

Pharmacokinetic study of Paeoniflorin, Paeonimetabolin-I and Glycyrrhetic acid in humans after oral administration of Paeony Root, Glycyrrhiza and Shakuyaku-kanzo-to (Shao-Yao-Gan-Cao-Tang)

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(Received September 16, 1999. Accepted December 13, 1999.)

Abstract

The pharmacokinetics of Paeoniflorin (PF), Paeonimetabolin-I (PM-I) and Glycyrrhetic acid (GA) after oral administration of the decoction of Paeony Root (PR) and Glycyrrhiza (GR) and Shakuyaku-kanzo-to (Shao-Yao-Gan-Cao-Tang; SK) were studied using human subjects. Concerning the intra- and inter-individual variability of PF and PM-I after oral administration of SK, each inter-individual coefficient of variation (CV) of the pharmacokinetic parameters was larger than its intra-individual CV. There was no significant correlation between each pharmacokinetic parameter and each subject's physiques. These results suggested that a large inter-individual variability were not caused by differences in the subjects' physiques. Regarding a comparison of the pharmacokinetic parameters, AUC and Cmax of PF of PR were significantly higher than those of SK, and MRT and Tmax of PM-I of SK were significantly longer than those of PR. AUC of GA of SK was significantly higher than those of GR. There is the possibility that PR would be better than SK when a stronger efficacy of PR is expected in a short time, and SK would be better than PR when a longer efficacy of PR or a stronger efficacy of GR is expected.

Key words Pharmacokinetics, Human, Paeoniflorin, Paeonimetabolin-I, Glycyrrhetic acid, Shakuyaku-kanzo-to.

Abbreviations AUC, area under serum concentration; AUMC, area under first moment; Cmax, peak serum concentration; CV, coefficient of variation; EIA, enzyme immunoassay; GA, Glycyrrhetic acid; GL, Glycyrrhizin; GR, Glycyrrhiza, 甘草; HPLC, high performance liquid chromatography; MRT, ratio of AUMC and AUC; PF, Paeoniflorin; PM-I, Paeonimetabolin-I; RIA, radioimmunoassay; PR, Paeony Root, 芍薬; SK, Shakuyaku-kanzo-to (Shao-Yao-Gan-Cao-Tang), 芍薬甘草湯; Tmax, time to Cmax.

Introduction

Shakuyaku-kanzo-to (combination of Paeony Root and Glycyrrhiza) is a traditional formulation, which was first described in the ancient textbook of

Shang-Han-Lun (about 200 A.D.). Since then, this formulation have been widely used for the purpose of anti-spasmodic and muscle relaxation effects. A large number of pharmacological studies with the individual crude drug (Paeony Root or Glycyrrhiza) was performed.^{1,8)} But there were very few pharmacological

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studies with the combination of these crude drugs,⁹⁻¹¹⁾ and specially no studies on humans. Therefore, the present study was performed to clarify the differences in pharmacokinetics after the oral administration of these two individual crude drugs and Shakuyaku-kanzo-to in humans.

Materials and Methods

Materials : Glycyrrhizin, the goat antiserum to rabbit IgG and normal rabbit serum were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 4-methylumbelliferyl β -D-galactoside and bovine serum albumin (BSA) was purchased from Sigma Chemicals (St.Louis, USA). Paeony Root in the Japanese Pharmacopoeia (thirteenth edition) (Shakuyaku (芍薬), Nara Pref.; Japan) and Glycyrrhiza (Kanzo (甘草), Inner Mongolia, 内蒙古; China) were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan).

Buffer A was 20mM phosphate buffered saline (pH7.3) containing 0.1 % BSA, 0.1 % NaN_3 and 0.001 % MgCl_2 , and buffer B was 20mM phosphate buffered saline (pH7.3) containing 0.1 % NaN_3 and 0.001 % MgCl_2 .

Subjects : Sixteen males aged from 18 to 28 years and weighting from 48 to 76 kg volunteered for this study. They were recruited from the student population of our university. All subjects received no other medication for at least 2 weeks before the beginning of and during the study. Written informed consent was obtained, and the protocol was approved by the Ethical Committee of the Toyama Medical and Pharmaceutical University.

Drug and dosages : The concentration of Paeoni-

florin (PF) and Glycyrrhizin (GL) were determined by HPLC, consisted of TOSOH 8020. The mobile phase was composed of H_2O with 0.1 % H_3PO_4 (A) and MeOH with 0.1 % H_3PO_4 , with gradient elution (A : B 1min 90 : 10, 15 min 50 : 50, 25 min 20 : 80, 30 min 0 : 100), and the flow rate was 1.0 ml/min. A TOSOH TSK-gel ODS-80Ts column (150×4.6 I.D.) was used. The chromatogram was monitored at wavelength of 254 nm on GL, and 238 nm on PF. The operating temperature was maintained at 40°C.

1) The decoction of Paeony Root (PR) :

5.0 g of PR was boiled in 200 ml of water for 30 minutes, and a 100 ml decoction was prepared for administration. This decoction contained 145 mg of PF.

2) The decoction of Glycyrrhiza (GR) :

5.0 g of GR was boiled in 200 ml of water for 30 minutes, and a 100 ml decoction was prepared for administration. This decoction contained 161 mg of GL.

3) Shakuyaku-kanzo-to (SK) :

SK was prepared as a 100 ml decoction, boiling the two crude drugs, 5.0 g of PR and 5.0 g of GR,¹²⁾ in 200 ml of water for 30 minutes before administration. This decoction contained 144 mg of PF and 150 mg of GL.

Procedures : Signed informed consent was obtained from all subjects. After overnight fasting, decoctions were orally administered at 8:30 a.m.. Breakfast, lunch and dinner, similar to daily meal, was given at 9:45 a.m., 1:00 p.m. and 9:00 p.m.. Although the subjects were not required to remain sedentary throughout the duration of the study, they were asked to refrain from strenuous activities. Whole blood samples (10 ml) were collected at the

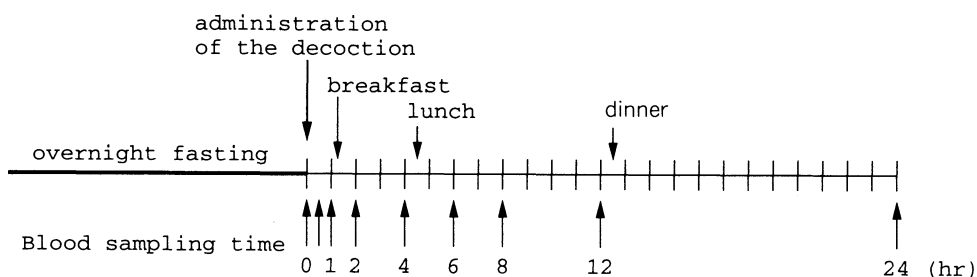


Fig. 1 Protocol in the present study

following times : prior to drug administration (at 8 : 30), and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hr after drug administration (Fig. 1). The samples were centrifuged at 2,000 rpm for 10 min at 4°C. The resulting serum was frozen and stored at -20°C or colder until analysis were performed.

For the study of intra-individual variability, the above protocol was performed on five subjects once a week, totaled to five times, for the decoction of SK. The coefficient of variation (CV) of five times on each subject was calculated, and CV of five subjects were averaged.

On the other hand, for the study of inter-individual variability and the difference in pharmacokinetics, the above protocol was performed on 16 subjects once a week for each decoction.

Drugs assays :

1) Determination of Paeonifrorin (PF) :

Concentration of PF in the serum were determined according to the enzyme immunoassay (EIA) method of Kanaoka *et al.*¹³⁾ The antiserum and labeled antigen were diluted with buffer A and B, respectively, to appropriate concentrations. A sample or standard solution (50 μ l) containing various amounts of PF was added to the 10000-fold diluted antiserum (50 μ l) and 1000-fold diluted labeled antigen (25 μ l). The mixture was kept for 2 hours at room temperature, then 10-fold diluted goat anti-rabbit IgG (50 μ l) and 100-fold diluted normal rabbit serum (20 μ l) were added, and the mixture was incubated at 4°C overnight. After the incubation, 1ml of buffer A was added and the solution was centrifuged at 3000rpm for 20min at 4°C. The supernatant was removed and the immunoprecipitate was washed with buffer A, followed by recentrifugation. The resulting immunoprecipitate was incubated with 0.1mM 4-methylumbelliferyl β -D-galactoside (150 μ l) at 30°C for 30min. Then, 3ml of 0.1M glycine-NaOH buffer (pH 10.3) was added to the reaction mixture, and the fluorescence intensity of 7-hydroxy-4-methylumbelliferon formed was measured spectrofluorometrically at wavelengths of 364 nm and 448 nm for excitation and emission, respectively.

2) Determination of Paeonimetabolin-I (PM-I) :

Concentration of PM-I in the serum was determined according to the EIA method of Hattori *et al.*¹⁴⁾ The antiserum and labeled antigen were diluted with

buffer A and B, respectively, to appropriate concentrations. A sample or standard solution (100 μ l) containing various amounts of PF was added to the 10000-fold diluted antiserum (100 μ l) and 1000-fold diluted labeled antigen (50 μ l). The mixture was kept for 2 hours at room temperature, then 10-fold diluted goat anti-rabbit IgG (50 μ l) and 100-fold diluted normal rabbit serum (20 μ l) were added, and the mixture was incubated at 4°C overnight. After the incubation, 1ml of buffer A was added and the solution was centrifuged at 3000rpm for 20min at 4°C. The supernatant was removed and the immunoprecipitate was washed with buffer A, followed by recentrifugation. The resulting immunoprecipitate was incubated with 0.1mM 4-methylumbelliferyl β -D-galactoside (150 μ l) at 30°C for 30min. Then, 3ml of 0.1M glycine-NaOH buffer (pH 10.3) was added to the reaction mixture and the fluorescence intensity of 7-hydroxy-4-methylumbelliferon formed was measured spectrofluorometrically at wavelengths of 364nm and 448 nm for excitation and emission, respectively.

3) Determination of Glycyrrhetic acid (GA) :

Concentration of GA in the serum was determined according to the RIA method of Kanaoka *et al.*¹⁵⁾ The antiserum and labeled antigen were diluted with buffer A and B respectively, to appropriate concentrations. A sample or standard solution (20 μ l) containing various amounts of GA was added to buffer A (80 μ l), the 50000-fold diluted antiserum (100 μ l) and labeled antigen (50 μ l, 10nCi/tube). The mixture was kept for 2 hours at room temperature, then 10-fold diluted goat anti-rabbit IgG (50 μ l) and 100-fold diluted normal rabbit serum (20 μ l) were added, and the mixture was incubated at 4°C overnight. After the incubation, 1ml of buffer A was added and the solution was centrifuged at 3000 rpm for 20 min at 4°C. The supernatant was removed and the immunoprecipitate was washed with buffer A, followed by recentrifugation. The resulting immunoprecipitate was dissolved in 1N NaOH (100 μ l) twice to measure the radioactivity by liquid scintillation counting.

4) Calibration curves for PF, PM-I and GA:

The serum sample were used directly without dilution. The calibration curves were prepared PF, PM-I and GA in the presence of serum from each subjects and constructed with the linearized lozit-log

plot. The calibration curves ranged 0.3 to 30ng/tube for PF, PM-I and GA.

Pharmacokinetic analysis: Pharmacokinetic analysis was performed by PK MOMENT, which is a package of macro programs for automatically calculate non-compartmental pharmacokinetic parameters on Microsoft Excel spreadsheets.¹⁶⁾ The following pharmacokinetic parameters were calculated for PF, PM-I and GA using non-compartmental techniques. The area under the serum concentration-time profile (AUC) was calculated using the trapezoidal method up to 24 hr after administration. The area under the first moment (AUMC) was calculated using the trapezoidal method up to 24 hr after administration. The ratio of AUMC to AUC (MRT) was calculated, which equals the sum of the mean residence time and the mean absorption time. The maximal serum concentration (Cmax) and the time in the maximal serum concentration (Tmax) were determined from individual profiles.

Statistical analysis: As a parameter of serum level deviation in individual subjects, we used the mean of the coefficient of variation (CV), which is defined as the ratio between the standard deviation and the mean of a numeric variable. The pharmacokinetic parameters of PF and PM-I after administration of PR, GR or SK were compared with the Wilcoxon matched pairs signed-rank test, and relationships between each subject's physiques and each pharmacokinetic parameter were investigated by using the Spearman rank correlation coefficient. Statistical significance was assumed when $p < 0.05$.

Results

Intra-individual variability

For the study of intra-individual variability, five male subjects were employed. Their mean age and weight were 21.2 years and 60.2 kg. The mean value of CV, the parameters of intra-individual variability, are shown in Table I. Each intra-individual CV of the parameter of PF ranged from 0.350 to 0.437, and that of PM-I ranged from 0.268 to 0.422.

Inter-individual variability and Pharmacokinetic parameters

Sixteen male subjects were employed for the elucidation of inter-individual variability and the pharmacokinetics of the two crude drugs. Their mean age and mean weight were 20.4 years and 75.2 kg. Their inter-individual CV values are shown in Table I. The inter-individual CV of each parameter of PF was within a range from 0.435 to 1.652, and that of PM-I from 0.362 to 2.013.

The mean values of each pharmacokinetic parameter of PF, PM-I and GA are shown in Table II, and that of PR or GR were compared with those of SK. Significant differences ($p < 0.05$) for PF were obtained in AUC (1780.6 and 629.1 ng·hr/ml) and Cmax (397.5 and 103.6 ng/ml) after oral administration of PR and SK, respectively. There were no significant differences for PF in MRT (5.1 and 4.7 hr) and Tmax (2.2 and 1.9 hr), nor for PM-I in AUC (504.4 and 221.1 ng·hr/ml) and Cmax (47.9 and 28.0 ng/ml). MRT (13.7 hr) and Tmax (9.5 hr) of PM-I after

Table I Intra- and inter-individual variability of pharmacokinetic parameters of Paeoniflorin and Paeonimetabolin after oral administration of Shakuyaku-kanzo-to

	Intra-individual variability (n=5)		Inter-individual variability (n=16)	
	mean CV of PF	mean CV of PM-I	mean CV of PF	mean CV of PM-I
AUC	0.357	0.310	1.652	1.601
MRT	0.437	0.268	0.435	0.362
Cmax	0.349	0.372	1.039	2.013
Tmax	0.433	0.422	0.889	0.982

PF: Paeoniflorin, PM-I: Paeonimetabolin-I

mean CV: mean value of coefficient of variance of each subject

AUC: Area under serum concentration-time, MRT: Mean residence time

Cmax: Maximal serum concentration

Tmax: Time in maximal serum concentration

Table II Pharmacokinetic parameters of Paeoniflorin, Paeonimetabolin-I and Glycyrrhetic acid after oral administration of Paeoniae Radix, Glycyrrhizae Radix and Shakuyaku-kanzo-to

	Paeoniflorin		Paeonimetabolin-I		Glycyrrhetic acid	
	PR	SK	PR	SK	GR	SK
AUC (ng·hr/ml)	1780.6±586.6	629.1±259.9*	504.4±283.4	221.1±88.5	4239.2±1365.7	10992.0±6575.2 †
MRT (hr)	5.1±0.50	4.7±0.51	9.3±1.57	13.7±1.24*	13.7±0.88	14.4±0.77
Cmax (ng/ml)	397.5±100.5	103.6±26.9*	47.9±24.1	28.0±11.9	445.2±145.9	609.9±323.2
Tmax (hr)	2.2±0.37	1.9±0.42	4.1±1.65	9.5±2.33*	9.9±1.73	14.0±1.97

PR : Paeoniae Root, SK : Shakuyaku-kanzo-to, GR : Glycyrrhiza

The others are the same as Table I

* $p < 0.05$ versus PR, † $p < 0.05$ versus GR

Table III Correlation between each pharmacokinetic parameter and subject's characteristics

			Age		Body Weight		Body Height		Body Mass Index	
			rho	p	rho	p	rho	p	rho	p
PF	PR	AUC	0.397	0.1238	0.075	0.7708	-0.114	0.6579	0.097	0.7070
		MRT	-0.068	0.7914	0.061	0.8123	0.220	0.3940	0.084	0.7451
		Cmax	0.307	0.2346	0.128	0.6192	-0.088	0.7344	0.118	0.6486
		Tmax	0.232	0.3695	0.214	0.4066	0.279	0.2802	0.173	0.5034
	SK	AUC	0.365	0.1578	0.183	0.4787	-0.169	0.5121	0.265	0.3053
		MRT	-0.234	0.3654	0.044	0.8661	0.288	0.2642	0.004	0.9864
		Cmax	0.393	0.1281	0.302	0.2416	-0.269	0.2979	0.391	0.1298
		Tmax	0.198	0.4423	0.128	0.6214	-0.406	0.1154	0.356	0.1684
PM-I	PR	AUC	-0.128	0.6214	0.170	0.5112	-0.062	0.8091	0.141	0.5845
		MRT	-0.291	0.2597	-0.019	0.9427	0.232	0.3695	-0.158	0.5395
		Cmax	0.190	0.4607	0.453	0.0794	-0.116	0.6526	0.418	0.1057
		Tmax	0.287	0.2661	0.086	0.7404	0.154	0.5515	0.052	0.8410
	SK	AUC	-0.228	0.3762	0.014	0.9567	-0.086	0.7385	-0.094	0.7153
		MRT	-0.478	0.0639	-0.078	0.7621	0.200	0.4375	-0.129	0.6162
		Cmax	0.229	0.3760	-0.153	0.5545	-0.249	0.3347	-0.167	0.5180
		Tmax	-0.144	0.5769	-0.068	0.7915	0.363	0.1603	-0.192	0.4583
GA	GR	AUC	-0.092	0.7307	0.242	0.3653	0.511	0.0557	0.021	0.9361
		MRT	-0.107	0.6903	-0.017	0.9492	-0.063	0.8128	0.141	0.5973
		Cmax	-0.070	0.7922	0.267	0.3173	0.477	0.0746	0.091	0.7331
		Tmax	-0.441	0.0986	-0.372	0.1637	-0.153	0.5665	-0.178	0.5061
	SK	AUC	-0.034	0.8980	0.384	0.1513	0.410	0.1249	0.218	0.4150
		MRT	-0.409	0.1257	-0.144	0.5910	-0.142	0.5950	-0.050	0.8513
		Cmax	-0.014	0.9569	0.425	0.1120	0.450	0.0923	0.264	0.3227
		Tmax	-0.460	0.0853	-0.105	0.6948	-0.074	0.7821	-0.068	0.7979

GA : Glycyrrhetic acid, rho : Spearman rank correlation coefficient

The others are the same as Table I and II

administration of SK were significantly longer than those after administration of PR (MRT : 9.3 hr, Tmax : 4.1 hr) ($p < 0.05$).

Significant differences of GA ($p < 0.05$) in AUC

(4239.2 and 10992.0 ng·hr/ml) were obtained after oral administration of GR and SK, respectively. There were no differences of GA in Cmax (445.2 and 609.9 ng/ml), MRT (13.7 and 14.4 hr) and Tmax (9.9 and

14.0 hr) after administration of GR and SK, respectively.

Table III depicts the relationship between each pharmacokinetic parameter and each subject's physiques, investigated by using the Spearman rank correlation coefficient. There were no correlations between all of parameters and any of the subjects' physiques.

Discussion

The present study was performed on healthy male volunteers while leading a daily normal life, since pharmacokinetics of various indicator components of the crude drugs would hardly be clarified. All subjects had standard meals at breakfast, lunch and dinner throughout the duration of the study. The pharmacokinetics at 24 hr were examined after the single administration of one day's decoction in the morning in the present study, although patients are usually given the decoction three times a day.

Concerning the intra- and inter-individual variabilities of Paeniflorin (PF) and Paeonimetabolin-I (PM-I) after oral administration of the decoction of Shakuyaku-kanzo-to (SK), each inter-individual coefficient of variation (CV) of the pharmacokinetic parameters was larger than each respective intra-individual CV, and specially the inter-individual CV of AUC and C_{max} were 3-6 times greater than their intra-individual CV counterparts. There was no significant correlation between each pharmacokinetic parameter and the each subject's physiques. These results suggest that there is a large inter-individual variability, which was not caused by difference in subjects' physiques, in the pharmacokinetics of PF and PM-I after oral administration of SK, and absorption and excretion of the components of these crude drugs would be different in each subject. Recent studies have shown that an intestinal bacteria was necessary for the metabolism of PF to PM-I,¹⁷⁻²¹⁾ and the ratio of metabolism varied in each human intestinal bacteria.²²⁾ This means that the differences of intestinal bacteria of each subject may be related to the prominent inter-individual variability. Clinically, it is probably possible that the 'Sho' (indication) of SK is concerned with this variability.

The pharmacokinetics of after administration of SK were different from that of PR or GR. Since PF levels of PR and SK, and Glycyrrhizin (GL) levels of GR and SK, administered orally in the present study, were similar, these significant differences were not due to the different volumes of PF and/or GL. It was reported that SK affected the gastric emptying rate, peristalsis of the small intestine, and consequently the rate of absorption of its components in mice,²³⁾ pointing to the possibility that orally administered SK suppressed intestinal peristalsis, and the volume of PF in the intestine was decreased. On the other hand, there were no significant differences in MRT and T_{max} of PF, and the dispositions of PM-I and GA, which were the respective metabolites from PF and GL by intestinal bacteria, were different. Therefore, it must be considered for reason of these significant differences that the existence of the components of PR and GR at the same time affects the metabolisms, the absorptions and the excretions of PF, PM-I and GA.

As to the pharmacological effects of PR, GR and SK, it was reported that an anti-inflammatory effect of SK derived from GR was decreased by adding PR, and an analgesic effect of SK derived from PR was increased by adding GR in mice,⁹⁾ on this report, it was expected that the concentrations of PF and PM-I of SK might be higher than those of PR, and the concentration of GA of SK might be lower than that of GR. But by contrast, AUC and C_{max} of PF or PM-I of SK were lower than those of PR, and AUC of GA of SK was higher than that of GR in the present study. Although the relationships between the concentrations of PF, PM-I or GA and the pharmacological effects were not be evaluated in the present study, it is possible that the effects of SK is not explained by the concentrations of the major components alone.

Clinically, these pharmacokinetic differences of PR and SK are very interesting. The significant high levels of PF and PM-I after oral administration of PR indicate that, PR should be used when a stronger efficacy of PR is expected in a short time. On the other hand, the significantly long internal residence time of PM-I and the significantly high level of GA of SK suggest that the efficacy of PM-I may continue for a long time, and the efficacy of GA may be in-

creased by the combination of PR and GR. That is to say, SK should be used when long efficacy of PR or strong efficacy of GR is expected.

Conclusion

In the present study, it was clarified that the inter-individual variabilities of PF and PM-I after administration of SK were larger than their intra-individual variabilities, AUC and Cmax of PF of PR and AUC of GA of SK were significantly higher, and MRT and Tmax of PM-I of SK were significantly longer. In other words, the oral administration of PR, GR and SK differed in the pharmacokinetics of PF, PM-I and GA.

Acknowledgments

We are grateful to New Drug Discovery Laboratories, Tsumura & Co. (Ibaraki, Japan) for the kind gift of Glycyrrhetic acid antibody.

和文抄録

ヒト健常人において芍薬・甘草・芍薬甘草湯経口投与時のペオニフロリン (PF), ペオニメタボリン-I (PM-I), グリチルレチン酸 (GA) の薬物動態の研究を施行した。芍薬甘草湯投与後の PF, PM-I の個体内・個体間変動については, 各々の個体間変動係数が個体内変動係数よりも大きく, また, 全ての薬物動態の各パラメータと被験者の体格との間には相関はみられなかった。芍薬・甘草と芍薬甘草湯との比較では, 芍薬の PF の AUC, Cmax が芍薬甘草湯のものに比較して有意に高値を示し, 芍薬甘草湯の PM-I の MRT, Tmax が芍薬に比較して有意に長かった。また, 芍薬甘草湯の GA の AUC は甘草単独投与のものに比較して有意に高値を示した。このことから, 短時間に芍薬のより強い効果を期待する際には芍薬単独で用い, 芍薬成分のより長い効果や甘草成分のより強い効果を期待する際には芍薬甘草湯として投与する方がよい可能性がある。

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