The Influence of the Sennosides on Absorption of Glycyrrhetic Acid in Rats

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In the course of our clinical studies of Kampo medicine (traditional Japanese medicines), we observed the pharmacokinetic interactions between two herbs. When Onpito (TJ-8117, Kampo medicine) containing licorice and rhubarb was administered orally to human subjects, we observed that the $AUC_{(0—lim)}$ and C_{max} of glycyrrhetic acid (GA) in plasma were lower than those treated with other Kampo medicines containing licorice. In this study, we demonstrate the pharmacokinetic interactions of GA derived from glycyrrhizinic acid (GL) in licorice and anthraquinones derived from rhubarb. To our knowledge, this is the first report to investigate the pharmacokinetic interactions between two herbs. When GL was orally co-administrated to rats with a non-effective dose of sennoside A having purgative activity, the $AUC_{(0—lim)}$ and C_{max} of GA decreased. In addition, sennoside A did not affect the metabolism of GL by the intestinal bacteria *in vitro*. In the examination using an *in situ* loop of rat colon, the remaining ratio of GA rose drastically by the co-administration of sennoside A, sennidin A and rhein. Observed inhibition activity of these anthraquinones on GA absorption depended on the concentration of the components added. The maximum inhibition ratio was approximately 75% by rhein, 60% by sennoside A and 25% by sennidin A. We conclude that the decrease of the pharmacokinetic parameters of GA in human plasma observed in the clinical study of TJ-8117 is attributable to an interactive action of absorption from the intestinal tract by anthraquinones contained in or derived from rhubarb.

Key words glycyrrhetic acid; interaction; absorption; rhein; licorice; rhubarb

Herbal medicines are increasingly used in the world, and herb–drug interactions are recognized as a clinically important problem.[1—5] Recently, various clinical and non-clinical studies including *in vitro* studies related to pharmacokinetic interactions between herbs and new drugs have been reported.[6—11] Durr *et al.*[12] reported that the administration of St. John's Wort (*Hypericum perforatum*) extract to 8 healthy male volunteers during 14 d resulted in an 18% decrease of digoxin exposure after a single digoxin dose (0.5 mg), in 1.4- and 1.5-fold increased expressions of duodenal P-glycoprotein/MDR1 and CYP3A4, respectively, and in a 1.4-fold increase in the functional activity of hepatic CYP3A4 in clinical study. However, to our knowledge, there has been no report dealing with the pharmacokinetic interactions between two herbs. In the course of our clinical studies of Kampo medicine, we observed changes in various pharmacokinetic parameters of a marker component of Kampo medicine. In this article, we demonstrate a pharmacokinetic interaction between two herbs by various non-clinical investigations of a representative marker component of a herb.

Glycyrrhizinic acid (GL), one of the main components of licorice root (*Glycyrrhizae radix*), shows various biological activities such as having an anti-inflammatory[13] and an anti-hepatitis effect[14] and is contained in many types of Kampo medicines. GL is a glycoside having two glucuronic acid molecules attached to the hydroxyl group at C-3 position of glycyrrhetic acid (GA) (Fig. 1). Since GA is derived from GL *via* metabolism as mentioned below, GA has been frequently used as a pharmacokinetic marker of Kampo medicines including licorice.

Kampo medicines are orally administered so that the chemical components contained in the Kampo medicines may be manipulated by the intestinal flora before being absorbed into the body. For example, the following hydrolysis, glycosides are transformed by intestinal flora to the corresponding aglycones.[15,16]

In a previous paper, we reported that only GA was detected instead of GL in the plasma when GL was orally administered to rats. In addition, we clarified that GA was not detected in the plasma after oral administration of GL to germ-free rats having no intestinal bacteria.[17] These results suggest that GL is hydrolyzed to GA by intestinal bacteria after oral administration of GL to rats, and is absorbed as GA from the intestinal tract. Absorption of GL might be smaller

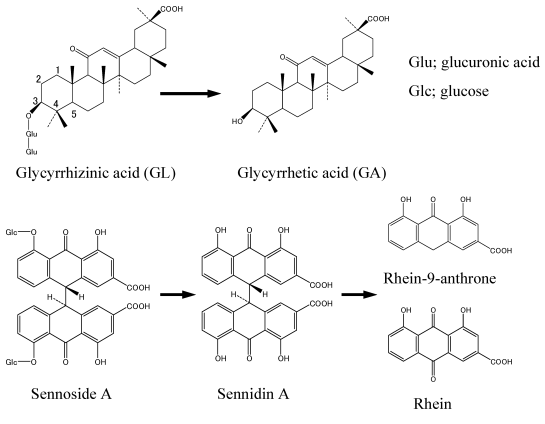

Fig. 1. The Metabolism of GL and Sennosides by Intestinal Bacteria

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than that of GA due to its hydrophilic property.[18] In contrast, Akao *et al.* reported that the elevation of the glutamic-oxalacetic transaminase (GOT) in serum induced by CCl_4 was inhibited in normal rats after oral administration of GL, and not observed in germ-free rats.[19] These results suggest that the hydrolysis of GL to GA by intestinal bacteria is determined by the pharmacological effect of orally administered GL.

Onpito (TJ-8117) is one Kampo medicine composed of five herbs, *Rhei rhizoma* (rhubarb), *Glycyrrhizae radix* (licorice), *Ginseng radix*, *Zingiberis rhizoma* and *Aconiti tuber*. In a clinical study of TJ-8117 in healthy human subjects, we observed that the pharmacokinetics parameters (the plasma concentration–time curve, $AUC_{(0—lim)}$ and the maximum peak plasma concentration, C_{max}) of GA, a marker substance, in plasma after oral administration of TJ-8117 were much lower than those after administration of the other Kampo medicines including licorice (Fig. 2). A detailed report of the clinical study of TJ-8117 is now in preparation, we will publish the report including pharmacokinetics of the other components soon after the data analyses.

He *et al.* reported a similar result, whereby the co-administration of antibiotics to rats such as amoxicillin and metronidazole decreased the *AUC* of GA after the administration of GL.[20] Thus, the influence on intestinal bacteria must be one of the reasons of decreased GA absorption. Moreover, diarrhea was also speculated to be for other reasons, because diarrhea affected the condition of intestinal bacteria and might shorten the retention time of intestinal content. Considering this point, diarrhea caused by anthraquinones (sennoside A, sennidin A, rhein and rhein-9-anthrone) contained in rhubarb were thought to be involved in the lowering of GA absorption in the clinical study of TJ-8117.

To elucidate the phenomenon in this clinical study, TJ-8117, we investigated the effect of the anthraquinones concerned as active principles for the purgative effect of rhubarb to the pharmacokinetics of GL and GA in rats.

MATERIALS AND METHODS

Chemicals GL was purchased from Wako Pure Chem. Industries (Osaka, Japan). GA and rhein purchased from Tokyo Kasei Co. (Tokyo, Japan) were purified by silica gel column chromatography and recrystallization. Sennoside A and sennidin A were purchased from EXTRASYNTHESE. Rhein-9-anthrone was synthesized from rhein according to the method reported by Witte *et al.*[21] Namely, 100 mg of rhein was suspended in 20 ml of acetic acid, and was refluxed. 3.5 ml of 40% $SnCl_2$-hydrochloric acid solution was dropped to this suspension under warm conditions (140 °C), and stirred for 2 h. This reaction mixture was left to stand overnight at 4 °C, and the insoluble precipitate was collected washed with water and crystallized from dichloromethane/methanol. Glycyrrhetic acid acetate was synthesized from GA by an ordinal method. β-galactosidase was purchased from Boehringer Mannheim GmbH.

Animals Male Sprague Dawley rats, weighing 235—306 g, were purchased from Charles River Japan (Atsugi, Japan). The rats were bred within one day, during a 12 h period, at a constant temperature (23±2 °C) and humidity (55±10%). The rats were fed standard laboratory chow with water freely available. The rats were fasted overnight prior to the experiment after acclimation for one week.

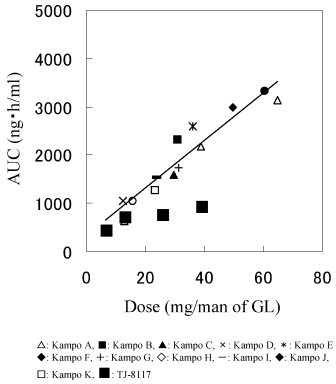

Fig. 2. Correlation of *AUC* of GA in Human Plasma and Dosage after Administration of Various Kampo Medicines to Human Subjects

Kampo A—J (not including rhubarb).

The Purgative Activity of Sennoside A in Rats Sennoside A suspended in 0.5% sodium carboxymethylcellulose (CMC) was orally administered to rats at doses of 2.5, 5, 10, and 20 mg/kg/10 ml. The frequency of diarrhea was recorded after administration. The condition of the feces was classified into four grades, normal feces (0), mucous feces (0.25), loose stool (0.5), and diarrhea (1). A probit plot of the corresponding feces score calculated the ED_{50} value of purgative activity.

The Simultaneous Administration of GL and Sennoside A Suspensions of GL at a dose of 5 mg/kg and sennoside A at a dose of 0, 1.6, 5.6 and 19.6 mg/kg were prepared with 0.5% CMC, and these suspensions were orally administrated to rats (7 weeks, each $n=6$). A dosage of 1.6 mg/kg of sennoside A is a non-effective dose, 5.6 mg/kg is equivalent to ED_{50} of purgative activity and 19.6 mg/kg causes diarrhea in rats. Approximately 300 μl of blood was collected from the femoral artery cannula treated in heparin at 1, 2, 4, 6, 9, 12, 18, 24 and 32 h after administration. The plasma was obtained by centrifugation (3000 rpm, 10 min, 4 °C) and was stored at −20 °C until analysis.

Elucidation of the Absorption of GA in Rats That Have Undergone Ileostomy Ileostomy was conducted in rats by the insertion of cannula (polyethylene tube O.D. 3 mm, Hibiki) at the ileocecum junction under anesthetic. GL suspended in 0.5% CMC was orally administered at a dose of 5 mg/kg to these operated on rats ($n=5$—6). Approximately 300 μl of blood was collected at 1, 2, 4, 6, 9, 12, 18, 24 and 32 h after administration from heparin-treated cannula inserted in the femoral artery. The plasma was obtained by centrifugation and stored at −20 °C until analysis.

The cannula (polyethylene tube O.D. 0.61 mm, Natsume factory) was inserted into the cecum from the ileocecum junction for direct administration to cecum. GL and sennoside A suspended in 0.5% CMC were administered in the cecum from this cannula at a dose of 5 mg/kg with or without 1.6 mg/kg of sennoside A to these treated rats ($n=5$—6). Blood samples were collected at the same time points and by the same method described above. The plasma obtained by centrifugation was stored at −20 °C until analysis.

GA Analysis of Rat Plasma by Enzyme Immuno Assay The plasma concentration of GA was measured by Enzyme

Immuno Assay (EIA), as reported by Kanaoka *et al.* with some modification.[22] The antiserum of GA was obtained by repeated administration of the synthesized *N*-glycyrrhetylglycine–BSA complex to rabbits. The cross reaction of antiserum for 11 analogs, following GL, 18α-GA, β-estradiol, aldosterone, sodium deoxycholate, hydrocortisone, progesterone, 5α-androstan-17β-ol-3-one, etiocholan-17β-ol-3-one, cholesterol and carbenoxolone was 1% or less. As a labeled antigen, the γ-glycyrrhetylaminobutylic acid β-galactosidase complex was synthesized.

The obtained plasma samples were diluted 16 times with buffer A (0.02 mol/l sodium phosphate buffer (pH 7.0) containing 0.1% of BSA, 0.1% of sodium azide and 1 mmol/l magnesium chloride). These samples (each 100 μl) were incubated with 100 μl of the GA antiserum solution diluted 60000 times with buffer A for 30 min at room temperature. Subsequently, 50 μl of the labeled antigen diluted 80000 times with buffer A were added to the above reaction mixtures, and incubated for 90 min at room temperature. Furthermore, 100 μl of MARCELLA10® (Non-dissolved secondary antibody: DAINIPPON PHARMACEUTICAL CO., LTD) diluted 5 times with buffer A was added to the reaction mixtures, and incubated for 40 min at room temperature. These reaction mixtures were washed with 1 ml of buffer A by removing the supernatant after centrifugation (3000 rpm, 15 min, 4 °C). This procedure was repeated twice. After washing the reaction mixtures, 100 μl of 4-methylumbelliferyl-β-D-galactoside aqueous solution (1.5×10^{-4} mol/l) was added to the precipitate and it was incubated for 30 min at 30 °C. Subsequently, the reaction was stopped by the addition of 2 ml of 0.1 mol/l glycine–NaOH (pH 10.3). The fluorescence intensity of the sample was measured by the fluorophotometer (F-4500 types a spectrum a fluorophotometer, Hitachi, Ltd.) at 365 nm for the excitation wavelength, and at 448 nm for the fluorescence wavelength. The lower limit of quantity in this method was 4 ng/ml.

Gastro Intestinal Absorption at the *in Situ* Loop Method The rats were used after fasting overnight. Each rat was anesthetized with Nembutal (1 ml/kg, i.p.). The loop of the colon (2 cm), duodenum (1 cm), jejunum (5 cm), ileum (5 cm), and cecum were prepared by the authorized method. The test compounds (GA (only), GA+sennoside A, GA+sennidin A, GA+rhein, GA+rhein-9-anthrone) were injected in a 0.5 ml volume into each loop under several combination patterns. At 30 min after injection, the intestinal loop was incised after blood collection with heparinized syringes from the inferior vena cava. The content of the each loop was recovered, and then the residual amount of GA was determined by HPLC.

The Determination of GA Content in the Intestines (HPLC) The content of GA in the each intestine was determined by HPLC. Each intestinal content was made uniform with physiological saline at 20 ml and homogenized by Polytron (NITI-ON, Japan). A total of 50 μl of ethanol and glycyrrhetic acid acetate solution (internal standard; i.s.: 10 μg/ml in methanol) were added to the obtained homogenates (100 μl). After the samples were stirred and centrifuged (15000 rpm, 5 min, 4 °C), 10 μl of the supernatant was injected into HPLC (Shimadzu, Japan; pump: LC-10AD, degasser: DGU-12A, auto sampler: SIL-10AXL, column oven: COT-10A, and UV detector: SPD-10A). HPLC conditions were as follows: column was Cosmosil Packed Column 5C18-300® (4.6×150 mm); the mobile phase was 75% methanol including 2% acetic acid, 1 ml/min of flow rate, column temperature at 45 °C, and detection wavelength at 254 nm. Ethanol solutions of GA (60—600 ng/ml) for the calibration curve were prepared separately. A total of 50 μl of i.s. solution and 100 μl of physiological saline were added to 50 μl of the obtained solutions. The lower limit of quantitation in this analysis was 150 ng/ml.

The Metabolism of GL by Intestinal Bacteria (*in Vitro*) The cecum of the rats (n=5) were promptly taken out after removing all blood and the cecum contents were moved into centrifuge tubes filled with N_2 gas. These samples were diluted 4 times with GAM broth. In order to extract several enzymes produced by intestinal microflora, the ultrasonic crusher treated the cecum contents for 3 min under cooling by ice.

These cecum contents (1.9 ml) in the tubes filled with N_2 gas were pre-incubated for several minutes at 37 °C. After the pre-incubation, 50 μl of the GL solution (final concentration; 0.0823 mg/ml) was added to the mixture with 50 μl of sennoside A solution (final concentration; 0.0086 and 0.0863 mg/ml) or 50 μl of sennidin A solution (final concentration; 0.0054 and 0.0538 mg/ml). These mixtures were incubated for 3 h at 37 °C. Then, 0.5 ml of 1 mol/l HCl was added to stop the reaction. The reaction mixtures were centrifuged (3000 rpm, 10 min, 4 °C), and then 0.5 ml of the supernatant was collected. Then, 20 μl of the internal standard solution (glycyrrhetic acid acetate: 100 μg/ml ethanol solution) and 2 ml of ethyl acetate were added to the collected supernatant and the mixtures were agitated for 10 min. After centrifugation (3000 rpm, 10 min, 4 °C), 1 ml of the upper layer was evaporated under a N_2 gas stream and dissolved in 200 μl of HPLC mobile phase. A total of 30 μl of this solution was injected into the HPLC (Shimadzu, Japan; pump: LC-6A, auto sampler: SIL-6A, column oven: CTO-6A, and UV detecter: SPD-6A). HPLC conditions were as follows: column was Cosmosil Packed Column 5C18-300® (4.6×250 mm); the mobile phase was methanol: 2% acetic acid (v:v=80:20), 1.1 ml/min flow rate, column temperature was at 45 °C and detection wavelength at 254 nm. The concentration of GA was calculated by the internal standard method (the ratio of the peak height of GA to glycyrrhetic acid acetate). The range of the calibration curve was from 200 ng/ml to 20 μg/ml.

Statistical Analysis The plasma concentration of GA was calculated by using a calibration curve program NEWCRA3J (Wakabayashi). The pharmacokinetic parameters were calculated by WinNonlin (non-compartment model; Pharsight Inc.). The peak plasma concentration (C_{max}), and time to reach the maximum plasma concentration (t_{max}) were calculated from the actual measurements. The area under the plasma concentration–time curve ($AUC_{(0—lim)}$) was computed by the trapezoid method.

Microsoft Excel calculated the mean value. Data was expressed as the mean±S.D. (n=5—6). The significant differences were evaluated using Dunnett's multiple comparison test or Student's t-test.

RESULTS

The Purgative Activity of Sennoside A in Rats The incidence of diarrhea after oral administration of sennoside A at doses of 2.5, 5, 10 and 20 mg/kg to rats was observed. After administration of sennoside A, diarrhea was observed after 3—4 h and reached a peak at 9 h in all groups. The frequency of diarrhea appearance at 9 h was 15, 50, 90, and 100%, respectively (Fig. 3). The grade of diarrhea was indicated as follows. In the 2.5 mg/kg group, mucous feces were observed in one, and loose stool was observed in one of the five rats. In the 5 mg/kg group, mucous feces were observed in two, loose stool was observed in two, and diarrhea was observed in one of the five rats. In the 10 mg/kg group, loose stool was observed in one rat, and diarrhea was observed in four of the five rats. In the 20 mg/kg group, all rats showed diarrhea. From these results, the ED_{50} value was computed as 5.6 mg/kg.

The Combined Treatment of Sennoside A and GL to Rats Based on the results of the purgative activity of sennoside A in rats, the optimal dose of sennoside A was determined. At doses of 1.6, 5.6, or 19.6 mg/kg (ratio: 3.5) of sennoside A orally administrated with 5 mg/kg of GL to rats. Then, the plasma concentration profile of GA was investigated (Fig. 4A). The pharmacokinetic parameters are shown in Table 1.

Although $AUC_{(0—lim)}$ and C_{max} of GA decreased significantly by co-administration with sennoside A compared to the group treated with GL (5 mg/kg) alone, no statistical significant difference was observed in t_{max}. Moreover, there was no other parameter showing a significant change among the co-administration groups.

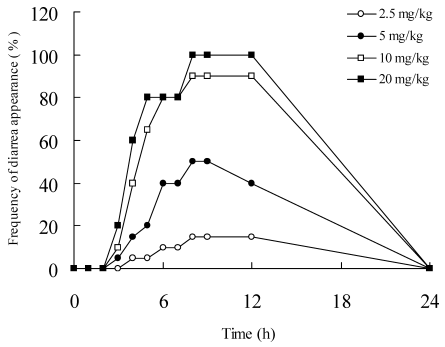

Fig. 3. Time Course of Diarrhea Appearance after Administration of Sennoside A (n=5)

Frequency of diarrhea appearance (%): total diarrhea score/maximum score (5)×100.

Examination of Absorption in Rats That Have Undergone Ileostomy In order to specify the part where the interaction of GL and sennoside A occurred, GL was intracecally administrated with or without sennoside A to normal rats and orally administrated to rats that had undergone ileostomy.

The plasma concentration of GA was determined by EIA. The plasma concentration profile of GA after intracecal or oral administration of GL to rats is shown in Fig. 4B. The pharmacokinetic parameters are shown in Table 1. The area under the curve ($AUC_{(0—lim)}$) of GA after intracecally administration of GL to rats was significantly reduced by sennoside A co-administration, similar to oral administration in rats. In addition, C_{max} also showed a tendency to decrease. Moreover, GA was not detected in the plasma after oral administration of GL to rats that had undergone ileostomy.

Loop Absorption Test *in Situ* In order to elucidate the interaction of GL and sennosides or its derivatives directly, the absorption of GA from *in situ* loops under various conditions was examined. The amount of remaining GA in the loops after co-administration of GA and sennosides or its derivatives is shown in Fig. 5.

Although the amount of remaining GA significantly in-

Table 1. Pharmacokinetic Parameters of GA after Administration of GL to Rats

Study	Dosing	$AUC_{(0—lim)}$ (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)
In vivo study 1	GL (control)	2401±1020	312±89	8.2±2.0
	GL+SA 1.6 mg/kg	1201±802*	175±92*	7.7±2.9
	GL+SA 5.6 mg/kg	1014±700**	174±106*	7.2±2.1
	GL+SA 19.6 mg/kg	742±429**	124±75**	6.7±2.0
In vivo study 2	Normal rat GL (i.c.)	3652±2131	310±240	7.0±5.9
	Normal rat GL+SA (i.c.)	1400±880*	176±138	5.2±3.6
	Ileostomized rat GL (*p.o.*)	—	—	—

GL: glycyrrhizinic acid, SA: sennoside A. i.c.: intracecal administration, *p.o.*: oral administration. *In vivo* study 1: oral administration of GL (5 mg/kg) with or without sennoside A to male rats (mean±S.D., n=5—6) $*p<0.05$ *vs.* control, $**p<0.01$ *vs.* control (Dunnet's multiple comparison test). *In vivo* study 2: oral administration of GL (5 mg/kg) to male ileostomized rats and after intracecal administration of GL with or without sennoside A (1.6 mg/kg) to male rats (mean±S.D., n=5—6) $*p<0.05$ *vs.* normale rat (Student's t-test).

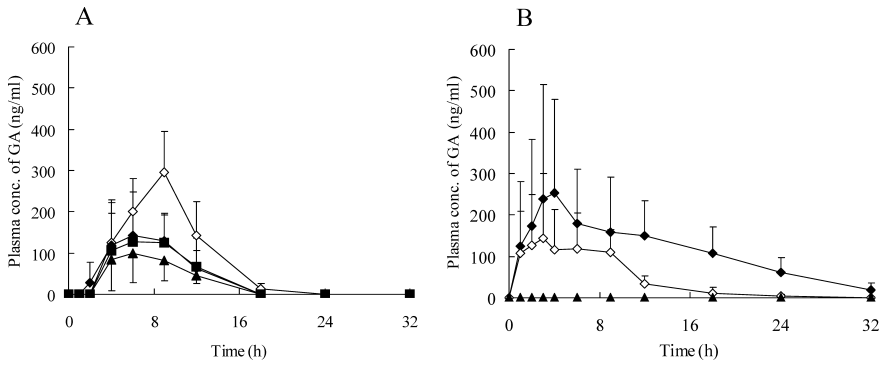

Fig. 4. (A) Effect of Sennoside A on Plasma Concentration of GA after Oral Administration of GL with or without Sennoside A to Male Rats (mean±S.D., n=6): ◇, GL (5 mg/kg); ◆, GL (5 mg/kg)+Sennoside A (1.6 mg/kg); ■, GL (5 mg/kg)+Sennoside A (5.6 mg/kg); ▲, GL+Sennoside A (19.6 mg/kg) and (B) Plasma Concentration of Glycyrrhetic Acid after Oral Administration of GL to Male Ileostomized Rats (▲) and after Intracecal Administration of GL with (◇) or without (◆) Sennoside A to Male Normal Rats (Mean±S.D., n=5—6)

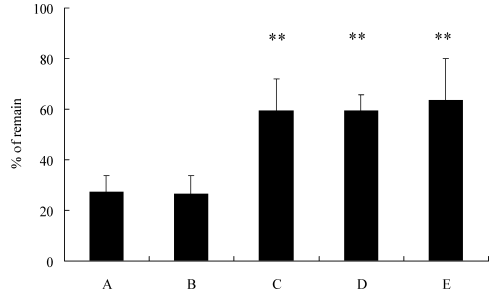

Fig. 5. Effect of Sennoside A, Sennidin A, Rhein-9-anthrone and Rhein on GA Absorption from Rat *in Situ* Loop at Colon (Mean±S.D., $n=5$)

A: Control (GA 0.3 mg/ml), B: GA 0.3 mg/ml+rhein-9-anthrone 0.095 mg/ml, C: GA 0.3 mg/ml+sennoside A 0.15 mg/ml, D: GA 0.3 mg/ml+sennidin A 0.095 mg/ml, E: GA 0.3 mg/ml+rhein 0.1 mg/ml. $**p<0.01$ *vs.* control (Dunnett's multiple comparison test) (mean±S.D., $n=5$).

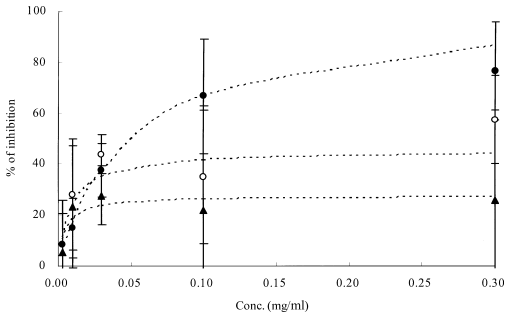

Fig. 6. Dose Dependency of Rhein (●), Sennoside A (○) and Sennidin A (▲) on GA Absorption from Rat *in Situ* Loop at Colon (Mean±S.D., $n=5$)

GA: 0.3 mg/ml. Dashed line: prediction curve (Langmuir isotherm).

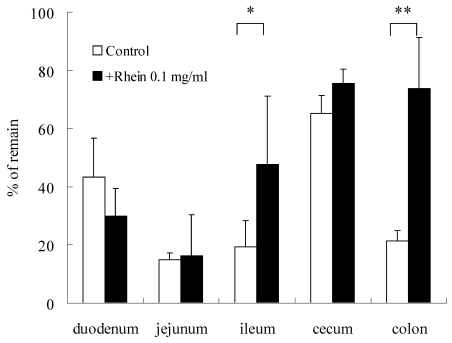

Fig. 7. Regional Differences of the Effect of Rhein on GA Absorption from Rat *in Situ* Loop (Mean±S.D., $n=5$)

Control: GA 0.3 mg/ml. $**p<0.01$ *vs.* control, $*p<0.05$ *vs.* control (Student's *t*-test).

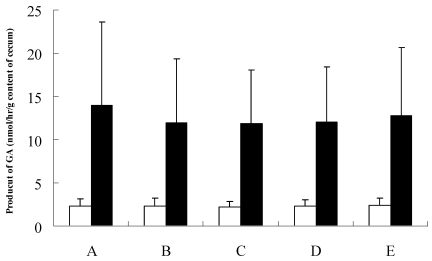

Fig. 8. The Metabolic Activity of GA in Rat Intestinal Bacteria after GL (0.0823 mg/ml) Incubated with Sennoside A or Sennidin A (Mean±S.D., $n=5$)

□: without sonication, ■: sonication. A: GL 0.0823 mg/ml (0.1 mmol/l), B: GL 0.0823 mg/ml+sennoside A 0.0086 mg/ml (0.01 mmol/l), C: GL 0.0823 mg/ml+sennoside A 0.0863 mg/ml (0.1 mmol/l), D: GL 0.0823 mg/ml+sennidin A 0.0054 mg/ml (0.01 mmol/l), E: GL 0.0823 mg/ml+sennidin A 0.0538 mg/ml (0.1 mmol/l).

creased by co-administration with sennoside A, sennidin A and rhein, it was not affected by the co-administration with rhein-9-anthrone.

When dosage dependency of sennoside A, sennidin A and rhein on the interaction was examined, the absorption (=dose−the amount of remaining GA) of GA decreased in a dosage-dependent manner in all compounds.

These inhibition activities were saturated at 75% in rhein, at 60% in sennoside A and at 25% in sennidin A. Figure 6 shows the observed difference of the maximum inhibition activity among each compound. Significant inhibition activity by rhein was observed only in the ileum and colon (Fig. 7).

The Metabolism of GL by Intestinal Bacteria (*in Vitro*) The metabolic activity of GL to GA incubated with or without sennoside A or sennidin A is shown in Fig. 8. Without ultrasonication treatment, the metabolic activity was constant in all treatments. In the experiments using ultrasonicated cecum content for the extraction of the internal enzymes contained in the intestinal bacteria, the metabolic activity of GA increased to five times that seen without sonication. However, no significant change was observed in each treatment group.

DISCUSSION

Since herbal medicines are generally prepared by extraction with hot water, they contain many water-soluble components such as glycosides. The intestinal bacteria often participate in the metabolism and absorption of these glycosides. Thus, the condition of the gastro-intestinal system and intestinal flora must be highly involved in the effectiveness of the herbal medicines. In our clinical study of TJ-8117, we observed a decrease in various pharmacokinetic parameters ($AUC_{(0—lim)}$ and C_{max}) of GA derived from GL in licorice (Fig. 2). In this case, we first speculated that the cause of this phenomenon might be due to diarrhea caused by rhubarb, because GA has been considered to be transformed from GL by intestinal bacteria.

In this study, we discovered that the plasma concentration of GA in rats decreased by concomitant oral administration of GL with sennoside A, one of the active principles of the purgative action of rhubarb. The frequency of diarrhea appearance after administration of sennoside A to rats was dose-dependent from 2.5 to 10 mg/kg. However, when GL was orally co-administrated to rats with a non-effective dose of sennoside A (1.6 mg/kg), the $AUC_{(0—lim)}$ and C_{max} of GA decreased when compared to those treated with GL alone. Moreover, there was not a significant change of these pharmacokinetics parameters of GA among the co-administration groups. These results suggest that the onset of diarrhea does not direct cause lowering the pharmacokinetic parameters of GA.

When GL was orally administrated to rats that had undergone ileostomy, GA was not detected in the plasma suggesting the involvement of intestinal flora in the hydrolysis of GL. Moreover, the $AUC_{(0—lim)}$ and C_{max} of GA decreased when GL was intracecally administrated to rats with sennoside A, when compared to those after administration of GL alone. These results indicate that the main absorption part of

GA is in the colon after transformation of GL to GA by intestinal bacteria, and that GA absorption in the colon is affected by sennoside A or its derivatives.

In this study, the purgative action was observed after the administration of sennoside A to the rats. However, sennoside A itself has been reported to have no purgative activity, with metabolites produced by intestinal bacteria causing the diarrhea.[23,24] Sennoside A is hydrolyzed by intestinal bacteria, and is transformed into sennidin A. Furthermore, it is reduced to rhein-9-anthrone under anaerobic conditions in the intestine and is oxidized to rhein.[25—27] Rhein-9-anthrone has been reported to be one of the main components related to the purgative activity caused by rhubarb.[28]

In order to elucidate the interaction of GL and sennosides or its derivatives directly, we investigated their direct influence on the absorption of GA using an *in situ* loop. Inhibition of GA absorption was observed in rhein (approximately 75%), sennoside A (approximately 60%) and sennidin A (approximately 25%), whereas rhein-9-anthrone showed no inhibitory effect. The significant inhibitory activity by rhein was observed in the ileum and colon suggesting the inhibition activity of rhein differs in parts. An *in vitro* study of the rat intestinal bacteria confirmed that sennoside A and sennidin A does not affect the transformation of GL to GA. Therefore, these results indicate that the inhibition of GA absorption occurs at the absorption phase of GA from the intestine by interaction with sennosides and its derivatives. However, the fact that the inhibition activity of rhein differed in parts is difficult to account for from these results.

In this study, it was clarified that sennosides and its derivatives inhibited the absorption of GA from the rat intestine. We conclude that a decrease of the pharmacokinetic parameters of GA in human plasma observed in the clinical study of TJ-8117 is attributable to the interactive action of absorption from the intestinal tract by anthraquinones contained in or derived from rhubarb. It has been reported that involvement of MCT1, monocarboxylic acid transporter, in the intestinal absorption of weak organic acid such as compound having carboxylic moiety.[29—31] Based on these reports, the MCT1 transporter may participate in the absorption of both rhein and GA from intestine. Thus, competitive inhibition of rhein and/or the other anthraquinones on GA transportation by MCT1 should be concerned as one of the reason to decrease GA absorption in human subjects. In addition, the induction of the metabolic enzyme or P-glycoprotein in an intestinal tract may be cause by lowing the absorption of GA. In order to clarify this hypothesis, further experiments related to MCT1/the other organic anion transporters, metabolic enzyme, and P-glycoprotein are required.

To our knowledge, this is the first report showing a pharmacokinetic interaction between two herbs. In the case of our clinical study of TJ-8117, since GA was a marker component of exposure, which might not directly relate to efficacy and safety, the observed decrease in the pharmacokinetic parameters was not considered a serious problem. However, attention should be paid to herbs with a low therapeutic range when they are administered with not only new drugs, but also with other herbs.

CONCLUSION

Based on this investigation, the decrease in GA absorption in human subjects in the clinical study of TJ-8117 is strongly suggested to be a direct interaction of GA absorption with sennosides and its derivatives. However, the mechanism of this interaction is still ambiguous. Thus, further studies to elucidate the mechanism are required.

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