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Comparison of oral bioavailability of genistein and genistin in rats

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Abstract

Genistein (GT) is an isoflavone from Leguminosae and has received much attention as a phytoestrogen. Genistin is a glycoside form of GT (genistein-7-*O*-β-D-glucopyranoside, GT-glu) is mainly found in soy-derived foods. In this study, we examined the pharmacokinetic properties and bioavailability of GT in rats and compared with those of GT-glu. In order to characterize and compare the pharmacokinetics of GT and GT-glu, these compounds were administered intravenously and orally. The plasma concentration of GT was determined by HPLC after enzymatic hydrolysis. After oral administration of GT with various doses (4, 20, 40 mg/kg), the bioavailability of GT was 38.58, 24.34 and 30.75%, respectively. The T_{max}, C_{max} and $AUC_{(0-\infty)}$ of GT after oral administration of GT (40 mg/kg), were 2 h, 4876.19 ng/ml, 31,269.66 ng h/ml, respectively. When smaller amount of GT was administered, the faster T_{max} was observed. Oral administration of GT-glu resulted in longer T_{max}, lower C_{max}, and greater bioavailability than that of GT. The pharmacokinetic parameters of GT following oral administration of GT-glu (64 mg/kg as GT-glu, 40 mg/kg as GT) were obtained as follows: 8 h (T_{max}), 3763.96 ng/ml (C_{max}), 51,221.08 ng h/ml ($AUC_{(0-\infty)}$) and 48.66% (absolute bioavailability), respectively. These results indicate that the oral bioavailability of GT-glu is greater than that of GT.

Keywords: Genistein; Genistin; Pharmacokinetics; Oral bioavailability

1. Introduction

Isoflavones, major dietary components of soybeans have received much attention because of their health-related and clinical benefits such as estrogenic and anti-oxidative activities as well as triggering of natural killer cell activity (Zhang et al., 1999). Particularly, genistein (GT), one of the major isoflavones in Leguminosae, has received great attention as a phytoestrogen demonstrating pharmacological effects such as prevention of hormone-related cancers and bone loss (Zhang et al., 1999).

There have been several detailed studies on absorption, distribution, metabolism and excretion of GT in rats and humans (Piskula et al., 1999; Coldham and Sauer, 2000; Mallis et al., 2003; Steensma et al., 2004; Pascual-Teresa et al., 2005). Although GT is rapidly absorbed in the small intestine, it has a low bioavailability due to its poor water solubility. GT exists in the systemic circulation after being absorbed as several molecular forms including glucuronide and sulfate conjugates, free GT, and protein-bound form (Shelnutt et al., 2002). Of these, the primary metabolite of GT is reported to be GT glucuronide and GT sulfate, and the metabolism is believed to occur mainly in the liver and epithelial cells of the intestinal wall (Coldham and Sauer, 2000). It has been reported that following infusion of the duodenum with GT, the major metabolite in portal blood is GT-7-*O*-glucuronide (Sfakianos et al., 1997). Thus, both the liver and intestine seem to contribute to the first pass effect. GT is known to be excreted as GT metabolites such as dihydroGT, 6′-OH-*O*-desmethylangolensin, trihydroxybenzene and 3′,4′,5,7-tetrahydroxyisoflavone through feces and urines (Coldham and Sauer, 2000; Heinonen et al., 2003) (Fig. 1).

There are several reports suggesting that the conjugates may either have biological activity themselves or serve as excellent sources of biologically active compounds (Shelnutt et al., 2002). For example, GT glucuronides may be active in vivo as they have

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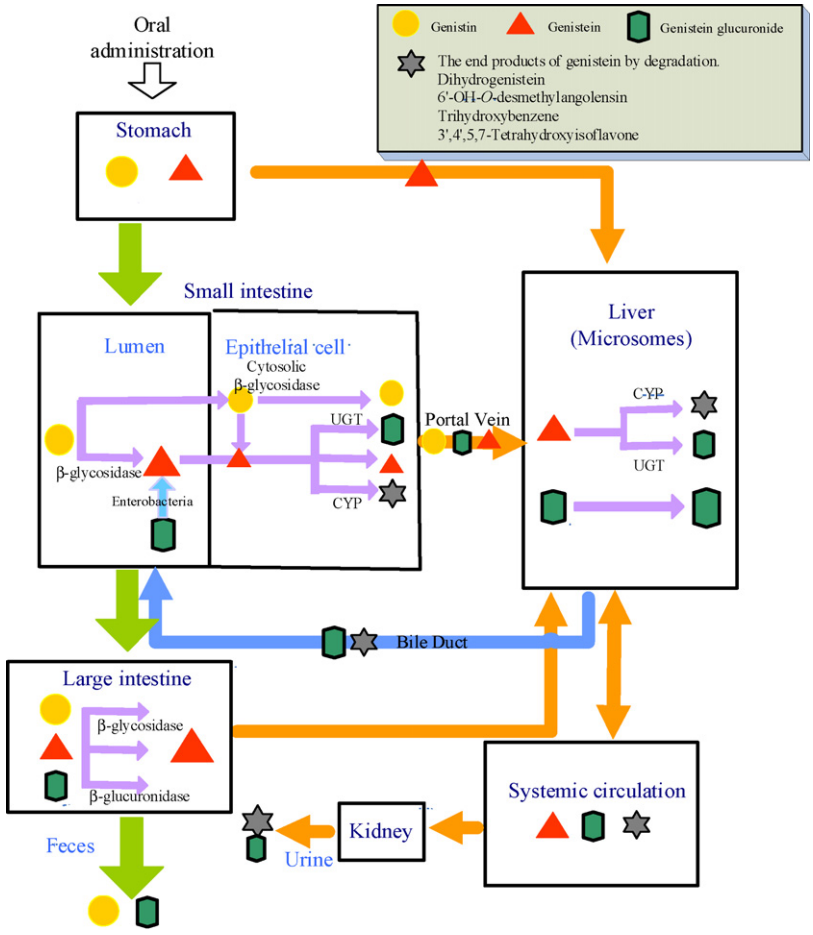

Fig. 1. A summary of absorption, distribution, metabolism and excretion of GT after oral administration of GT and its glycoside form (GT-glu) (CYP, cytochrome P 450; UGT, UDP-glucuronosyl-transferase).

been shown to have weak estrogenic activity and can activate human natural killer cells in vitro (Zhang et al., 1999). Additionally there is a report that GT-7,4-di-*O*-sulfate competitively inhibits sterol sulfatase in hamster liver microsomes (Wong and Keung, 1997). It is possible, therefore, that sulfate conjugated isoflavones are also active in vivo or are expected to be primary sources of free cellular GT after enzymatic hydrolysis in target tissues.

Soybeans and soy-derived foods contain GT, which are present and ingested mainly as genistin, a glycoside form of GT (genistein-7-*O*-β-D-glucopyranoside, GT-glu) (Reinli and Block, 1996). Nonetheless, in general, little is known about the pharmacokinetic characteristics and bioavailability of GT-glu. Particularly, it is still unknown whether GT-glu can be absorbed in its intact form and the degree to which it is hydrolyzed to its respective aglycone form during absorption process. Furthermore, pharmacokinetic parameters of GT-glu have not been revealed yet.

Thus, in this study, we evaluated the oral pharmacokinetic properties and bioavailability of GT-glu in comparison with GT in rats. With the results obtained from our study, we have been able to determine which compound is more orally bioavailable between GT and GT-glu.

2. Materials and methods

2.1. Materials

GT and GT-glu were obtained from Rexgene Biotech Co. (Seoul, Korea). 4-Hydroxybenzophenone (internal standard) and ammonium formate were purchased from Sigma Chemical Company (St, Louis, MO). Sulfatase extracted from *Helix pomatia* was also a product of Sigma Chemical Company and was used to hydrolyze GT-glu and the glucuronide and sulfate conjugates of GT. HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ). Ethyl acetate, sodium carboxylmethylcellulose (Na-CMC), dimethyl sulfoxide (DMSO), formic acid and other reagents were reagent grade and purchased from Junsei Chemical Company (Tokyo, Japan). Distilled and deionized water was used for the preparation of all solutions.

2.2. Activity test of sulfatase toward glucuronide and sulfate conjugates of GT and GT-glu

The sulfatase from *H. pomatia* that exhibits deglucuronidation and desulfatation activities toward the conjugates of GT was

used to determine total GT concentration in plasma (Shelnutt et al., 2002). To determine the amount of sulfatase required to deglucuronidate and desulfate GT conjugates, we examined the sulfatase activity toward the GT conjugates. Rat plasma samples were obtained 3 h after oral administration of GT (40 mg/kg). Plasma samples (50 μl) were incubated in a shaking incubator with various amounts of the sulfatase at 37 °C for 5 h. Then each sample was prepared as described below in Section 2.4 and analyzed by HPLC.

Also, the sulfatase was used to hydrolyze GT-glu to GT in plasma since it was reported that the sulfatase contains β-glycosidase activity toward GT-glu (Ioku et al., 1998). To determine the amount of sulfatase required to deglycosidate GT-glu, a 50 μl GT-glu stock solution (5 μg/ml) was incubated with various amounts of the sulfatase at 37 °C for 5 h.

2.3. Animals and drug administration

Male Sprague-Dawley rats, 8-week old and weighing 260–310 g, had free access to isoflavone-free diet and water until 12 h prior to being used in the experiments. The four experiments, oral and intravenous administrations of GT and GT-glu, were performed to estimate their bioavailabilities. Rats were anesthetized with chloroform before cannulation. Polyethylene cannulas were inserted in the femoral vein for intravenous administration and the femoral artery was cannulated for blood sampling. The oral doses of GT suspended in a 0.2% Na-CMC solution were 4, 20 and 40 mg/kg, and that of GT-glu was 40 mg/kg as GT. In cases of GT and GT-glu oral administration, plasma samples were collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h for GT and 0, 2, 4, 6, 7, 8, 9, 10, 12, 14, 18, 24, 30, 36 and 48 h for GT-glu, respectively. The intravenous dose of GT solubilized in 50% DMSO was 1 mg/kg. Plasma samples were collected after intravenous administration at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 h. Then, each blood sample was centrifuged at 12,000 rpm for 10 min. The plasma samples (approximately 0.2 ml) were stored at −20 °C until analyzed by HPLC.

2.4. Sample preparation

Plasma samples (50 μl) were incubated with 500 unit of the sulfatase at 37 °C for 5 h. Then 4-hydroxybenzophenone (internal standard) and 5 ml of ethyl acetate (extraction solvent) were added to samples. Extraction was conducted by vortexing vigorously for 50 min and centrifuged at 3000 rpm for 15 min. The supernatant was transferred to glass tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted with 0.2 ml of 50% MeOH.

2.5. HPLC assay

GT and 4-hydroxybenzophenone were separated on an ODS column (150 mm 4.6 mm Capcell Pak, Shiseido, Tokyo, Japan) maintained at 40 °C. The mobile phase used was a 6:4 mixture of acetonitrile and 50 mmol ammonium formate buffer solution (pH 3.5) (A line) and a 2:8 mixture of acetonitrile and 50 mmol ammonium formate buffer solution (pH 3.5) (B line). The elution conditions were as follows: for 0–4 min, A line was changed from 65 to 51% and B line from 35 to 49%; for 4–8 min, A line from 51 to 65% and B line from 49 to 35%; for 8–10 min, A line 65%, B line 35% were maintained for column stability. Flow rate was set at 1.5 ml/min and detection was performed at 262 nm.

2.6. HPLC method validation

The accuracy and precision of the HPLC method was evaluated in the concentration range of 100–4000 ng/ml. The within-run accuracy and precision values were determined in six replicates at the concentrations of 100, 200, 500, 1000, 2000 and 4000 ng/ml. The between-run accuracy and precision were determined for 5 different days, and the mean concentrations and the coefficient of variation were calculated.

2.7. Pharmacokinetic analysis

Pharmacokinetic analysis was carried out using BA Calc 2002 pharmacokinetic analysis computer program (Korea Food & Drug Administration, Korea). Area under the curve (AUC), maximum plasma concentration (C_{max}) and the time needed to reach the maximum plasma concentration (T_{max}) were determined by the program. The elimination rate constant (K_{el}) was obtained from the terminal slope using regression analysis, and the half-life ($t_{1/2}$) of the drug was calculated by a relationship of $0.693/K_{el}$. The absolute bioavailability (AB) of GT and GT-glu were calculated using the following equation:

$$\text{AB}\,(\%) = \frac{\text{AUC oral}}{\text{AUC iv}} \times \frac{\text{iv dose}}{\text{oral dose}} \times 100$$

where oral is the oral administration of GT or GT-glu and iv is the intravenous administration of GT or GT-glu.

3. Results

3.1. Activity of sulfatase toward glucuronide and sulfate conjugates of GT and GT-glu

The amount of sulfatase required to deglucuronidate and desulfate GT conjugates was shown in Fig. 2. The sulfatase activity to deglucuronidate and desulfate was saturated at sulfatase amounts exceeding 200 unit. When 200 unit of sulfatase was added, level of GT in plasma was 3100 ng/ml and it was not increased significantly at sulfatase amounts greater than 200 unit.

The β-glycosidase activity of the sulfatase toward GT-glu was also shown in Fig. 2. One hundred units of the sulfatase were capable of producing 2253 ng/ml of GT from GT-glu and an increase in the amount of the sulfatase caused a slight increase in GT level produced by hydrolysis of GT-glu. Based on the relationship shown in Fig. 2, 500 unit of the sulfatase was considered to be sufficient to deglycosidate GT-glu and deconjugate GT metabolites.

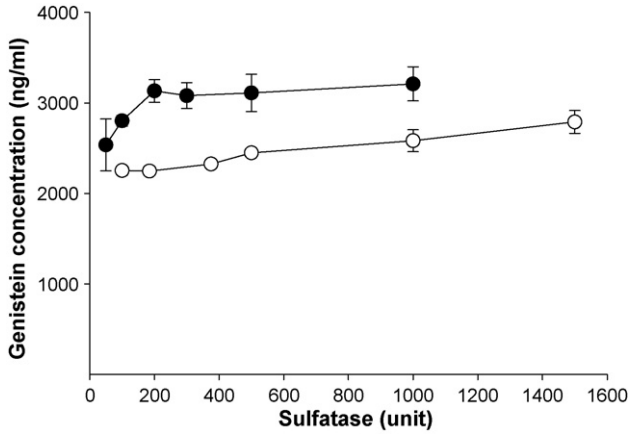

Fig. 2. The activity test of sulfatase from *Helix pomatia* toward GT conjugates and GT-glu. Filled circle (●) shows the glucuronidase and sulfatase activities of the sulfatase used in the study toward the conjugates of GT and empty circle (○) demonstrates the β-glycosidase activity of the sulfatase toward GT-glu (mean ± S.E., $n = 3$).

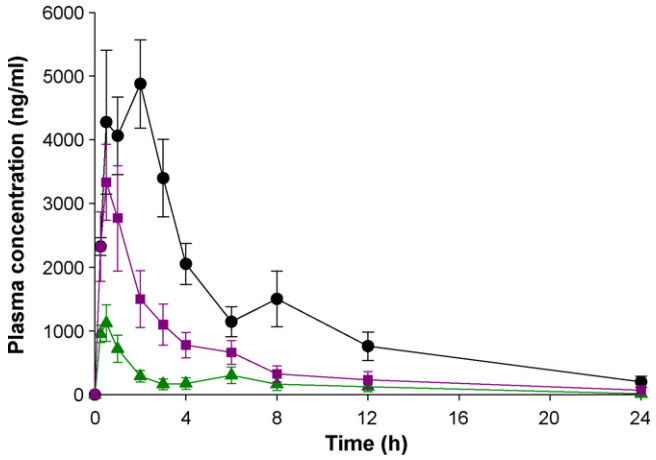

Fig. 3. Mean (±S.E.) plasma concentration vs. time curves after oral administration of GT suspension at various doses. (▲) 4 mg/kg ($n = 6$); (■) 20 mg/kg ($n = 5$); (●) 40 mg/kg ($n = 5$).

3.2. Validation

Standard curves were plotted from the ratio of peak height of GT to that of internal standard. The standard curves exhibited excellent linearity and correlation coefficient was about 0.9998. The linear equation obtained was $y = 0.4472x + 0.0343$. The inter-intra validation data are shown in Table 1.

3.3. Oral and intravenous administration of GT

Mean plasma concentration–time profiles of GT after oral administration of GT (4, 20 and 40 mg/kg) and intravenous administration (1 mg/kg) are shown in Figs. 3 and 4, respectively, and pharmacokinetic parameters are listed in Table 2. These results indicate plasma levels of GT determined from free and conjugated forms. After oral administration of GT 40 mg/kg, T_{max} of GT was 2 h and almost eliminated from the systemic circulation after 24 h. On the other hand, the T_{max} values were approximately 0.5 h after oral administration of GT 4 and 20 mg/kg, which were 1.5 h faster than that obtained with oral dose of 40 mg/kg of GT. The second small peak appeared at 6–8 h.

After oral administration of GT at doses of 4, 20 and 40 mg/kg, the $AUC_{(0-\infty)}$ values of GT were 3923.03 ± 1531.09, 12,376.88 ± 3183.47, 31,269.66 ± 5548.44 ng h/ml, respec-

Table 1
Summary of the precision and accuracy of the HPLC method for GT

Nominal concentration (ng/ml)	Intra-day validation		Inter-day validation	
	Precision (%CV)	Accuracy (%)	Precision (%CV)	Accuracy (%)
100	27.50	102.56	20.49	92.14
200	13.24	111.15	12.97	109.95
500	15.06	110.86	10.61	95.53
1000	5.55	96.84	3.13	95.93
2000	6.81	104.31	3.13	99.31
4000	5.29	101.23	2.36	99.28

CV, coefficient of variation.

Table 2
Pharmacokinetic parameters of GT following the oral and intravenous administration of GT in rats (mean ± S.E., $n = 5$–6)

	GT			
	$Oral_{(0-24\,h)}$			$IV_{(0-12\,h)}$
Dose as GT (mg/kg)	**4**	**20**	**40**	1
$AUC_{(terminal)}$ (ng h/ml)	3832.18 ± 1404.69	11,931.76 ± 2625.13	29,618.14 ± 5216.64	2339.03 ± 197.42
$AUC_{(0-\infty)}$ (ng h/ml)	3923.03 ± 1531.09	12,376.88 ± 3183.47	31,269.66 ± 5548.44	2542.06 ± 203.11
C_{max} (ng/ml)	1124.49 ± 236.40	3330.08 ± 647.76	4876.19 ± 926.21	2943.64 ± 146.02
T_{max} (h)	0.5 ± 0.10	0.5 ± 0.10	2 ± 0.30	
$t_{1/2}$ (h)	4.53 ± 1.40	4.41 ± 1.21	5.25 ± 0.92	0.196 ± 0.02
AB (%)	**38.58**	**24.34**	**30.75**	

$AUC_{(0-\infty)}$, area under the plasma concentration–time curve from 0 h to infinity; C_{max}, peak concentration; T_{max}, time to reach peak concentration; $t_{1/2}$, terminal half-life; AB (%), absolute bioavailability.

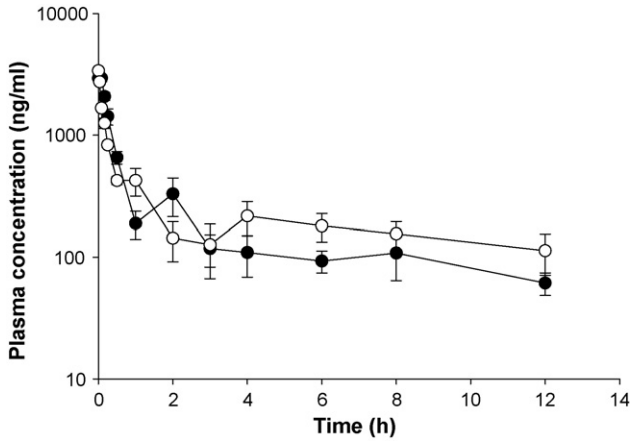

Fig. 4. Mean (±S.E.) plasma concentration–time profiles of GT after intravenous administration of GT and GT-glu. Filled circle (●) shows the plasma concentration of GT after the injection of GT solution (1 mg/kg) and empty circle (◯) shows the plasma concentration of GT after the injection of 1.6 mg/kg of GT-glu (equivalent to 1 mg/kg GT) solution ($n = 5$).

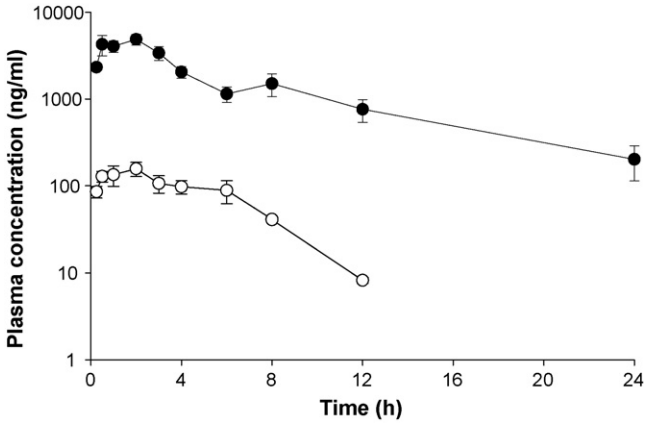

Fig. 5. Mean (±S.E.) plasma concentration–time curves after oral administration (40 mg/kg) of GT suspension. Filled circle (●) shows the plasma concentration of GT after enzymatic hydrolysis and empty circle (◯) demonstrates the concentration of GT without enzymatic hydrolysis ($n = 5$).

tively. Absolute bioavailabilities of each dose were 38.58, 24.34, and 30.75%, respectively.

After oral administration of GT at a dose of 40 mg/kg, the pharmacokinetic profiles of GT obtained with or without sulfatase hydrolysis are shown in Fig. 5 and Table 3. The mean plasma level of GT obtained without sulfatase hydrolysis was much lower than that obtained after sulfatase hydrolysis. The maximum plasma concentration of GT without sulfatase hydrolysis was 157.01 ± 25.68 ng/ml and this was only 3.2% of that measured with sulfatase hydrolysis. The $AUC_{(0-\infty)}$ of GT obtained after sulfatase treatment was 37-fold greater than that found without sulfatase hydrolysis. T_{max} was observed at 2 ± 0.41 h for both groups.

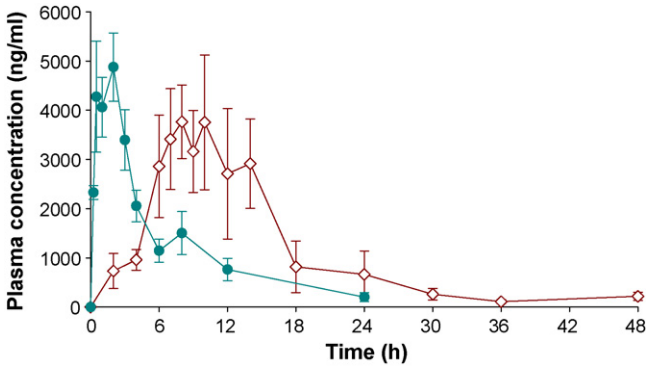

Fig. 6. Mean (±S.E.) plasma concentration–time curves of GT after oral administration of GT (40 mg/kg) and GT-glu (64 mg/kg, equivalent to 40 mg/kg GT). Filled circle (●) shows the plasma concentration of GT after oral administration of GT and empty diamond (◊) demonstrates the concentration of GT after oral administration of GT-glu ($n = 5$).

Table 4
Pharmacokinetic parameters of GT following the oral and intravenous administration of GT-glu in rats (mean ± S.E., $n = 5$)

	GT-glu	
	$Oral_{(0-48\,h)}$	$IV_{(0-12\,h)}$
Dose as GT (mg/kg)	**64**[a]	1.6[a]
$AUC_{(terminal)}$ (ng h/ml)	48,866.13 ± 12,450.48	2600.93 ± 390.33
$AUC_{(0-\infty)}$ (ng h/ml)	51,221.08 ± 12,358.58	2631.41 ± 393.28
C_{max} (ng/ml)	3763.96 ± 1007.65	3392.10 ± 73.61
T_{max} (h)	8 ± 1.03	
$t_{1/2}$ (h)	7.47 ± 0.65	0.19 ± 0.01
AB (%)	**48.66**	

$AUC_{(0-\infty)}$, area under the plasma concentration–time curve from 0 h to infinity; C_{max}, peak concentration; T_{max}, time to reach peak concentration; $t_{1/2}$, terminal half-life; AB (%), absolute bioavailability.

[a] GT-glu doses of 64 and 1.6 mg/kg are equivalent to GT doses of 40 and 1 mg/kg, respectively.

3.4. Oral and intravenous administrations of GT-glu

The profile of total GT plotted against time after oral administration of GT-glu at a dose of 40 mg/kg as GT is illustrated in Fig. 6 and the pharmacokinetic parameters are shown in Table 4. The plasma profile of total GT after oral administration of GT-glu was considerably different from that of orally administered GT at the same dose. Plasma level of GT steadily increased when

Table 3
Comparison of pharmacokinetic parameters of total GT obtained with or without sulfatase hydrolysis after oral administration of GT (40 mg/kg; mean ± S.E., $n = 5$)

	$AUC_{(0-24\,h)}$ (ng h/ml)	$AUC_{(0-\infty)}$ (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)
EH	29,618.14 ± 5216.64	31,269.66 ± 5548.44	4876.19 ± 926.21	2	5.25 ± 0.92
Non-EH	786.30 ± 116.33	786.30 ± 116.33	157.01 ± 25.68	2	2.69 ± 0.08

$AUC_{(0-\infty)}$, area under the plasma concentration–time curve from 0 h to infinity; C_{max}, peak concentration; T_{max}, time to reach peak concentration; $t_{1/2}$, terminal half-life; EH, total GT measured after enzymatic hydrolysis; non-EH, free GT measured without enzymatic hydrolysis.

GT-glu was orally administered until 8 h and was maintained for about 2 h. Most GT was eliminated 48 h after administration. The $AUC_{(0–48\,h)}$ and $AUC_{(0–\infty)}$ of GT measured after oral administration of GT-glu were estimated to be 48,866.13 ± 12,450.48 and 51,221.08 ± 12,358.58 ng h/ml, respectively. The maximum plasma concentration of GT obtained with oral administration of GT-glu was 3763.96 ± 1007.65 ng/ml and it appeared approximately at 8 h after oral administration of GT-glu. When considering the $AUC_{(0–\infty)}$ determined following intravenous administration of GT-glu, the absolute bioavailability of GT-glu was 48.66%, considerably higher than that obtained with oral administration of GT.

4. Discussion

GT was absorbed much faster than that of GT-glu when administered orally to rats. T_{max} of 40 mg/kg dose of GT was four times faster than that of equivalent dose of GT derived from GT-glu. This is in line with previous report in healthy premenopausal women (Setchell et al., 2001). The faster absorption of GT is considered to be due mainly to its higher hydrophobicity and lower molecular weight than that of GT-glu (Spencera et al., 1999).

As demonstrated by other flavonoid glycosides such as rutin in rats, GT-glu showed a delayed absorption than its aglycone (Manach et al., 1997). This can be explained mainly by the fact that hydrolysis of GT-glu by β-glycosidase present in the jejunum and ileum, and by enterobacterias in the colon took significant amount of time before the absorption had occurred.

The $AUC_{(0–\infty)}$ of GT in rats following oral administration of GT-glu was 50% greater than that of direct GT. The result was similar to previous report that the bioavailability of GT-glu is greater than that of GT (Setchell et al., 2001). The reason for this can be attributed to different absorption and metabolic behavior of GT and GT-glu. Andlauer et al. (2000) has shown that only about 15% of administered GT-glu is converted to GT in the small intestine and Steensma et al. (2004) has also shown that 15% of GT-glu is converted to GT in the large intestine. Thus, based on these literatures, we can assume that about less than 50% of GT-glu administered was converted to GT, rather than all GT-glu administered were changed to GT. Therefore, after the oral administration of GT-glu, it is speculated that both GT-glu and GT are available for absorption in the small and large intestine. As mentioned above, although the conversion of GT-glu to GT occurs gradually (Taylor et al., 2005), GT converted from GT-glu would rapidly be absorbed throughout the intestinal tract. Additionally, GT-glu would also be absorbed in its intact form. The mechanism of the absorption of GT-glu has previously been reported to be different from that of GT. GT-glu can passively be transported across the small intestinal membrane (Andlauer et al., 2000; Steensma et al., 2004) and enter the systemic circulation via the sodium dependent glucose transporter (SGLT1) localized in the small intestinal brush border membrane (Toggenburger et al., 1982; Gee et al., 1998). Thus, GT-glu can steadily be absorbed in the form of both GT and GT-glu as long as they are available for absorption in the small and large intestinal tract. Inside the cellular compartment, cytosolic β-glycosidases can cleave some of GT-glu to GT, and GT-glu, GT and its metabolites (GT-glucuronide and GT-sulfate) could be transported into the blood stream (Day et al., 1998). The above explanation may be used to support a greater bioavailability of GT-glu compared to that of GT.

The secondary peak found in the GT concentration–time profiles is considered as a result of the enterohepatic recirculation. Several pharmacokinetic studies of GT have also found a similar second maximal peak (Supko and Malspeis, 1995; Coldham and Sauer, 2000) due to extensive biliary excretion of GT metabolites (Yasuda et al., 1996; Sfakianos et al., 1997). Enterohepatic recirculation would increase the duration of exposure in the body and thus provide a possibility to prolong pharmacological action (Dobrinska, 1989). The reason why the plasma concentration profile of GT after oral administration of GT-glu did not show apparently a second peak was probably because the absorption of GT transformed from GT-glu was very slow and continuously occurred as judged by the slower T_{max} value.

There were differences in plasma concentration–time profiles and pharmacokinetic parameters of GT among individual rats after oral administration of GT-glu. This may be attributed to that the activity of β-glycosidase in the small intestine and exhibited by enterobacteria in the colon of the individual rat was highly different from each other.

A primary site of glucuronidation is likely to be the intestinal wall based on a report that following infusion of the duodenum with [^{14}C] GT, the major metabolite in the portal vein is GT-7-*O*-β-glucuronide (Sfakianos et al., 1997). Another work also reported that in the perfusion model using rats, it was found that GT is metabolized into GT-7-*O*-β-glucuronide at the jejunal compartment of the rat gut within 2 h of perfusion (Steensma et al., 2004).

The predominant appearance of GT-7-*O*-β-glucuronide and sulfate in the systemic circulation is consistent with the studies carried out in humans (Adlercreutz et al., 1995; Coward et al., 1996) demonstrating that β-glucuronide and sulfate esters of GT accounted for 95% of the GT in the circulation. In our experiment, GT conjugated with β-glucuronide and sulfate accounted for 97% of the GT in the systemic blood.

In conclusion, we compared the bioavailability and pharmacokinetic profiles of GT after oral administration of GT and GT-glu. The study clearly demonstrated that GT-glu was more bioavailable than GT. The greater bioavailability of GT-glu than that of GT appears to have resulted from the ability of GT-glu to be absorbed in its intact form and GT after being hydrolyzed in the small and large intestinal tract. The sulfatase treatment was necessary since it had a significant impact on the measurement of level of GT in plasma samples. The study on the bioavailability of GT and GT-glu in rats may be used as standard data when the study on the improvement of bioavailability of GT and GT-glu need to be performed.

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References

Adlercreutz, H., Fotsis, T., Kurzer, M.S., Wahala, K., Makela, T., Hase, T., 1995. Isotope dilution gas chromatographic–mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. Anal. Biochem. 225, 101–108.

Andlauer, W., Kolb, J., Fürst, P., 2000. Absorption and metabolism of genistin in the isolated rat small intestine. FEBS Lett. 475, 127–130.

Coldham, N.G., Sauer, M.J., 2000. Pharmacokinetics of [^{14}C]genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. Toxicol. Appl. Pharmacol. 164, 206–215.

Coward, L., Kirk, M., Albin, N., Barnes, S., 1996. Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring–mass spectrometry. Clin. Chim. Acta 247, 121–142.

Day, A.J., DuPont, M.S., Ridley, S., Rhodes, M., Rhodes, M.J.C., Morgan, M.R.A., Wiliamson, G., 1998. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett. 436, 71–75.

Dobrinska, M.R., 1989. Enterohepatic circulation of drugs. J. Clin. Pharmacol. 29, 577–580.

Gee, J.M., DuPont, M.S., Rhodes, M.J.C., Johnson, I.T., 1998. Quercetin glucosides interact with the intestinal glucose transport pathway. Free Radic. Biol. Med. 25, 19–25.

Heinonen, S.-M., Hoikkala, A., Wähälä, K., Adlercreutz, H., 2003. Metabolism of the soy isoflavones daidzein, genistein and glycitein in human subjects. Identification of new metabolites having an intact isoflavonoid skeleton. J. Steroid Biochem. Mol. Biol. 87, 285–299.

Ioku, K., Pongpiriyadacha, Y., Konishi, Y., Takei, Y., Nakatani, N., Terao, J., 1998. β-Glucosidase activity in the rat small intestine toward quercetin monoglycerides. Biosci. Biotechnol. Biochem. 62, 1428–1431.

Mallis, L.M., Sarkahian, A.B., Harris, H.A., Zhang, M.-Y., McConnell, O.J., 2003. Determination of rat oral bioavailability of soy-derived phytoestrogens using an automated on column extraction procedure and electrospray tandem mass spectrometry. J. Chromatogr. B. 796, 71–86.

Manach, C., Morand, C., Demigne, C., Texier, O., Regerat, F., Remecy, C., 1997. Bioavailability of rutin and quercetin in rats. FEBS Lett. 409, 12–16.

Pascual-Teresa, S., Hallund, J., Talbot, D., Schroot, J., Williams, C.M., Bugel, S., Cassidy, A., 2005. Absorption of isoflavones in humans: effects of food matrix and processing. J. Nutr. Biochem. 17, 257–264.

Piskula, M.K., Yamakoshi, J., Iwai, Y., 1999. Daidzein and genistein but not their glucosides are absorbed from the rat stomach. FEBS Lett. 447, 287–291.

Reinli, K., Block, G., 1996. Phytoestrogen content of foods: a compendium of literature values. Nutr. Cancer 26, 123–148.

Setchell, K.D.R., Brown, N.M., Desai, P., Zimmer-Nechemias, L., Wolfe, B.E., Brashear, W.T., Kirschner, A.S., Cassidy, A., Heubi, J.E., 2001. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J. Nutr. 131, 1362–1375.

Sfakianos, J., Coward, L., Kirk, M., Barnes, S., 1997. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. J. Nutr. 127, 1260–1268.

Shelnutt, S.R., Cimino, C.O., Wiggins, P.A., Badger, T.M., 2002. Pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein in men and women after consumption of a soy beverage. Am. J. Clin. Nutr. 76, 588–594.

Spencera, J.P.E., Chowrimootoo, G., Choudhury, R., Debnam, E.S., Srai, S.K., Rice-Evans, C., 1999. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett. 458, 224–230.

Steensma, A., Bienenmann-Ploum, M.E., Noteborn, H.P.J., 2004. Intestinal uptake of genistein and its glycoside in the rat using various isolated perfused gut segments. Environ. Toxicol. Pharmacol. 17, 103–110.

Supko, J.G., Malspeis, L., 1995. Plasma pharmacokinetics of genistein in mice. Int. J. Oncol. 7, 847–854.

Taylor, J.I., Grace, P.B., Bingham, S.A., 2005. Optimization of conditions for the enzymatic hydrolysis of phytoestrogen conjugates in urine and plasma. Anal. Biochem. 341, 220–229.

Toggenburger, G., Kessler, M., Semenza, G., 1982. Phloridzin as a probe of the small-intestinal Na^+, D-glucose cotransporter. A model. Biochim. Biophys. Acta 688, 557–571.

Wong, C.-K., Keung, W.M., 1997. Daidzein sulfoconjugates are potent inhibitors of sterol sulfatase. Biochem. Biophys. Res. Commun. 233, 579–583.

Yasuda, T., Mizunuma, S., Kano, Y., Saito, K.-I., Ohsawa, K., 1996. Urinary and biliary metabolites of genistein in rats. Biol. Pharmacol. Bull. 19, 413–417.

Zhang, Y., Song, T.T., Cunnick, J.E., Murphy, P.A., Hendrich, S., 1999. Daidzein and genistein glucuronides in vitro are weakly estrogenic and activate human natural killer cells at nutritionally relevant concentrations. J. Nutr. 129, 399–405.