in rat faeces and AUC or t_{lag} were investigated. As shown in Figure 5, the activity of the transformation of PF into PM-I was significantly ($P < 0.001$) reduced by the treatment with antibiotics, suggesting the possibility that the intestinal bacteria capable of producing PF-metabolizing enzymes were eliminated by the co-administered antibiotics. This reduction in the transforming activity would have resulted in the marked decrease in the AUC of PM-I and the prolongation of its absorption delay, while it could have led to the increase in the AUC of PF.

Since it is PM-I, not PF itself, that is the key compound for the analgesic and antispasmodic effects of orally administered SGT, the concomitant use of the antibiotics tested here, which induced a drastic reduction in the absorption of PM-I, might markedly diminish the desired therapeutic effects of SGT.

The intestinal bacteria are an extremely important factor influencing the metabolism of constituents in crude drugs (Kobashi et al 1992), and are also a crucial cause of the variation in therapeutic efficacy of some natural glycoside drugs (Alam et al 1988). The present findings further reveal the key role of intestinal bacteria in the pharmacokinetics of

the important bioactive components in traditional Chinese formulations. It is worth mentioning that some other antibiotics also influence the intestinal bacteria and thus are likely to affect the pharmacokinetic behaviour of the constituents of traditional Chinese formulations. Therefore, the role of intestinal bacteria should be taken into account in studies of drug–drug interactions between traditional Chinese formulations and co-administered synthetic drugs.

In summary, in order to clarify the feasibility of administering traditional Chinese formulations together with some antibiotics, the influence of co-administered AMPC-MET and ofloxacin on the pharmacokinetics of PF and PM-I from SGT were examined. The co-administered antibiotics significantly reduced the C_{max} and AUC of PM-I, whereas they significantly increased those of PF, but did not influence the absorption rate or elimination rate of these two compounds from SGT in both single and multiple regimens. The pharmacokinetic alterations were owing to a significant reduction in the PF-metabolizing activity by the antibiotics, but not related to the processes of absorption and elimination. The present findings suggest that it is not appropriate to use SGT together with some antibiotics such as AMPC-MET or ofloxacin to relieve pain in the treatments of peptic ulcer or urinary diseases. Further studies regarding the time required for recovery of the PF-metabolizing activity of the intestinal bacteria eliminated by the antibiotics, as well as of the optimal dosing regimen of SGT to avoid the influence of antibiotics, are necessary.

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