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# 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

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**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 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 32 healthy volunteers receiving single oral doses (2, 4, 8, and 12 capsules).

**Results :** After a single oral administration, ECG and RH exhibited linear pharmacokinetics in AUC and  $C_{max}$ , while GA did not exhibit linear pharmacokinetics. A cross-over study was conducted to evaluate the effect of food at a single dose of 4 capsules. The effect of food was observed for the plasma concentrations of ECG and RH, while not for GA. The potential accumulations of  $\delta$ -(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone (VL-2), a metabolite of ECG and RH were not observed. GA was not detected in urine.

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

**Key words :** clinical pharmacokinetics, TJ-8117, Unpito, healthy male volunteers, single 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<sup>1-4</sup>. Unpito has been utilized in patients with chronic renal failure<sup>5,6</sup>.

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. ECG is a component of Rhei Rhizoma, reportedly one of the active components of Unpito which has been shown to improved the plasma levels of uremic toxins and various parameters of renal function in rats with adenine-induced renal failure<sup>7,8</sup>. Moreover, ECG suppresses proliferating changes in glomeruli in 5/6 nephrectomized rats<sup>9</sup>.

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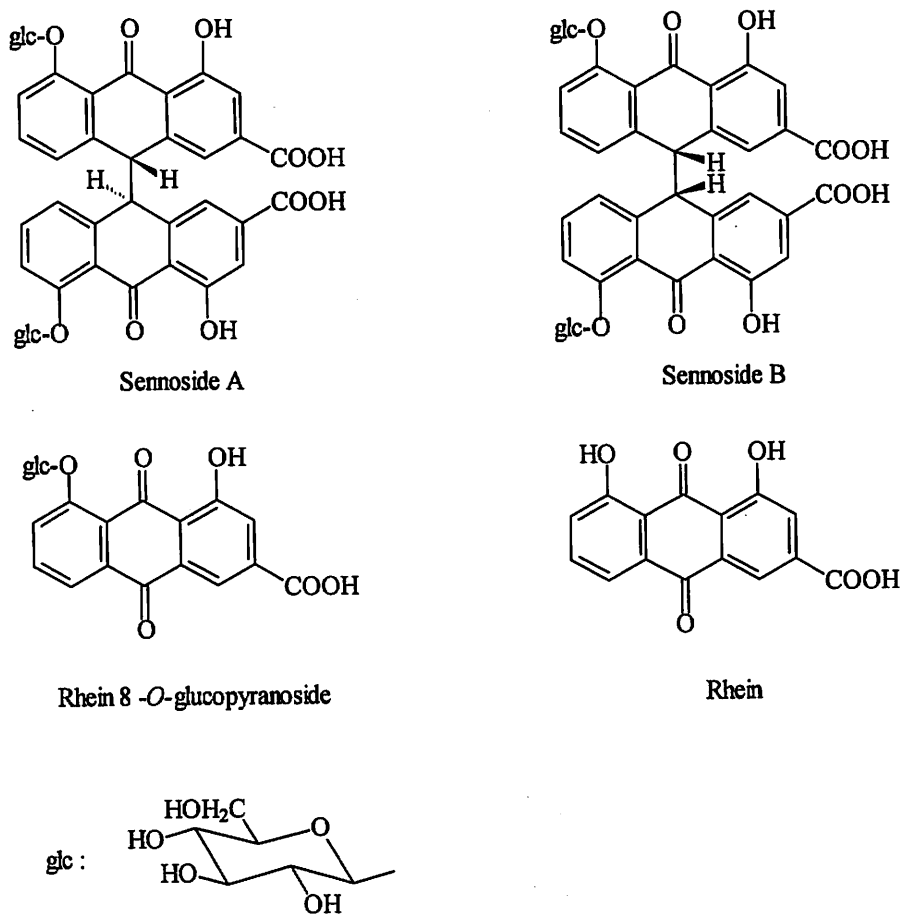


Fig. 1 Structure of anthranoids in Rhei Rhizoma

GA is a metabolite of glycyrrhizin (GL). GL is a major component of *Glycyrrhizae Radix*, and exerts various pharmacological activities, such as anti-inflammatory and hepatoprotective effects<sup>9)</sup>. GL is predominantly metabolized to GA by human intestinal flora<sup>10,11)</sup>. This conversion takes place in the gut prior to absorption. Rhein is a metabolite of anthranoids that are major components of *Rhei Rhizoma*. Anthranoids (Fig. 1), including sennoside A, sennoside B, rhein 8-*O*-glucopyranoside and rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid) cause diarrhea, a side effect of Unpito. Sennosides A and B are homodianthrone diglucosides of rhein and are known to have a laxative effect on the colon. The active compounds are not sennosides themselves but their metabolite rheinanthrone<sup>12)</sup>. Sennosides A and B are transformed to rhein by bacterial enzymes in association with subsequent oxidation by intestinal flora<sup>13,14)</sup>. Rhein 8-*O*-glucopyranoside is the monoanthrone glucoside of rhein and is likely to be also transformed to rhein by bacterial enzymes. From a safety

perspective, it is very important to control the contents of sennosides A and B in the manufacturing process of Unpito.

ECG, sennoside A, B and glycyrrhizin have been adopted as marker compounds for quality control in the manufacturing process of this medicine in our facility. Dosages of these three compounds derived from Unpito are clearer than other components, thus they were judged to be suitable indicators for the pharmacokinetic investigation after Unpito administration.

Recently, the biotransformation of ECG *in vitro* in human and rat fecal suspensions is reported<sup>15)</sup>.  $\delta$ -(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone (VL-2; Fig. 2, III) and (-)-epicatechin (EC; Fig. 2, II) were identified in addition to twelve other metabolites. Since ECG was not excreted in the urine in humans, VL-2, an alternative of ECG, was selected as a urinary marker compound. Additionally, RH and GA were selected as urinary marker compounds.

The disposition of the marker compounds was

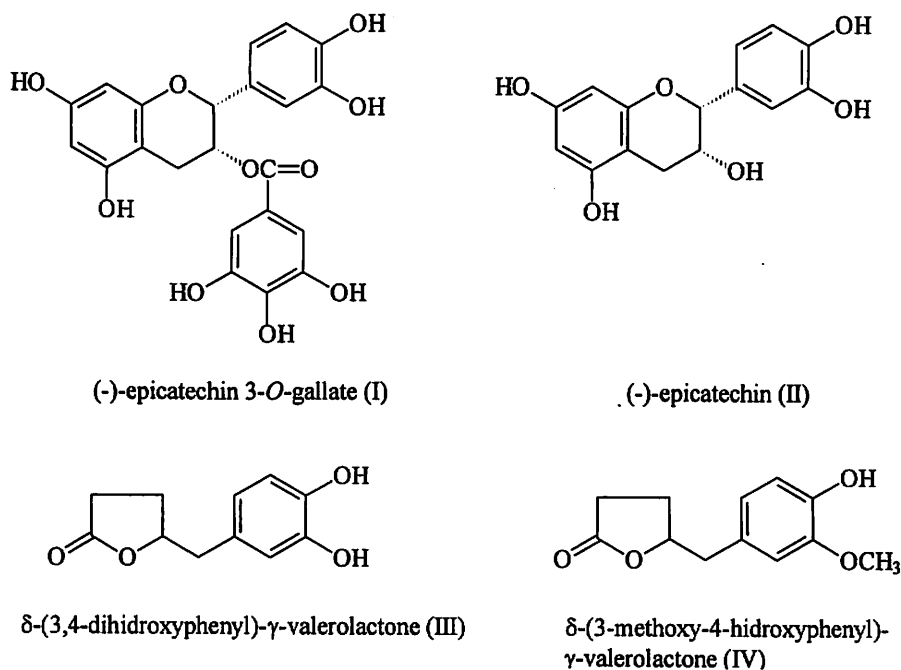


Fig. 2 Structure of (-)-epicatechin 3-O-gallate and its metabolites

evaluated in healthy volunteers who received single oral doses (2, 4, 8, and 12 capsules). This study was conducted as part of a phase I study of Unpito with healthy volunteers.

## Materials and Methods

### 1. Subjects

The study involved 32 healthy male volunteers receiving a single dose. All subjects were male and their age ranged from 20 to 34 years. The body weight ranged from 51.0 to 81.4 kg. The height ranged from 160.3 to 186.9 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.

GL is a corrigent often mixed in drugs and foods. ECG and catechins are major constituents of tea and some foods<sup>16,17</sup>. Thus, foods and drinks not

containing 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 spray-dried powder 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 single dose, randomized single blind study, each subject received Unpito or placebo 2, 4, 8, and 12 capsules prior to breakfast with water. After administration, blood samples were taken before and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h to determine ECG and RH, or at 4, 6, 8, 10, 12, 14, 24, and 48 h after administration to determine GA. The effects of food were estimated in an open two occasion cross-over study with at least a week between occasions, receiving a single oral dose of Unpito 4 capsules prior to or after breakfast with water.

Blood samples were collected in tubes containing heparin. The samples were centrifuged and plasma was stored at  $-80^{\circ}\text{C}$  until analysis.

Urine was collected before drug administration,

**Table 1** The dose of Unpito and its marker compounds in the single dose study of healthy volunteers

Step	Dose	Unpito (capsules)	Extracted powder of Unpito (mg)	ECG (mg)	GA (mg eq.)	RH (mg eq.)
1		2	665.5	2.30	3.68	6.20
2	(fasted)	4	1331	4.60	7.37	12.40
Cross-over study	(fed)	4	1331	4.60	7.37	12.40
3		8	2662	9.20	14.73	24.81
4		12	3993	13.80	22.10	37.21

**Table 2** Analytical methods of the marker compounds during phase I trials

Compounds	Matrix	Instruments	Column	Mobile phase	Flow rate (mL/min)	Lower limit of quantitation (ng/mL)	Range of standard curve (ng/mL)
(-)-Epicatechin 3-O-gallate	Plasma	LC/MS/MS	YMC-Pack MB-ODS AM (2.1 mmI. D.*150 mm)	CH <sub>3</sub> OH/0.1% TFA (4/6, v/v)	0.2	1.0	1.0-200
VL-2	Urine	LC/MS/MS		CH <sub>3</sub> OH/5 mM HCOONH <sub>4</sub> /HCOOH (400/600/2, v/v/v)	0.2	1000	1000-200000
Glycyrrhetic acid	Plasma	HPLC-UV	Cosmosil 5C18-300 (4.6 mmI. D.*250 mm)	CH <sub>3</sub> OH/2%CH <sub>3</sub> COOH (8/2, v/v)	1	10	10-1000
	Urine	HPLC-UV				50	50-5000
Rhein	Plasma	HPLC-UV	Cosmosil 5C18-AR (4.6 mmI. D.*150 mm)	CH <sub>3</sub> OH/0.5%H <sub>3</sub> PO <sub>4</sub> / CH <sub>3</sub> COOH	1	10	10-1000
	Urine	HPLC-UV		(300/700/15, v/v/v)		100	100-50000

at 0-6, 6-12, 12-24, 24-48 h after administration of a single dose. 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.

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). Standard curves were prepared over the plasma concentration ranges of 1.0 to 200 ng/mL, 10 to 1,000 ng/mL and 10 to 1,000 ng/mL for ECG, GA and RH, respectively. In addition,

plasma control samples were prepared and analyzed in relation to the subject samples from the pharmacokinetic studies. The accuracy of the plasma control samples was within ±15% except for the lower limit of quantitation (LLOQ) at which it was within ±20%. Stability studies showed that ECG, GA and RH in plasma were hardly degraded when frozen (-80°C) during a period up to the last analysis of the samples. Standard curves were linear over the tested concentration range.

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 controls 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  $\pm 20\%$ .

### 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  $\beta$ -glucuronidase and sulfatase using the methods previously developed in our laboratories (Table 2). Standard curves were prepared over the urine concentration ranges of 1,000 to 200,000 ng/mL, 50 to 5,000 ng/mL and 100 to 50,000 ng/mL for VL-2, GA and RH, respectively. In addition, urine control samples were prepared and analyzed in relation to the subject samples from the pharmacokinetic studies. Stability studies showed that there was minimal degradation of each compound in urine when frozen during a period up to the last analysis of the samples. The control samples were assayed at the same time when the subject samples were assayed. Standard curves were linear over the tested concentration range.

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. HPLC

#### 1) ECG and VL-2

The HPLC system consisted of a 626 solvent delivery system, a 717 plus autoinjector (Waters Associates, Milford, MA), and a TSQ7000 spectrometer (FinniganMAT/Thermoquest, San Jose, CA). Chromatography was performed on a YMC-Pack MB-ODS AM C18 column (2.1 mm  $\times$  150 mm, 5  $\mu$ m) with a mobile phase containing a mixture of 0.1% (v/v) TFA (solvent A) and methanol (solvent B). The mobile phase was composed of solvent A/solvent B (6 : 4). A flow rate of 0.2 mL/min was used for all analyses. Quantification of the metabolite, VL-2, was carried out by the same HPLC system, with a mobile phase containing a mixture of 5 mM ammoniumformate/formic acid/methanol (600/2/400).

LC/MS/MS was conducted with a TSQ7000 spectrometer equipped with an electrospray ion source. The electrospray interface was operated at 4,500 V, and the mass spectrometer was operated in the positive ion mode with the collision energy of 30 eV (ECG) or 35 eV (VL-2) and a pressure of approximately 2.0 mTorr. Multiple reaction

monitoring transitions for ECG, VL-2 and IS were 443/123, 209/103 and 445/125, respectively.

#### 2) GA

The HPLC system consisted of a LC-10AD solvent delivery system (Shimadzu Ltd., Kyoto, Japan), SIL-10AXL auto injector (Shimadzu), and a SPD-10A UV spectrometer (Shimadzu) set at 254 nm. Chromatography was performed on a Cosmosil 5C18-300 column (4.6 mm  $\times$  250 mm) with a mobile phase containing a mixture of 2% (v/v) acetic acid (solvent A) and methanol (solvent B). The mobile phase was composed of solvent A/solvent B (20 : 80). A flow rate was 1 mL/min.

#### 3) RH

The HPLC system consisted of a LC-10AD solvent delivery system (Shimadzu), SIL-10A auto injector (Shimadzu), and a SPD-10A UV spectrometer (Shimadzu) set at 430 nm. Chromatography was performed on a Cosmosil 5C18-AR-300 column (4.6 mm  $\times$  150 mm) with a mobile phase consisted of a 700/15/300 (v/v/v) mixture of 0.5% phosphoric acid, acetic acid, and acetonitrile. A flow rate of 1 mL/min was used.

### 7. Quantitation of ECG in plasma

Aliquots of plasma (1,000  $\mu$ L) were pipetted into tubes. Then, 4,000  $\mu$ L of citric acid buffer (pH 2.0) and 30  $\mu$ L internal standard (IS) [2,3-D] ( $\pm$ )epicatechin 3-O-gallate was added (500 ng/mL in 75 : 25 ethanol/water). Before extraction of samples, a Bond Elut C18 (Varian Associates, Inc., Palo Alto, CA) was conditioned with 3,000  $\mu$ L methanol, 3,000  $\mu$ L water, followed by 3,000  $\mu$ L of citric acid buffer (pH 2.0). Plasma samples were applied to the preconditioned extraction columns. The column was washed with 6,000  $\mu$ L of citric acid buffer (pH 2.0), 6,000  $\mu$ L water, and 6,000  $\mu$ L n-hexane. Samples were eluted into tubes with 6,000  $\mu$ L of 1% (v/v) acetic acid/acetonitrile (3/7). The eluate was evaporated under nitrogen until dryness. Samples were reconstituted in 150  $\mu$ L mobile phase/water (2 : 1) and 40  $\mu$ L was injected into the HPLC column.

### 8. Quantitation of VL-2 in urine

Aliquots of urine (490  $\mu$ L) were pipetted into tubes. Then, 10  $\mu$ L of 75 : 25 ethanol/water was added. Aliquots of the samples (50  $\mu$ L) were incubated at 37°C with 50  $\mu$ L  $\beta$ -glucuronidase (20

mg/mL) and 200  $\mu$ L 0.1 M sodium acetate (pH 5). The reaction was terminated after 2 h by the addition of 150  $\mu$ L acetonitrile and 50  $\mu$ L IS (50  $\mu$ g/mL). After centrifugation, the supernatants (200  $\mu$ L) were mixed with 800  $\mu$ L water and 4,000  $\mu$ L 0.1 M sodium acetate (pH 5). Solid phase extraction was performed under the same condition as quantitation of ECG in plasma. The eluate was evaporated under nitrogen until dryness. Samples were reconstituted in 150  $\mu$ L mobile phase/water (2 : 1) and 10  $\mu$ L was injected into the HPLC column.

### 9. Quantitation of GA in plasma

Aliquots of plasma (250  $\mu$ L) were pipetted into tubes. Then, 500  $\mu$ L of 0.1 M sodium dihydrogen phosphate buffer (pH 3.0), 10  $\mu$ L IS (3-*O*-acetylglycyrrhetic acid; 25  $\mu$ g/mL in methanol) and 5 mL diethylether were added. The mixture was centrifuged at 3,000 rpm for 5 min after vortex mixing for 5 min. The diethylether extract was transferred; 5 mL diethylether was added to the water phase, and the mixture was centrifuged at 3,000 rpm for 5 min after vortex mixing for 5 min. The diethylether extract was mixed with the second extract and dried under nitrogen. The residues were reconstituted with 400  $\mu$ L of chloroform. Before the solid phase extraction of samples, a Bond Elut SI (Varian Associates, Inc.) was conditioned with a 2,000  $\mu$ L 7/3 (v/v) mixture of chloroform and methanol, 2,000  $\mu$ L ethyl acetate, followed by 4,000  $\mu$ L of chloroform. The samples were applied to the preconditioned extraction columns. The column was washed with 5,000  $\mu$ L of ethyl acetate, and an 800  $\mu$ L 7/3 (v/v) mixture of chloroform and methanol. Samples were eluted into tubes with 800  $\mu$ L of a 7/3 (v/v) mixture of chloroform and methanol. The eluate was evaporated under nitrogen until dryness and reconstituted in 500  $\mu$ L acetonitrile. The samples were centrifuged after mixing with 1,000  $\mu$ L n-hexane. The samples were evaporated under nitrogen until dryness after n-hexane extract was removed and reconstituted in 150  $\mu$ L mobile phase, followed by 70  $\mu$ L injection into the HPLC column.

### 10. Quantitation of GA in urine

Aliquots of urine (100  $\mu$ L) were incubated at 37°C with 200  $\mu$ L  $\beta$ -glucuronidase (8,000 Unit/mL

in 0.1 M sodium acetate (pH 5)). The reaction was terminated after 2 h by the addition of 5,000  $\mu$ L diethylether and 50  $\mu$ L IS (2.5  $\mu$ g/mL). The mixture was centrifuged at 3,000 rpm for 5 min after vortex mixing for 10 min. The diethylether extract was transferred and dried under nitrogen. The residues were reconstituted with 400  $\mu$ L of chloroform. Solid phase extraction was performed under the same condition as quantitation of GA in plasma. The eluate was evaporated under nitrogen until dryness and reconstituted in a 200  $\mu$ L mobile phase, followed by 50  $\mu$ L injection into the HPLC column.

### 11. Quantitation of RH in plasma

Before solid phase extraction of samples, a Bond Elut C8 (Varian Associates, Inc.) was conditioned with 1,000  $\mu$ L methanol, followed by 2,000  $\mu$ L of 0.1 M citric acid buffer (pH 2.0). Aliquots of plasma (300  $\mu$ L), 600  $\mu$ L of citric acid buffer (pH 2.0) and 100  $\mu$ L IS (2.5  $\mu$ g/mL of alizarin in 50% N,N-dimethylacetamide) were applied to the preconditioned extraction columns. The column was washed with 2,000  $\mu$ L of citric acid buffer (pH 2.0) and 1,000  $\mu$ L n-hexane. Samples were eluted into tubes with 1,000  $\mu$ L of acetonitrile. The eluate was evaporated under nitrogen until dryness. Samples were reconstituted in the 300  $\mu$ L mobile phase and 100  $\mu$ L was injected into the HPLC column.

### 12. Quantitation of RH in urine

Aliquots of urine (100  $\mu$ L) were incubated at 37°C with 200  $\mu$ L  $\beta$ -glucuronidase (8,000 Unit/mL in 0.1 M sodium acetate (pH 5)). The reaction was terminated after 2 h by the addition of 5,000  $\mu$ L 3/2 (v/v) mixture of diethylether and dichloromethane, and 100  $\mu$ L IS (5.0  $\mu$ g/mL). The mixture was centrifuged at 3,000 rpm for 5 min after vortex mixing for 10 min. The organic layer extract was transferred and the lower water layer was centrifuged at 3,000 rpm for 5 min after vortex mixing for 10 min with 5,000  $\mu$ L 3/2 (v/v) mixture of diethylether and dichloromethane. The organic layer extracts were mixed and dried under nitrogen. The residues were reconstituted with 20% N,N-dimethylacetamide and 80% mobile phase, followed by 70  $\mu$ L injection into the HPLC column.

### 13. 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 the 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)  $\times 100$ . 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 plasma concentrations-time curves of ECG, GA and RH after single oral dosages of Unpito are given in Figures 3, 4, and 5, respectively. The pharmacokinetic parameters of ECG, GA and RH are

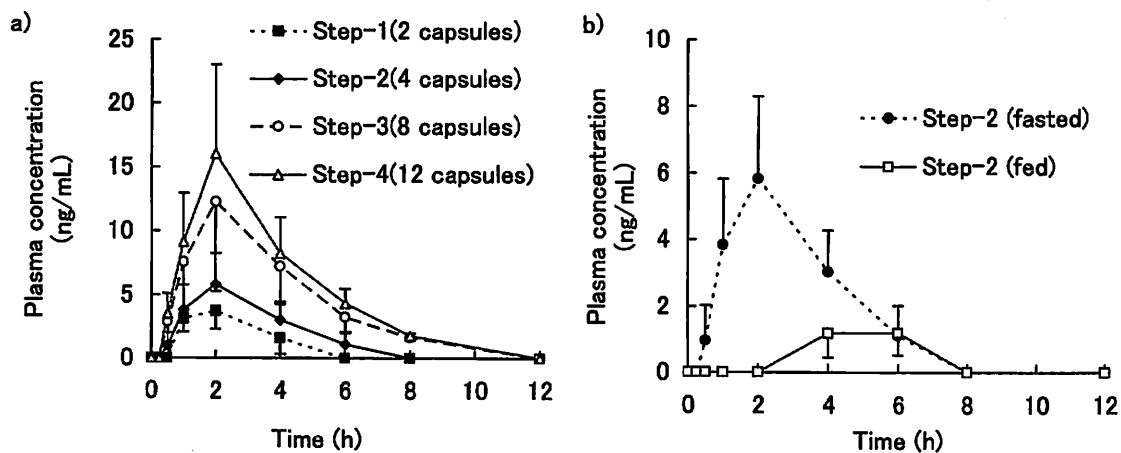


Fig. 3 Mean plasma concentration-time curves of (-)-epicatechin 3-O-gallate (ECG)  $\pm$  s.d. in 6 healthy subjects after single oral administration of Unpito; dose dependency (a), food effect (b)

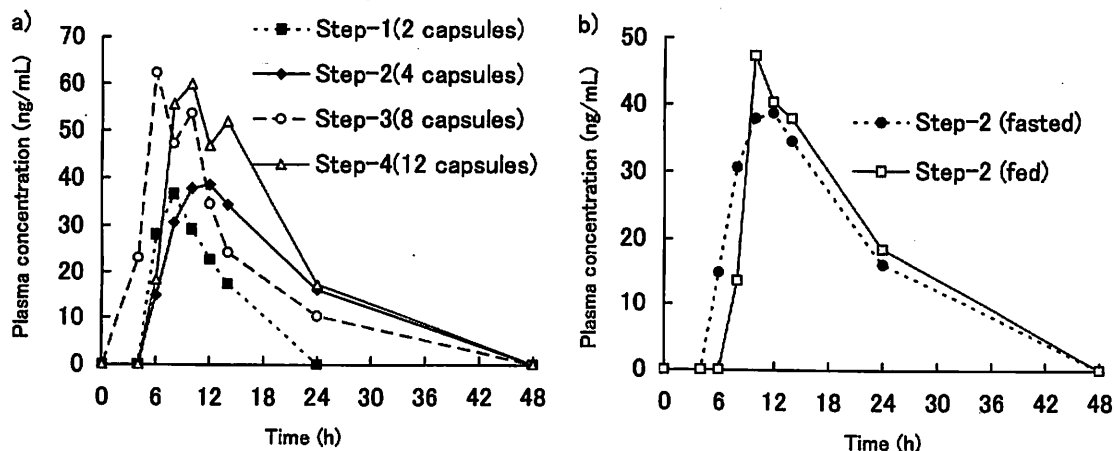


Fig. 4 Mean plasma concentration-time profiles of glycyrrhetic acid (GA) in 6 healthy subjects after single oral administration of Unpito; dose dependency (a), food effect (b)



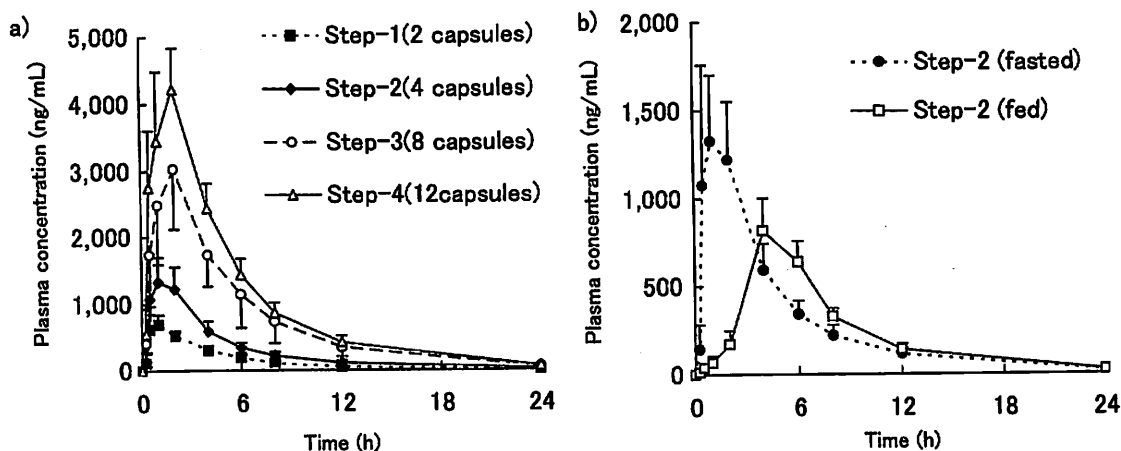


Fig. 5 Mean plasma concentration-time profiles of rhein (RH)  $\pm$  s. d. in 6 healthy subjects after single oral administration of Unpito; dose dependency (a), food effect (b)

Table 3 Mean plasma pharmacokinetic parameters of (-)epicatechin 3-O-gallate in 6 healthy subjects after single oral administration of Unpito

Step	Dose		Parameter (mean $\pm$ s. d., n=6)			
	(capsules)	(mg of ECG)	AUC (0-24) (ng·h/mL)	$C_{max}$ (ng/mL)	$t_{max}$ (h)	$t_{1/2}$ (h)
1	2	2.3	12.47 $\pm$ 6.00	4.00 $\pm$ 1.21	1.67 $\pm$ 0.52	N. C.
2	(fasted)	4	21.35 $\pm$ 9.55	5.93 $\pm$ 2.42	1.83 $\pm$ 0.41	2.00 $\pm$ 0.18 <sup>a)</sup>
	(fed)	4	4.77 $\pm$ 2.30	1.48 $\pm$ 0.47	4.67 $\pm$ 1.03	N. C.
3	8	9.2	52.96 $\pm$ 20.82	13.43 $\pm$ 4.21	2.17 $\pm$ 0.98	2.15 $\pm$ 0.41 <sup>b)</sup>
4	12	13.8	64.43 $\pm$ 16.28	16.03 $\pm$ 6.97	2.00 $\pm$ 0.00	1.98 $\pm$ 1.06

N. C. : not calculated, <sup>a)</sup>n=4, <sup>b)</sup>n=5

Table 4 Mean plasma pharmacokinetic parameters of glycyrrhetic acid in 6 healthy subjects after single oral administration of Unpito

Step	Dose		Parameter (mean $\pm$ s. d., n=6)			
	(capsules)	(mg eq. of GA)	AUC (0-48) (ng·h/mL)	$C_{max}$ (ng/mL)	$t_{max}$ (h)	$t_{1/2}$ (h)
1	2	3.68	455.53 $\pm$ 166.66	49.03 $\pm$ 21.36	9.33 $\pm$ 3.50	4.00 $\pm$ 2.31 <sup>a)</sup>
2	(fasted)	4	723.88 $\pm$ 332.50	44.18 $\pm$ 14.77	10.67 $\pm$ 2.42	11.58 $\pm$ 4.07 <sup>b)</sup>
	(fed)	4	742.82 $\pm$ 182.31	54.65 $\pm$ 25.08	13.33 $\pm$ 5.47	8.02 $\pm$ 1.45 <sup>a)</sup>
3	8	14.73	786.85 $\pm$ 181.77	85.92 $\pm$ 28.16	8.33 $\pm$ 3.67	7.23 $\pm$ 6.37 <sup>b)</sup>
4	12	22.10	966.85 $\pm$ 588.96	72.67 $\pm$ 29.63	11.33 $\pm$ 2.42	8.24 $\pm$ 8.78 <sup>a)</sup>

N. C. : not calculated, <sup>a)</sup>n=4, <sup>b)</sup>n=5,

summarized in Tables 3, 4 and 5, respectively.

The AUC and  $C_{max}$  of ECG after single doses of Unpito were increased with the increase of dose, but the half life,  $t_{1/2}$  of 2 h were approximately invariable (Table 3). The pharmacokinetic parameters of GA after single doses of Unpito were not

dose proportional (Table 4). The AUC and  $C_{max}$  of RH after single doses of Unpito were dose proportional with approximately invariable half life,  $t_{1/2}$  of 4-5 h (Table 5). ECG and RH exposure were affected by food intake, with a decrease of  $C_{max}$  and a delay of  $t_{max}$  (Fig. 3, Fig. 5). The effect of food

**Table 5** Mean plasma pharmacokinetic parameters of rhein in 6 healthy subjects after single oral administration of Unpito

Step	Dose		Parameter (mean±s.d., n=6)			
	(capsules)	(mg eq. of RH)	AUC (0-24) ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	$C_{\text{max}}$ ( $\mu\text{g}/\text{mL}$ )	$t_{\text{max}}$ (h)	$t_{1/2}$ (h)
1	2	6.20	3.36±0.46	0.77±0.25	1.00±0.55	4.07±1.28
2 (fasted) (fed)	4	12.40	6.84±1.39	1.58±0.33	1.17±0.68	4.86±0.50
	4	12.40	5.47±0.78	0.84±0.16	4.33±0.82	4.33±0.41
3	8	24.81	18.17±3.63	3.14±0.76	2.08±1.11	4.20±0.19
4	12	37.21	24.19±3.65	4.38±0.70	1.83±0.41	4.26±0.29

**Table 6** Cumulative urinary excretions of VL-2 after single oral administration of Unpito in healthy volunteers

Step	Dose		Amount excreted (0-48 h) (mg)	Excretion ratio (0-48 h) (%)
	(capsules)	(mg eq. of VL-2)		
1	2	1.08	1.74±1.14	160.69±104.98
2 (fasted) (fed)	4	2.17	2.99±1.96	138.16±90.49
	4	2.17	3.12±1.88	144.01±86.74
3	8	4.33	4.76±2.22	109.85±51.28
4	12	6.50	3.06±3.71	47.09±57.17

Mean±s.d., n=6

**Table 7** Cumulative urinary excretions of rhein after single oral administration of Unpito in healthy volunteers

Step	Dose		Amount excreted (0-48 h) (mg)	Excretion ratio (0-48 h) (%)
	(capsules)	(mg eq. of rhein)		
1	2	6.20	4.18±0.24	67.33±3.93
2 (fasted) (fed)	4	12.40	8.45±0.79	68.10±6.40
	4	12.40	8.07±0.77	65.05±6.24
3	8	24.81	16.67±0.82	67.19±3.30
4	12	37.21	24.23±1.41	65.11±3.79

Mean±s.d., n=6

was not observed in the GA plasma concentrations (Fig. 4).

The amounts of VL-2 and RH excreted in the urine after a single dose of Unpito are given in Tables 6 and 7, respectively.

The cumulative urinary excretion ratio of VL-2 decreased with the increase in dose of Unpito, though without statistical significance. The concentrations of VL-2 were below the LLOQ up to 24 h after dosing. GA was not detected in urine of any subject. The total urinary excretion ratio of RH was approximately constant: 65.05–67.33% in all doses; >97% of the total excretion of RH was completed in 24 h after dosing. No food effect was observed in the urinary excretions of VL-2 and RH (Table 6, Table 7).

The plasma and urine of the subjects in all of the placebo groups in the single dose study were analyzed similar to the other subjects given Unpito, in terms of the marker compounds. No marker compounds were detected in plasma and urine of

placebo groups except for one subject whose urinary sample contained a small amount of VL-2 prior to dosing of placebo capsules.

There were 11 and 7 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 the 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 pharmacokinetics derived from an herbal medicinal in detail after a single dose in humans. ECG is one of the constituents in tea as well as Rhei Rhizoma. Since green tea has been shown to exhibit cancer-preventive activities in preclinical studies, pharmacokinetic studies of tea catechins have been performed in human volunteers<sup>18,19</sup>. Principal active components in tea include epigallocatechin

gallate (EGCG), epigallocatechin (EGC), epicatechin (EC) and ECG, of which EGCG is the most abundant and possesses the most potent antioxidative activity. Plasma concentrations of individual tea catechins at a relative high dose (1.5 mmol) after a single oral dose in humans are reported<sup>20</sup>. In the report,  $t_{1/2}$  of the enzymatically deconjugated ECG was 6.9 h which was longer than that of unchanged ECG from the present study. Because of the relatively lower level of ECG in plasma at a normal consumption of tea, its pharmacokinetics after administration of tea in human has not been evaluated sufficiently compared with other catechins such as EGCG<sup>21,22</sup>.

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.

For the past two decades, pharmacokinetic studies of Kampo medicines in healthy volunteers have been conducted in our laboratory. Thirteen Kampo medicines containing the extract of *Glycyrrhizae Radix* were evaluated in terms of GA plasma concentrations after single oral dosing in healthy volunteers (results not shown). Except for Unpito, AUCs of GA were doseproportional. Since *Rhei Rhizoma* is contained in Unpito that differs from the other twelve herbal medicines, it was

suggested that some compounds in *Rhei Rhizoma* potentially contributed to the non-linear pharmacokinetics of GA after Unpito dosing.

The non-linear pharmacokinetics of GA was also observed in a pre-clinical study in rats after oral administration of Unpito (results not shown). Moreover, GA plasma concentrations were decreased with an increase in dose of sennoside A, one of the anthranoids in *Rhei Rhizoma*, after the concomitant administration of sennoside A with GL in rats (results not shown). At the dose of sennoside A at which the laxative effect did not appear, the linearity of the GA pharmacokinetics was lost, suggesting that the laxative effect was not a dominant factor affecting the GA pharmacokinetics. Additional studies are needed to elucidate the mechanism of the interaction between GA and anthranoids in the pharmacokinetics of GA in humans.

Since the food effect on the plasma concentrations was observed in terms of ECG, an active component of Unpito, to decrease its exposure, it was recommended to administer this medicine prior to the food intake in subsequent trials.

The influence of renal functions on the pharmacokinetics of RH after a single administration of diacerein, an anti-inflammatory, analgesic drug, was reported<sup>23</sup>. Diacerein is completely metabol-

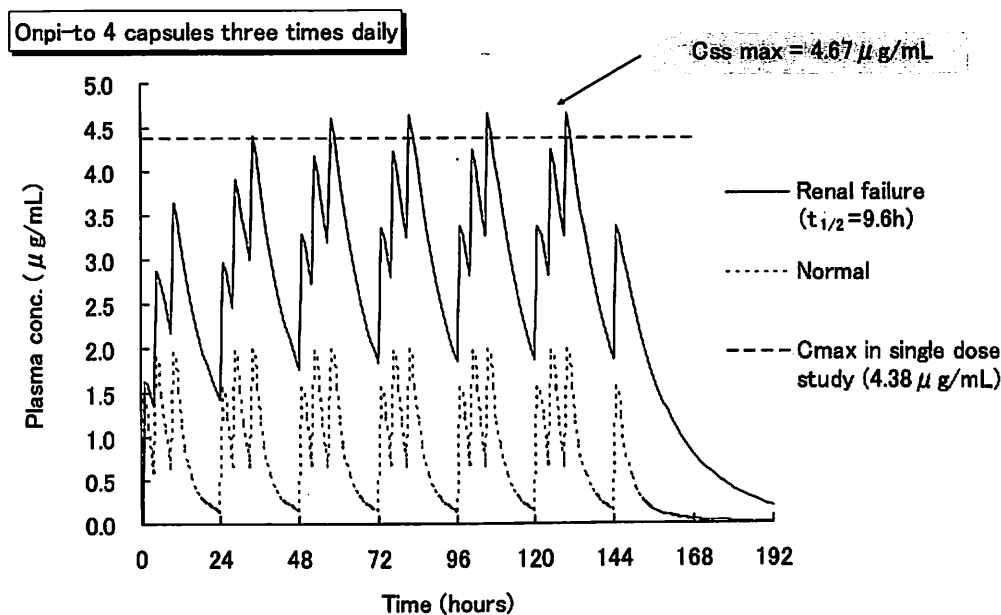


Fig. 6 Simulation curves of rhein plasma concentrations after multiple dose of Unpito 4 capsules three times a day in a patient with renal failure compared with a healthy subject

ized in animals and humans into RH. In the report,  $t_{1/2}$  of RH was 4.3 h in subjects with normal renal function and 6.9 and 9.6 h in uraemic patients with mild and severe renal failure, respectively. The  $t_{1/2}$  of the normal subjects was consistent with that in our study, 4.07–5.65 h after single or multiple dose of Unpito. Since it might be useful to predict RH exposure in the patients of renal failure in early-phase II clinical trials, simulation of RH in plasma with multiple dosing in the patients was estimated under some assumptions. The simulation curves after administration of Unpito 4 capsules three times a day are shown in Figure 6, on the assumption that no change of pharmacokinetic parameters was observed except for  $t_{1/2}$  which was 9.6 h, compared with the parameters of healthy volunteers after a single dose of Unpito of 4 capsules. From the simulations, it was indicated that the  $C_{max}$  of the steady state ( $C_{ss\ max}$ ;  $4.67\ \mu\text{g}/\text{mL}$ ) in patients was about the same as that ( $4.38\ \mu\text{g}/\text{mL}$ ) after a single dose of Unpito 12 capsules which was the highest exposure of plasma RH in the present study.

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. This is the first study presenting pharmacokinetics of ECG, GA and RH derived from Unpito, an herbal medicine in healthy volunteers. In the single dose study, linear pharmacokinetics of ECG and RH were observed, however, GA appeared non-linear. Potential accumulations were not indicated by the trough plasma concentrations or by the urinary excretion of the marker compounds.

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