Pharmacokinetics of （−）Epicatechin 3-*O*-Gallate, Glycyrrhetic Acid and Rhein in Healthy Male Volunteers after Multiple Dose Administration of TJ-8117 （Unpito）, a Japanese Traditional Medicine for Renal Failure

Yukiho TAKIZAWA[*1]   Yasuharu MIZUHARA[*1]   Toshimi MORITA[*1]
Yoshio KASE[*1]   Shuichi TAKEDA[*1]   Masaki ABURADA[*1,2]
and Ken'ichi MIYAMOTO[*3]

[*1] Pharmacology Research Department, Central Research Laboratories, R & D Division,
Tsumura & Co., Ibaraki, Japan
[*2] Faculty of Pharmacy, Musashino University, Tokyo, Japan
[*3] Hospital Pharmacy, School of Medicine, Kanazawa University, Ishikawa, Japan

Aims : Unpito, an herbal medicine extracted from a mixture of five crude medicines (Rhei Rhizoma, Glycyrrhizae Radix, Ginseng Radix, Zingiberis Rhizoma and Aconiti Tuber), has been developed as a drug for chronic renal failure. In general, it is difficult to estimate the absorption and excretion of herbal medicines due to the presence of a wide variety of components. The purpose of the current study was to examine the systemic pharmacokinetics and elimination of Unpito in healthy volunteers following repeated administration as part of the clinical study of the medicine.

Methods : Three compounds, （−）epicatechin 3-*O*-gallate (ECG), glycyrrhetic acid (GA) and rhein (RH) were selected as markers, to examine the clinical pharmacokinetics of Unpito based on their levels in this medicine. The disposition of each compound was evaluated in 24 healthy volunteers receiving multiple oral doses (4, 6, and 8 capsules three times a day).

Results : After repeated administration, plasma ECG and GA concentrations were lower than those simulated. RH plasma concentrations were consistent with the simulation, indicating linear pharmacokinetics of RH. The potential accumulations of marker compounds were not observed from the roughly constant plasma concentrations of troughs at 72, 120, and 144 h after the first administration nor from the urinary excretions.

Conclusions : This is the first study presenting pharmacokinetics of ECG, GA and RH derived from Unpito, an herbal medicine, in healthy volunteers after multiple dose administration.

Key words : clinical pharmacokinetics, TJ-8117, Unpito, healthy male volunteers, multiple dose

Introduction

Unpito, a Japanese traditional herbal medicine (Kampo medicine) consisting of five herbal drugs (Rhei Rhizoma, Glycyrrhizae Radix, Ginseng Radix, Zingiberis Rhizoma and Aconiti Tuber), enhances renal functions in rats displaying renal failure[1-4]. Unpito has been utilized in patients with chronic renal failure[5,6].

In general, it is difficult to estimate the absorption and excretion of herbal medicines because of the presence of various components. We selected three compounds, i. e. （−）epicatechin 3-*O*-gallate (ECG), glycyrrhetic acid (GA), and rhein (RH) as markers to examine the clinical pharmacokinetics of Unpito based on their levels in this medicine as well as in view of their efficacy and safety. A criterion for the selection was reported in the previous paper in detail[7].

The disposition of the marker compounds was evaluated in healthy volunteers receiving multiple oral doses (4, 6, and 8 capsules three times a day). This study was conducted as part of a phase I study of Unpito with healthy volunteers.

Table 1 Pharmacokinetic parameters of $(-)$epicatechin 3-O-gallate at Day 7 following the multiple administrations of Unpito three times a day compared with single dosing

Step		Dose (capsules)	(mg of ECG)	Parameter			
				AUC (0-24) (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)
Day 7 of multiple dosing	1	4	4.6	16.23±4.21	4.58±1.34	2.00±0.00	N.C.
	2	6	6.9	23.30±8.10	6.85±0.97	1.83±0.41	2.02±0.52[a]
	3	8	9.2	20.00±5.06**	5.82±1.04**	2.00±0.00	2.04±0.50[b]
Single dosing	2	4	4.6	21.35±9.55	5.93±2.42	1.83±0.41	2.00±0.18[a]
	3	8	9.2	52.96±20.82	13.43±4.21	2.17±0.98	2.15±0.41[b]

Mean±s.d., n=6, N.C. : not calculated, [a]n=4, [b]n=5, ** : $p < 0.01$ vs. single dosing with t-test

Materials and Methods

1. Subjects

The study involved 24 healthy male volunteers receiving multiple doses. All subjects were male and their age ranged from 20 to 24 years. The body weight ranged from 48.9 to 83.1 kg. The height ranged from 162.2 to 182.4 cm. The subjects were in good health based on medical history and physical examination, including electrocardiogram and standard laboratory tests (hematology, blood chemistry, hepatitis B surface antigen, HIV antigen and urine analysis) performed prior to the study and at the end of the interval of each study. In all the subjects, there was no history or signs of prevailing active diseases, allergies or hypersensitivities, alcohol or drug exposure. The subjects were not allowed to consume alcohol, nicotine, caffeine or any other drugs and were advised not to undertake physical exercise or major dietary changes during the entire study period. Glycyrrhizin (GL) is a corrigent often mixed in drugs and foods. ECG and catechins are major constituents of tea and some foods[8,9]. Thus, foods and drinks not containg such effective compounds were selected to avoid any effects on the pharmacokinetic study of Unpito.

2. Study medication

TJ-8117 (Unpito) encapsulated formulation of a dry powder spray of hot water extracts from the five crude drugs was obtained from Tsumura & Co. (Tokyo, Japan). The contents of total potential ECG, RH and GA as mg equivalent are summarized in Table 1.

3. Study design

In the multiple dose, randomized single blind study, each subject received 4, 6 and 8 capsules of Unpito or placebo with water three times a day, 2 h prior to each meal for seven days. Each meal was served at 10 : 30, 14 : 30, and 19 : 30 every seven days.

After multiple dosing, blood samples were taken before and at 0.5, 1, 2, 4, 6, 8, 12, and 14 h after the first administration of Unpito on Day 1, and at 0, 0.5, 1, 2, 4, 6, 8, 12, 14, 24, 48 h after the first administration of Unpito on Day 7 to determine ECG and RH. Samples were taken each 0 and 12 h after the first administration of Day 2, Day 4 and Day 6 to determine ECG and RH.

After multiple dosing, blood samples were taken before and at 4, 6, 8, 10, 12, and 14 h after the first administration of Unpito on Day 1 and at 0, 4, 6, 8, 10, 12, 14, 24, 48 h after the first administration of Unpito on Day 7 for GA detection. Samples were taken each 0 h of Day 2, Day 4 and Day 6 to determine GA.

Blood samples were collected in tubes containing heparin. The samples were centrifuged and plasma was stored at $-80°C$ until analysis.

Urine was collected before drug administration, at 0-6, 6-12, 12-24, 24-48 h after administration of a single dose, while at 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-156, 156-168, 168-192 h during and after multiple administration of Unpito three times a day for 7 days. A portion of each urine sample was stored at $-80°C$ until analysis.

Vital signs (heart rate, blood pressure, respiration) and adverse events were assessed before each drug administration and several times post administration.

Table 2 Pharmacokinetic parameters of glycyrrhetic acid at Day 7 following the multiple administrations of Unpito three times a day compared with single dosing

Step		Dose (capsules)	Dose (mg eq. of GA)	Parameter			
				AUC (0-48) (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)
Day 7 of multiple dosing	1	4	7.37	N.C.	N.C.	N.C.	N.C.
	2	6	11.05	N.C.	N.C.	N.C.	N.C.
	3	8	14.73	353.39±297.83*	35.61±21.48**	8.67±3.27	N.C.
Single dosing	2	4	7.37	723.88±332.50	44.18±14.77	10.67±2.42	11.58±4.07[a]
	3	8	14.73	786.85±181.77	85.92±28.16	8.33±3.67	7.23±6.37[a]

Mean±s.d., n=6, N.C. : not calculated, [a]n=5, * : $p<0.05$, ** : $p<0.01$ vs. single dosing with t-test

Table 3 Pharmacokinetic parameters of rhein at Day 7 following the multiple administrations of Unpito three times a day compared with single dosing

Step		Dose (capsules)	Dose (mg eq. of RH)	Parameter			
				AUC (0-lim)[a] (μg·h/mL)	C_{max} (μg/mL)	t_{max} (h)	$t_{1/2}$ (h)
Day 7 of multiple dosing	1	4	12.40	8.54±1.08*	1.65±0.34	1.25±0.61	4.74±0.45
	2	6	18.60	13.59±2.77	2.53±0.72	1.50±0.55	5.65±1.05
	3	8	24.81	16.75±1.68	3.03±0.59	1.92±1.20	4.65±0.38*
Single dosing	2	4	12.40	6.84±1.39	1.58±0.33	1.17±0.68	4.86±0.50
	3	8	24.81	18.17±3.63	3.14±0.76	2.08±1.11	4.20±0.19

Mean±s.d., n=6, [a]single dosing : AUC (0-24), multiple dosing : AUC (0-48), * : $p<0.05$ vs. single dosing with t-test

All subjects gave a written, informed consent and the protocol was approved by the independent Tsumura Protocol Review Committee and the Kitasato Institute Ethics Review Committee.

4. Analysis of ECG, RH and GA in plasma

ECG, RH and GA concentrations in the plasma from the subjects receiving Unpito were determined by the methods previously developed in our laboratories (Table 2), as reported in brief in the previous study[7].

The overall analytical methods were validated for accuracy and precision prior to the phase I trials by analyzing the blank plasma spiked with known amounts of the compounds. Inter-day and intra-day coefficients of variation for the plasma control samples were 15% or less except for LLOQ, at which they were 20% or less. Inter-day and intra-day accuracies for the plasma control samples were within ±15% except for LLOQ, at which it was within ±20%.

5. Analysis of VL-2, RH and GA in urine

VL-2, RH and GA concentrations in the urine from subjects receiving Unpito were determined after hydrolysis with β-glucuronidase and sulfatase using the methods previously developed in our laboratories (Table 3), reported in brief in the previous study[7].

The overall analytical methods were validated for accuracy and precision prior to the phase I trials with the same criteria displayed in plasma analysis.

6. Data analysis

Pharmacokinetic parameters for ECG, RH and GA in the plasma were calculated using non-compartmental methods by the computer program PAG-CP (Asmedica, Osaka, Japan). The measured values were directly used as maximum concentration in the plasma (C_{max}) and the time to attain C_{max} (t_{max}). The area under the concentration-time curve (AUC) from time zero to 24 or 48 h was calculated using the trapezoidal rule. The half-life was calculated by dividing 0.693 by the absolute

value of slope.

The cumulative urinary excretion ratios (% of dose) were calculated as follows : (the cumulative excretion of each compound divided by the dose of each compound estimated from the content of potential ECG, RH or GA) ×100. Unpito included 0.35%, 0.55% and 0.93% potential ECG, GA and RH, respectively (Table 2).

The plasma concentration curves were simulated by the nonlinear least-squares regression analysis to achieve best fit with a compartment model by the computer program PAG-CP (Asmedica, Osaka, Japan).

Statistical differences in the pharmacokinetic parameters were determined, as appropriate, using a t-test or analysis of variance with the value set a priori at $p < 0.05$ and $p < 0.01$.

Results

The experimental data of ECG, GA and RH plasma concentrations obtained during the repeated administration of Unpito and multiple-dosing simulations using the pharmacokinetic model are shown in Figures 1, 2, and 3, respectively.
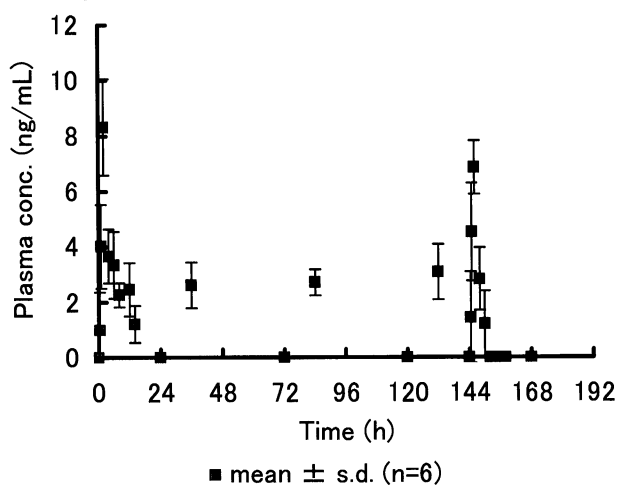
The ECG and GA plasma concentrations at each time point were lower than those simulated. On the other hand, the RH plasma concentrations were consistent with the simulation. The trough plasma concentrations of the three compounds were determined just prior to the first administration on Day 3, Day 5, and Day 6 ; as a result, the concentrations were constant and found to reach steady state. Only a slight variation of peak-trough plasma concentrations was observed during daily administration.

The pharmacokinetic parameters of ECG, GA and RH at Day 7 after multiple dosing of Unpito are summarized in Tables 1, 2 and 3, respectively, where they are compared with those of single dosing. Comparison of the pharmacokinetic parameters at Day 7 of multiple dosing with single dosing indicates a statistically significant difference in terms of the C_{max} and AUC (0-lim) values for ECG and GA at a dose of 8 capsules. For ECG and GA, multiple dosing groups had a lower C_{max} and a lower AUC (0-lim) than the single dosing group. No significant difference in the t_{max} and $t_{1/2}$ values for ECG and GA was observed between the groups. For RH, a statistically significant difference was observed in terms of the AUC (0-lim) and $t_{1/2}$

a) 4 capsules three times a day

······Simulation   ■mean ± s.d. (n=6)

b) 6 capsules three times a day

■ mean ± s.d. (n=6)

c) 8 capsules three times a day
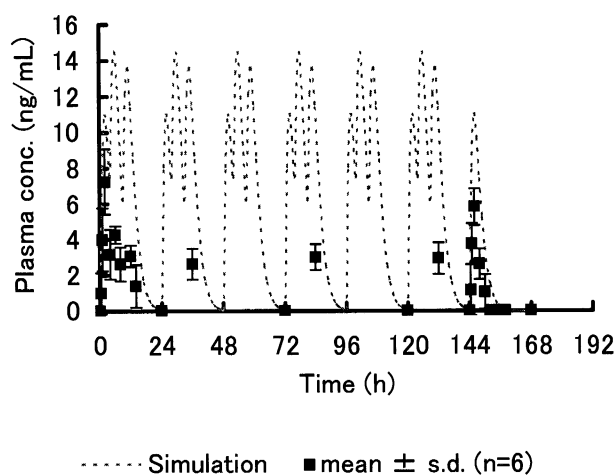

······Simulation   ■mean ± s.d. (n=6)
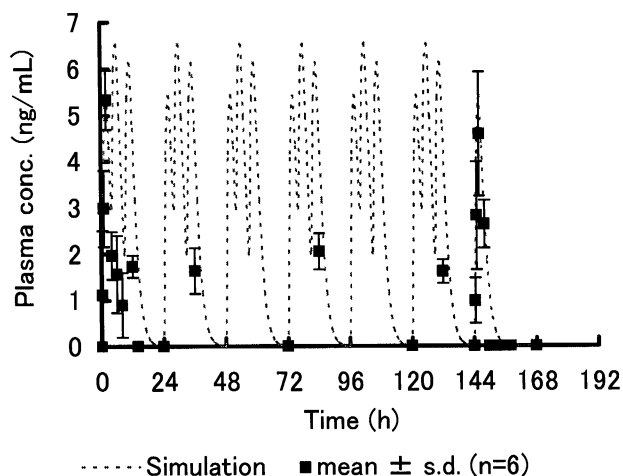
Fig. 1   Plasma concentration-time curve of (−) epicatechin 3-O-gallate during and after multiple dosing of Unpito three times a day

Dotted lines indicate simulations by the single-dose PK model. Each point and bar represents mean±s. d..

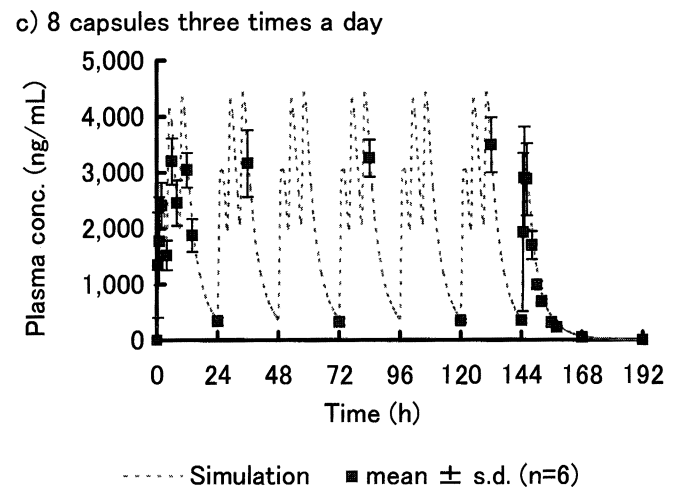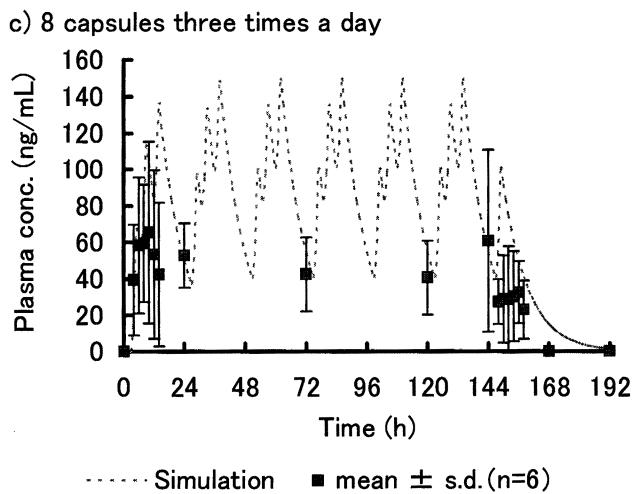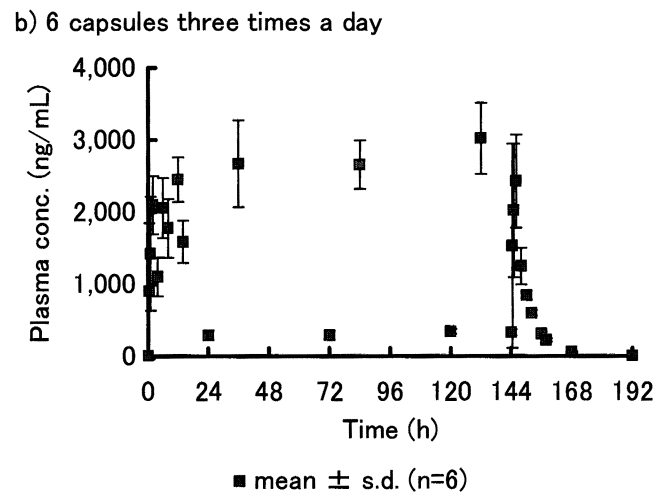a) 4 capsules three times a day

----- Simulation   ■ mean ±s.d. (n=6)

b) 6 capsules three times a day

■ mean ± s.d. (n=6)

c) 8 capsules three times a day
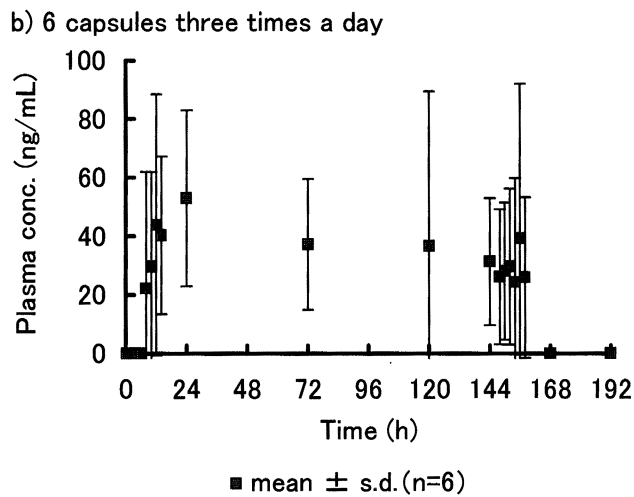

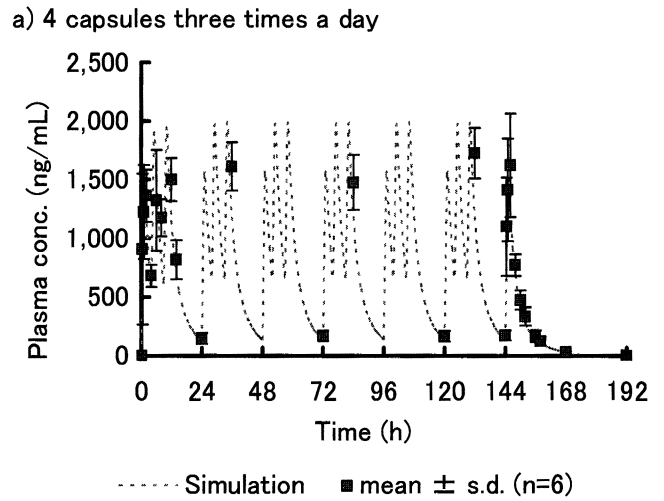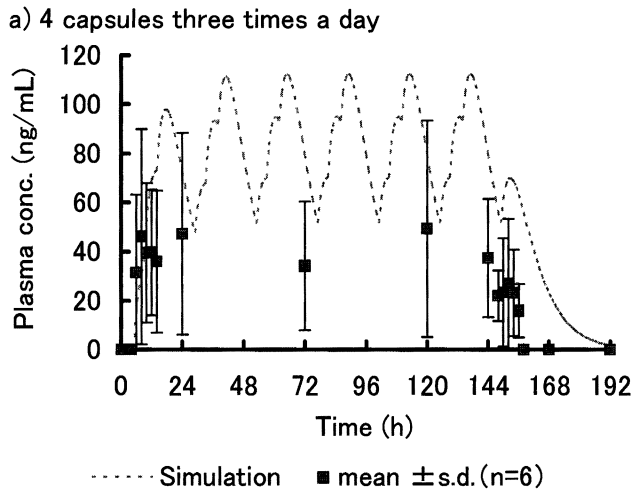----- Simulation   ■ mean ± s.d. (n=6)

Fig. 2 Plasma concentration-time curve of glycyrrhetinic acid during multiple dosing of Unpito three times a day

Dotted lines indicate simulations by the single-dose PK model. Each point and bar represents mean±s. d..

a) 4 capsules three times a day

----- Simulation   ■ mean ± s.d. (n=6)

b) 6 capsules three times a day

■ mean ± s.d. (n=6)

c) 8 capsules three times a day

----- Simulation   ■ mean ± s.d. (n=6)

Fig. 3 Plasma concentration-time curve of rhein during multiple dosing of Unpito three times a day

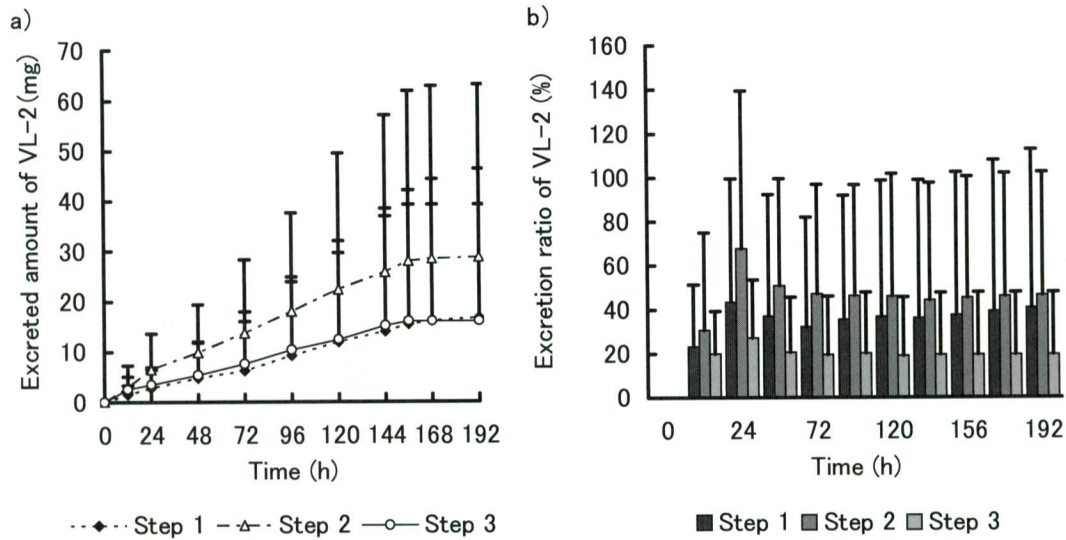Dotted lines indicate simulations by the single-dose PK model. Each point and bar represents mean±s. d..

a)

b)

··◆·· Step 1 −△−· Step 2 —○— Step 3　　　■ Step 1 ■ Step 2 □ Step 3

Fig. 4　Cumulative excreted amount of VL-2+s. d. (a) and excretion ratio+s. d. within each interval (b) following the multiple administrations of Unpito three times a day

a)

b)

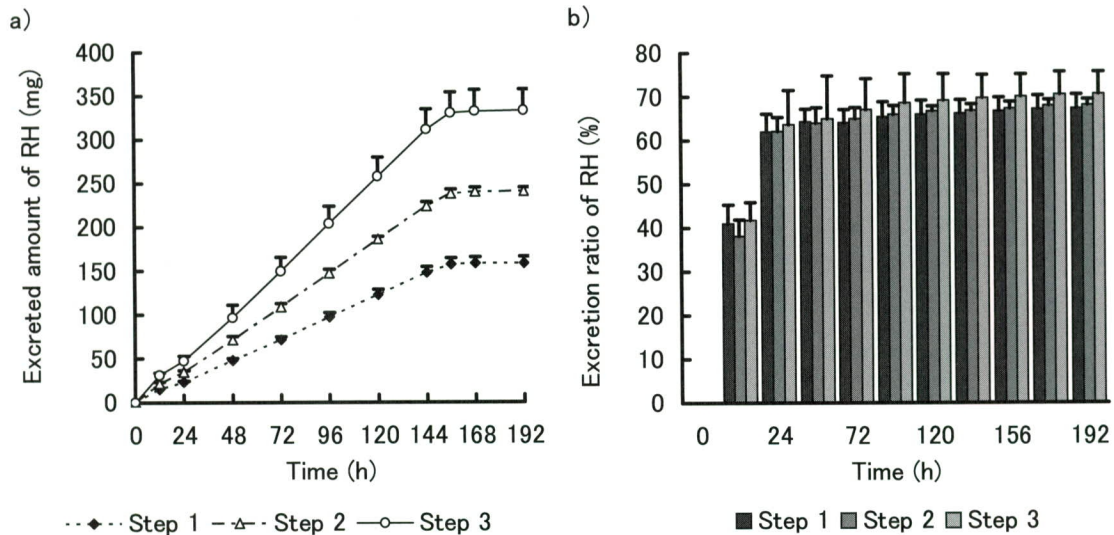··◆·· Step 1 −△−· Step 2 —○— Step 3　　　■ Step 1 ■ Step 2 □ Step 3

Fig. 5　Cumulative excreted amount of rhein+s. d. (a) and excretion ratio+s. d. within each interval (b) following the multiple administrations of Unpito three times a day

values at a dose of 4 and 8 capsules, respectively.

Except for RH, the AUC (0-24) and C_{max} at Day 7 was not dose proportional.

The amounts of VL-2 and RH excreted in the urine after multiple doses of Unpito and the cumulative excretion ratio of the two compounds are shown in Figures 4 and 5, respectively.

The cumulative excretion ratio of VL-2 after multiple dosing of 8 capsules three times a day was lower than the ratio observed in the lower dosing. The cumulative excretions of VL-2 for every 24 h were almost invariable from Day 2 to Day 7. The excretion of VL-2 ended within 24 h after the last

dosing at Day 7.

The cumulative excretion ratio of RH was constant at multiple dosing of 4, 6 and 8 capsules three times a day. The cumulative excretions of RH for every 24 h were almost invariable from Day 2 to Day 7. The excretion of RH ended mostly within 24 h after the last dosing at Day 7.

Since GA was not detected in urine in almost all of the subjects, except for two subjects whose cumulative excretion was 0.01% at multiple dosing of 4 capsules three times a day and one subject whose cumulative excretion was 0.01% at multiple dosing of 8 capsules three times a day.

There were 16 and 6 subjects who reported loose stool and diarrhea, respectively, as a subjective or objective symptom during this study. Since Rhei Rhizoma exerts pharmacological activities which cause diarrhea, the administration of Unpito may have relevance to the loose stool and diarrhea in the studies.

Discussion

The present study is the first report which evaluates ECG, RH and GA pharmacokinetics derived from Unpito in detail after multiple doses in humans.

It was reported that ECG and RH exposure appeared to be dose-proportional in the single dose study, indicating their linear pharmacokinetics. On the other hand, GA did not exhibit linear pharmacokinetics[7].

After the multiple dosing of Unpito, the ECG and GA plasma concentrations were lower than those simulated. Moreover no dose proportional pharmacokinetics was observed in terms of ECG and GA. In the multiple dose study, every food was served at 2 h after the Unpito administration. As ECG pharmacokinetics were affected by food intake in the single dose study, it was presumed that the food effect contributed to the lower concentrations of ECG than those simulated. On the other hand, since GA pharmacokinetics was not linear in the single dose study, it was reasonable that the simulation curves on the assumption of linear pharmacokinetics were not consistent with the observation. Since diarrhea was observed as a side effect of Unpito at the high doses in the multiple study, ECG and GA absorptions were possibly affected by the diarrhea.

RH plasma concentrations were consistent with the simulation and dose proportion, indicating linear pharmacokinetics of RH after multiple dosing of Unpito similar to the single dose study.

Potential accumulations of the three compounds were not observed from the roughly constant plasma concentrations of troughs at 72, 120, and 144 h after the first administration. Significant change in $t_{1/2}$ was not observed between single dosing and Day 7 of multiple dosing except for RH. In the case of RH, it may likely have caused the apparent increase of $t_{1/2}$ two detection points (14, 48 h) which were added in the elimination phase in the

multiple dose study compared with the single dose study.

Urinary excretion of VL-2 ended within 48 h after the last dosing in the multiple dose study as well as in the single dose study. Because the flavan-3-ols, like ECG and other catechins were metabolized to a significant extent by ring-scission in human intestinal bacteria[10,11], it was suggested that the decreasing urinary excretion ratio of VL-2 due to an increase in dose was due to a change of intestinal flora caused by an increase in dose or repeated dosing. The cumulative urinary excretion ratio of RH was constant throughout the single and multiple studies. RH excretions in urine were ended in 24 h in almost all the subjects. GA was not detected in the urine any of the studies. The potential accumulation any of the compounds was not indicated in these results.

In conclusion, the pharmacokinetics of ECG, GA and RH derived from Unpito, an herbal medicine, was examined in healthy volunteers as part of the clinical study of the medicine. In the single dose study, linear pharmacokinetics of ECG and RH were observed, however, GA appeared non-linear. In the multiple dose study, RH exhibited linear pharmacokinetics, while ECG and GA in plasma were lower than the simulation curves. Potential accumulations were not indicated by the trough plasma concentrations or by the urinary excretion of the marker compounds.

References

1) Fujitsuka N, Kurogi A, Hattori T, Shindo S. Effects of onpi-to (TJ-8117) and (−)epicatechin-3-O-gallate on the proliferating changes in glomeruli of 5/6 nephrectomized rats. *Nippon Jinzo Gakkai Shi* 1997 ; **39** : 693-700 (in Japanese).

2) Hattori T, Shindo S, Hisada T, Fujitsuka N, Hibino T, Terazono Y, Maruno M. Effects of Onpi-to (TJ-8117) on mesangial injury induced by anti Thy-1 antibody. *Nippon Yakurigaku Zasshi* 1995 ; **105** : 63-75 (in Japanese).

3) Hattori T, Fujitsuka N, Kurogi A, Shindo S. Protective effect of onpi-to (TJ-8117) on the expression of apoptosis in 5/6 nephrectomized rats. *Nippon Jinzo Gakkai Shi* 1997 ; **39** : 377-86 (in Japanese).

4) Hattori T, Fujitsuka N, Kurogi A, Shindo S. Effect of Onpi-to (TJ-8117) on TGF-beta 1 in rats with 5/6 nephrectomized chronic renal failure. *Nippon Jinzo Gakkai Shi* 1996 ; **38** : 475-83 (in Japanese).

5) Mitsuma T, Yokozawa T, Oura H, Terasawa K. Rhubarb therapy in patients with chronic renal failure (Part 2). *Nippon Jinzo Gakkai Shi* 1987 ; **29** : 195-207 (in Japanese).

6) Mitsuma T, Yokozawa T, Oura H, Terasawa K, Narita M. Clinical evaluation of kampo medication, mainly with wen-pi-tang, on the progression of chronic renal failure. *Nippon Jinzo Gakkai Shi* 1999 ; **41** : 769-77 (in Japanese).

7) Takizawa Y, Mizuhara Y, Morita T, Kase Y, Takeda S, Aburada M, Miyamoto K. Pharmacokinetics of (−) epicatechin 3-*O*-gallate, glycyrrhetic acid and rhein in healthy male volunteers after a single dose administration of TJ-8117 (Unpito), a Japanese traditional medicine for renal failure. *J Clin Pharmacol Ther* 2005 ; **37** : 21-32.

8) Yang CS, Chen L, Lee MJ, Balentine DA, Kuo MC, Schantz S. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomark Prev* 1998 ; **7** : 351-4.

9) Arts IC, van de Putte B, Hollman PC. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000 ; **48** : 1746-51.

10) Meselhy MR, Nakamura N, Hattori M. Biotransformation of (−)-epicatechin 3-O-gallate by human intestinal bacteria. *Chem Pharm Bull* 1997 ; **45** : 888-93.

11) Spencer JP. Metabolism of tea flavonoids in the gastrointestinal tract. *J Nutr* 2003 ; **133** : 3255S-61S.