

Pharmacokinetics of Rhein from Onpi-to, an Oriental Herbal Medicine, in Rats

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Onpi-to, an herbal medicine composed of five crude drugs (Rhei Rhizoma, Glycyrrhizae Radix, Ginseng Radix, Zingiberis Rhizoma and Aconiti Tuber), was administered orally to rats. Onpi-to includes 1.240% of total potential rhein derived from sennoside A, sennoside B, rhein 8-*O*-glucopyranoside and rhein. Plasma, urinary and biliary levels of rhein were determined by an HPLC-UV method. The plasma levels displayed curves characterized by maximum peaks at 8.3 ± 5.2 min, 8.3 ± 5.2 min and 20.0 ± 21.9 min following dosages of 125, 250 and 500 mg/kg with mean concentrations of 1302.5 ± 926.4 , 2973.6 ± 684.3 and 3118.8 ± 1701.2 ng/ml, respectively, followed by a subsequent decline. Area under the concentration–time curve (*AUC*)(0–48 h) at doses of 125, 250 and 500 mg/kg were 752.3 ± 321.5 , 2443.3 ± 554.4 and 4443.2 ± 2641.3 ng·h/ml, respectively. In female rats, rhein plasma levels showed curves which had a maximum peak at 45.0 ± 16.4 min after a dosage of 250 mg/kg with mean concentration of 3058.0 ± 1533.7 ng/ml, followed by a subsequent decline. *AUC*(0–48 h) was 5537.7 ± 1876.0 ng·h/ml. The cumulative urinary excretion of rhein and of conjugated rhein was $3.14 \pm 1.56\%$ and $38.21 \pm 18.87\%$ of dose, respectively, 48 h after dosing at 500 mg/kg of Onpi-to in male rats. The cumulative biliary excretion of rhein was $1.34 \pm 0.44\%$ of dose 48 h after dosing at 500 mg/kg of Onpi-to in male rats.

Key words Onpi-to; rhein; pharmacokinetic; rat; HPLC; anthranoid

Onpi-to, an herbal medicine composed of five crude drugs (Rhei Rhizoma, Glycyrrhizae Radix, Ginseng Radix, Zingiberis Rhizoma and Aconiti Tuber), has been reported to improve renal function in rats with renal failure¹⁾ and has also been used in patients with chronic renal failure.²⁾ One of the components of Onpi-to, Rhei Rhizoma, is widely used as a laxative. The primary constituents of Rhei Rhizoma are anthranoids (Fig. 1), including sennoside A, sennoside B, rhein 8-*O*-glucopyranoside and rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid). Sennoside A and sennoside B are homodianthrone diglucosides of rhein and are known to exert their laxative effect on the colon. The active compounds are not sennosides themselves but their metabolite rheinanthrone.³⁾ Sennosides A and B are transformed to rhein by bacterial enzymes in association with subsequent oxidation in intestinal flora.^{4,5)} Rhein 8-*O*-glucopyranoside is the monoanthrone glucoside of rhein and is probably 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 Onpi-to. There is little quantitative data in the literature concerning absorption and excretion of sennosides and its metabolite rhein.^{6–10)}

In general, it is difficult to estimate the absorption and excretion of an herbal medicines because of the presence of various components. To examine the pre-clinical pharmacokinetics of Onpi-to, three compounds were selected as markers based on their levels in this medicine as well as in view of efficacy and safety. The first substance was (–)epicatechin 3-*O*-gallate (ECG), a component of Rhei Rhizoma, reportedly one of the active components of Onpi-to which improved the plasma levels of uremic toxins and various parameters of renal function in rats with adenine-induced renal failure.^{11,12)} Moreover, ECG suppressed proliferating changes in glomeruli in 5/6 nephrectomized rats.¹³⁾ The second substance was glycyrrhetic acid (GA), a metabolite of glycyrrhizin (GL). A major component of Glycyrrhizae Radix, GL has various pharmacological activities, including anti-inflammatory and

hepatoprotective effects.¹⁴⁾ The third substance was rhein, a metabolite of anthranoids, major components of Rhei Rhizoma. Anthranoids contribute to the diarrhea effect of Onpi-to. ECG, sennoside A, B and glycyrrhizin were adopted as marker compounds for quality control in the manufacturing process of this medicine in our facility. Dosages of these three compounds derived from Onpi-to are clearer than other components, thus they were judged to be suitable indicators for the pharmacokinetics investigation following Onpi-to administration.

We selected rhein as one of the characteristics of the pharmacokinetics of Onpi-to. Pharmacokinetics of rhein derived from Onpi-to was examined in rats *via* its detection in plasma, urine and bile following a single oral administration of the medication utilizing an HPLC-UV method. This experiment was part of the pre-clinical pharmacokinetic study

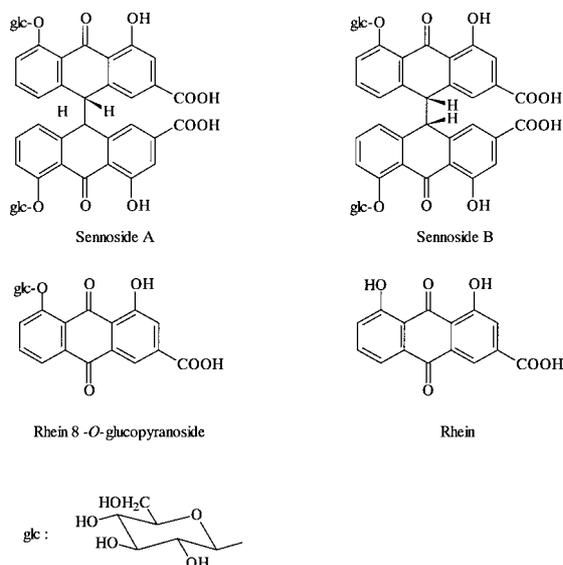


Fig. 1. Structure of Anthranoids in Rhei Rhizoma

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of Onpi-to.

MATERIALS AND METHODS

Chemicals Onpi-to, a spray-dried powder of hot water extracts from the five crude drugs, was obtained from Tsumura and Co. (Tokyo, Japan). Contents of dominant anthranoids in Onpi-to and the content of total potential rhein as μmol equivalent rhein/g are summarized in Table 1. Onpi-to includes 1.240% of total potential rhein derived from sennoside A, sennoside B, rhein 8-*O*-glucopyranoside and rhein. Rhein was purchased from Funakoshi, Ltd. (Tokyo). Alizarin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Additional reagents and solvents used in this study were commercial products of analytical or HPLC grade.

Animals Male Sprague-Dawley rats, obtained at 7 weeks of age from Charles-River Japan (Tokyo) were used in the study after at least 6 d of acclimatization.

The rats were kept in an animal room at constant temperature ($23 \pm 2^\circ\text{C}$) and humidity ($55 \pm 10\%$) with 12 h of light per day (from 07:00 to 19:00) and were allowed access to water and a commercial pellet diet (MF, Oriental Yeast Co., Tokyo) *ad libitum*. The rats were fasted overnight prior to and for 8 h after dosing.

Dosage form and Administration to Animals Onpi-to was suspended in water at 125, 250 and 500 mg/10ml for oral administration to rats at 10 ml/kg.

Pharmacokinetic Studies in Rats Under light anesthesia with ether, the femoral artery was cannulated with polyethylene tubing (SP 10; Natsume Seisakusho, Tokyo) filled with 200 U/ml heparin in saline. After the rats recovered from anesthesia, dosing solutions were given by gastric intubation on a single occasion at doses of 125, 250 and 500 mg/kg. Blood samples (approx. 300 μl) were collected in tubes at 0 (predose), 5, 15, 30 min, 1, 2, 4, 8, 12, 18, 24, 32 and 48 h after administration. Plasma samples were immediately separated by centrifugation from these arterial blood samples.

In order to collect the urine, rats were kept in metabolic cages. Urine samples were collected predose and at 0–2, 2–4, 4–8, 8–12, 12–24 and 24–48 h after drug administration. The dosing solutions were given by gastric intubation on a single occasion at a dose of 500 mg/kg.

To collect the bile, the bile duct was cannulated with polyethylene tubing (SP 10; Natsume Seisakusho) under light anesthesia with ether. After the rats recovered from anesthesia, dosing solutions were given by gastric intubation on a single occasion at a dose of 500 mg/kg. Bile samples were collected predose and at 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12, 12–24 and 24–48 h after the drug administration.

The plasma, bile and urine samples were stored at -20°C until analysis.

Analytical Procedure for Rhein in Plasma and Bile The HPLC system was consisted of a LC-6A pump (Shimadzu, Kyoto, Japan), a SIL-10A auto-injector (Shimadzu), a DG-100 degasser (Eicom, Tokyo), a CTO-6A column oven (Shimadzu) and a SCL-10A system controller (Shimadzu) equipped with a SPD-10A detector (Shimadzu). A 150 mm \times 4.6 mm i.d. Cosmosil 5C18-AR-300 column (Nacalai

Table 1. Contents of Anthranoids in Onpi-to

Constituent	Content (%)	Equiv. rhein ^{a)} ($\mu\text{mol/g}$)
Sennoside A	0.1431	3.317
Sennoside B	0.1412	3.273
Rhein 8-glucopyranoside	1.2470	27.937
Rhein	0.2584	9.092
Potential rhein ^{b)}	1.240	43.620

^{a)} Content of μmol equivalent to rhein/g. ^{b)} Potential rhein means the sum of rhein present as corresponding anthranoids.

Tesque, Tokyo) equipped with a guard column packed with a 10 mm \times 4.6 mm i.d. Cosmosil 5C18-AR-300 (Nacalai Tesque) was used. The mobile phase consisted of 0.5% v/v $\text{H}_3\text{PO}_4/\text{CH}_3\text{CN}/\text{CH}_3\text{COOH}$ (680 : 320 : 15, v/v). Wavelength, column temperature, injection volume and flow rate were 258 nm, 40°C , 20 μl and 1.0 ml/min, respectively, for plasma. For bile, injection volume was 10 μl . Peak areas were measured with a Chromatopac C-R4AX (Shimadzu).

Before applying the plasma and bile samples to HPLC analysis, solid-phase extraction was performed with disposable Bond Elut C8 cartridges (100 mg/1 ml; Varian, California, U.S.A.). Cartridges were preconditioned sequentially with methanol (1 ml) and 0.1 M citric acid (1 ml). A 100- μl aliquot of plasma or bile was loaded onto the cartridges with 800 μl of 0.1 M citric acid and 100 μl of 500 ng/ml alizarin (internal standard), washed with 1 ml of 0.1 M citric acid, water and *n*-hexane, and eluted with 1 ml of acetonitrile. The eluent was evaporated to dryness under vacuum with centrifugal freeze-drying, and reconstituted with 100 μl of mobile phase solution for HPLC. Aliquots were injected onto the HPLC column.

Analytical Procedure for Rhein in Urine The HPLC system was consisted of a LC-10AD pump (Shimadzu), a SIL-10A auto-injector (Shimadzu), a DGU-4A degasser (Shimadzu), a CTO-6A column oven (Shimadzu) and a SCL-10A system controller (Shimadzu) equipped with a SPD-10A detector (Shimadzu). A 150 mm \times 4.6 mm i.d. Cosmosil 5C18-AR-300 column (Nacalai Tesque) equipped with a guard column packed with a 10 mm \times 4.6 mm i.d. Cosmosil 5C18-AR-300 (Nacalai Tesque) was used. The mobile phase was 0.5% v/v $\text{H}_3\text{PO}_4/\text{CH}_3\text{CN}/\text{CH}_3\text{COOH}$ (700 : 300 : 15, v/v). The wavelength, column temperature, injected volume and flow rate were 258 nm, 40°C , 10 μl and 1.0 ml/min, respectively. Peak areas were measured with a Chromatopac C-R4AX (Shimadzu).

Urine samples were diluted 11 fold with water. A 100 μl aliquot of the diluted urine was incubated at 37°C for 1 h with a 100 μl aliquot of 0.1 M sodium acetate buffer (pH 5.0) and 20 μl of 1 mg/ml β -glucuronidase and sulfatase in 0.2% NaCl to hydrolyze the conjugates of rhein. Determination of the unconjugated form was undertaken in the absence of β -glucuronidase and sulfatase, and incubation at 37°C was not conducted. Following the introduction of a 100 μl aliquot of 2 $\mu\text{g/ml}$ alizarin (internal standard) in $\text{CH}_3\text{CN}/N,N$ -dimethylacetamide (47 : 3, v/v) to the resultant mixture, the sample was mixed and centrifuged at 3000 rpm for 10 min. A 10 μl aliquot of the supernatant was injected onto the HPLC column.

Calibration Curves Quantitation was done using peak

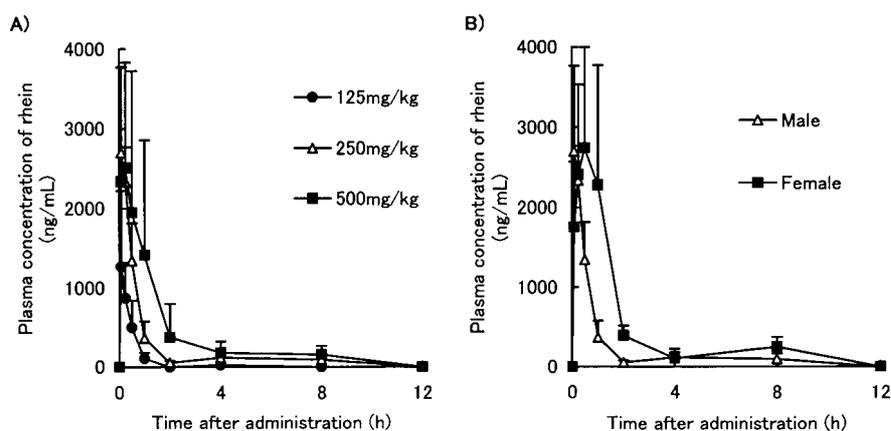


Fig. 2. Mean Plasma Concentrations of Rhein after Single Oral Administration of 125, 250 or 500 mg/kg of Onpi-to in Male Rats (A) and 250 mg/kg of Onpi-to in Male and Female Rats (B)

Each point represents the mean \pm S.D. of 6 rats.

area ratios from calibration curves with a weighted ($1/Y^2$) linear, least-squares regression.

For the calibration curve, rat blank plasma spiked with known amounts of rhein to give final concentrations of 10–3927 ng/ml was used. The lower limit of quantitation (LOQ) was 10 ng/ml for rhein in plasma.

Rat blank urine spiked with known amounts of rhein to give final concentrations of 50–10000 ng/ml was used for the calibration curve. The LOQ was 50 ng/ml for rhein in urine.

Rat blank bile spiked with known amounts of rhein to give final concentrations of 50–5000 ng/ml was used. The LOQ was 50 ng/ml for rhein in bile.

Standard curve correlation coefficients (r) for all components were ≥ 0.997 .

Pharmacokinetic Analysis Pharmacokinetic parameters for rhein in plasma were calculated using non-compartmental methods with the computer program PAG-CP (Asmedica, Osaka, Japan). The maximum concentration in plasma (C_{max}) and the corresponding time (t_{max}) were determined from each of the individual rat plasma concentration–time curves. The area under the concentration–time curve (AUC) from time zero to 48 h was calculated using the trapezoidal rule.

The cumulative urinary or biliary excretion ratios (% of dose) were calculated as ((the cumulative rhein excretion/the dose of rhein estimated by the content of potential rhein) \times 100). Onpi-to included 1.240% potential rhein.

RESULTS

Plasma Levels of Rhein from Onpi-to Onpi-to was administered to male rats at oral dosages of 125, 250 and 500 mg/kg. The plasma concentration *versus* time curves are presented in Fig. 2A. Pharmacokinetic parameters of rhein are provided in Table 2. The plasma concentration of rhein reached C_{max} of 1302.5 ± 926.4 , 2973.6 ± 684.3 and 3118.8 ± 1701.2 ng/ml at 8.3 ± 5.2 min, 8.3 ± 5.2 min and 20.0 ± 21.9 min after administration at doses of 125, 250 and 500 mg/kg, respectively. Subsequently, plasma levels declined to below LOQ (10 ng/ml) for all samples at 12, 18 and 18 h at doses of 125, 250 and 500 mg/kg, respectively. From 4 to 8 h after administration, plasma concentration–time curves revealed sec-

Table 2. Pharmacokinetic Parameters of Rhein after Oral Administration of Onpi-to in Rats

Dose (mg/kg)	$AUC(0-48)$ (ng·h/ml)	C_{max} (ng/ml)	t_{max} (min)
125 (Male)	752.3 ± 321.5	1302.5 ± 926.4	8.3 ± 5.2
250 (Male)	2443.3 ± 554.4	2973.6 ± 684.3	8.3 ± 5.2
500 (Male)	4443.2 ± 2641.3	3118.8 ± 1701.2	20.0 ± 21.9
250 (Female)	5537.7 ± 1876.0	3058.0 ± 1533.7	45.0 ± 16.4

Each value represents the mean \pm S.D. of 6 rats.

ondary peaks or shoulders. $AUC(0-48)$ values at doses of 125, 250 and 500 mg/kg were 752.3 ± 321.5 , 2443.3 ± 554.4 and 4443.2 ± 2641.3 ng·h/ml, respectively, suggesting linear pharmacokinetics of rhein in plasma. However, C_{max} did not show linear pharmacokinetics.

Onpi-to was administered to female rats at an oral dosage of 250 mg/kg. The plasma concentration *versus* time curves are shown in Fig. 2B, which compares male and female rats. Pharmacokinetic parameters of rhein in female rats are exhibited in Table 2. In female rats, the plasma concentration of rhein reached C_{max} of 3058.0 ± 1533.7 ng/ml at 45.0 ± 16.4 min after administration at a dose of 250 mg/kg; subsequently, plasma levels declined to below LOQ (10 ng/ml) at 18 h for all samples. $AUC(0-48)$ value at a dose of 250 mg/kg was 5537.7 ± 1876.0 ng·h/ml in females.

Urinary Excretion Onpi-to was administered to male rats at an oral dosage of 500 mg/kg. Data regarding the cumulative urinary excretion of rhein are shown in Fig. 3. The cumulative urinary excretion of rhein and conjugated rhein was 3.14 ± 1.56 and $38.21 \pm 18.87\%$, respectively, 48 h following dosing at 500 mg/kg. Excretion was completed by 24 h after administration.

Biliary Excretion Onpi-to was administered to male rats at an oral dosage of 500 mg/kg. Figure 4 shows the cumulative biliary excretion of rhein which was $1.34 \pm 0.44\%$ 48 h following the above dosage.

DISCUSSION

Pharmacokinetics of ^{14}C -labelled rhein in rats was reported by Lang.¹⁰ The extent of bioavailability of approxi-

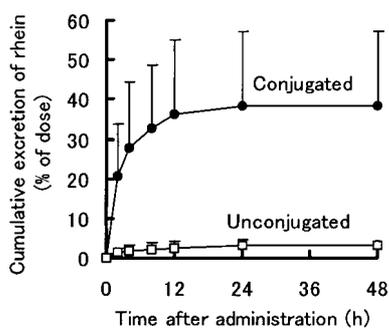


Fig. 3. Cumulative Excretion of Rhein in Urine after Oral Administration of 500 mg/kg of Onpi-to in Rats

Each point represents the mean \pm S.D. of 5 rats.

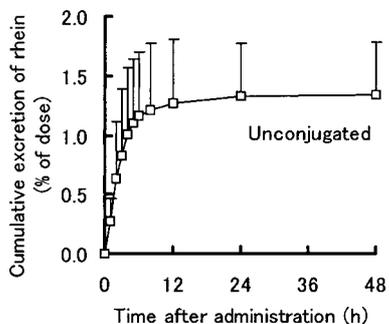


Fig. 4. Cumulative Excretion of Rhein in Bile after Oral Administration of 500 mg/kg of Onpi-to in Rats

Each point represents the mean \pm S.D. of 4 rats.

mately 30–35% calculated from the ratio of AUC from radioactivity following intravenous and oral application, and absorption of approximately 50–60% following oral administration are described in the report. However, plasma levels of rhein following its administration in rats have not been reported. After a single oral administration of rhein, the extent of bioavailability was 63.6% calculated from the ratio of AUC after intravenous and oral application (data not shown). On the other hand, after a single oral administration of sennoside A and rhein 8-glucopyranoside, the extent of bioavailability calculated from the ratio of AUC of rhein levels after intravenous and oral application was 7.4 and 75.7%, respectively (data not shown).

After a single oral administration of each component, *i.e.*, rhein or rhein 8-*O*-glucopyranoside at doses of 3.100 or 4.869 mg/kg, respectively, which were equivalent to 1.240% as rhein in 250 mg/kg of Onpi-to, rhein plasma levels displayed curves similar to those obtained after a single oral administration of Onpi-to at a dose of 250 mg/kg. AUC and C_{max} were 62–73% and 57–75%, respectively, of those values observed after Onpi-to administration (data not shown). On the other hand, after a single oral administration of sennoside A at a dose of 4.705 mg/kg, which was equivalent to 1.240% rhein in 250 mg/kg of Onpi-to, rhein plasma levels exhibited a curve which had a peak at 7.3 h after dosing. AUC and C_{max} were 7 and 1%, respectively, of those after Onpi-to administration (data not shown). These results suggested that rhein 8-*O*-glucopyranoside dominated rhein levels in plasma after dosing of Onpi-to based on the content in Onpi-to; sennosides contributed little.

The maximum rhein plasma level was attained rapidly

after oral administration of Onpi-to, then subsequently declined for 2–4 h thereafter. At 4–8 h after administration, the plasma concentration–time curves revealed a secondary peak or shoulder, followed by a subsequent decline. This suggested an enterohepatic recirculation of rhein. Rhein appeared in plasma presumably derived from anthranoids such as sennoside A, sennoside B, rhein 8-*O*-glucopyranoside and rhein in Onpi-to. Their contents in Onpi-to were 0.1431, 0.1412, 1.2470 and 0.2584%, respectively. Thus, rhein 8-*O*-glucopyranoside was determined to be the predominant anthranoid component of Onpi-to.

Following oral administration of this drug, $AUC(0–48\text{ h})$ levels of rhein increased linearly in proportion to dose, while C_{max} was not in proportion to dose, suggesting the non-linear pharmacokinetics of rhein derived from Onpi-to.

In female rats, $AUC(0–48\text{ h})$ level was higher and t_{max} was later than those readings in male rats. C_{max} did not differ in male and female rats. These results suggested the existence of sex differences in rhein pharmacokinetics after oral administration of Onpi-to to rats. Since no such sex difference has been reported, the reason for this difference is not yet known. In the process of metabolism of sennoside A, B and rhein 8-*O*-glucopyranoside to rhein in the intestinal flora, and in the process of the absorption of rhein, sex differences likely do not exist. In the process of rhein conjugation in the liver, it is possible that activity differs between males and females. Sex differences with respect to glucuronidation have not been extensively reported; however, Nakagomi *et al.* noted such differences in glucuronidation of pirlmenol metabolite in rats.¹⁵ Further experiments are necessary to elucidate these differences pertaining to rhein pharmacokinetics in these animals.

Cumulative urinary and biliary excretions of rhein at 48 h after administration of Onpi-to were 3.14 ± 1.56 and $1.34 \pm 0.44\%$, respectively, while cumulative urinary excretion of conjugated rhein was $38.21 \pm 18.87\%$ at 48 h after its administration. Conjugated rhein in bile was not investigated in the present study, however, it may also occur in bile after oral administration of Onpi-to. It was reported that two metabolites hydrolyzed by glucuronidase/arylsulfatase to rhein were detected in bile samples after oral administration of ^{14}C -rhein.¹⁰ After oral administration of sennoside A to rats, 1.86 and 4.87% rhein reportedly were found in urine at doses of 5 and 17 mg, respectively,⁷ and after oral administration of rhein to rats, it was reported that 6% rhein could be detected in urine.⁸ Following ^{14}C -rhein administration, excretion of ^{14}C -activity amounting to 37.2 and 50% in urine was documented by de Witte⁹ and Lang,¹⁰ respectively. These findings are in agreement with the results of our investigations following Onpi-to administration considering the main products in urine were rhein and its two conjugates.¹⁰

In conclusion, pharmacokinetics of rhein derived from Onpi-to, an herbal medicine, was examined in rats as part of the pre-clinical pharmacokinetic study of the medicine. After Onpi-to administration, rhein rapidly appeared in plasma; moreover, plasma concentration–time curves revealed secondary peaks. The pharmacokinetics of rhein was nonlinear and the urinary and biliary excretion of unconjugated rhein did not dominate. We have obtained pharmacokinetic data regarding rhein derived from TJ-8117 in human healthy subjects and these data will be reported elsewhere.

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