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Dose-Dependent Absorption, Metabolism, and Excretion of Genistein in Rats

SIYUAN ZHOU,† YUZHEN HU,§ BANGLE ZHANG,† ZENGHUI TENG,† HONGQUAN GAN,† ZHIFU YANG,† QINGWEI WANG,† MENGLEI HUAN,† AND QIBING MEI*,†

Department of Pharmacology, School of Pharmacy, and Department of Physiology, School of Preclinical Medicine, Fourth Military Medical University, Xi'an 710032, China

Genistein (4′,5,7-trihydroxyisoflavone), a naturally occurring phenolic compound, possesses well-known preventive activity in breast and prostate cancer, cardiovascular diseases, and postmenopausal problems. The aim of this study is to investigate the distribution and dose-dependent absorption, metabolism, and excretion of genistein in rats. Genistein was orally administered to rats at different doses. At various time intervals, blood, bile, and urine samples were collected and incubated with glucuronidase to hydrolyze the glucuronidated genistein. Genistein was detected by HPLC. High levels of glucuronidated genistein were detected in the plasma, bile, and urine after genistein administration. When genistein was administered to rats at 6.25, 12.5, and 50 mg·kg^{-1} doses, the $AUC_{(0-t)}$ values for genistein were 23.5, 80.9, and 177.9 mg·min·L^{-1}; the oral absolute bioavailabilities were 21.9, 33.5, and 19.0%; the $AUC_{(0-t)}$ values of glucuronidated genistein were 173.8, 470.7, and 1721.2 mg·min·L^{-1}, respectively. The cumulative biliary excretion of genistein respective to each dose was 42.6 ± 6.5, 75.2 ± 18.9, and 126.6 ± 34.8 μg; the cumulative biliary excretion of glucuronidated genistein was 108.5 ± 35.2, 423.5 ± 158.3, and 853.7 ± 320.8 μg for each dose, respectively. The cumulative urinary excretion of genistein was 34.8 ± 10.8, 187.3 ± 67.0 and 213.6 ± 30.6 μg for each dose, respectively; the cumulative levels of glucuronidated genistein excreted in the urine were 217.8 ± 52.1, 583.1 ± 106.9, and 1108.4 ± 88.1 μg, respectively. These results indicated that at high doses absorption, biotransformation, and excretion of genistein occurred in a nonlinear dose-dependent manner. Therefore, the results of these pharmacokinetic studies raise important questions about the therapeutic significance of consuming large quantities of genistein, genistein analogues, or soy-based neutraceuticals.

KEYWORDS: Genistein; bioavailability; biliary excretion; urine excretion; pharmacokinetics

INTRODUCTION

Genistein (4′,5,7-trihydroxyisoflavone), the predominant phytoestrogen found in soy-based products, is an isoflavone with structural features similar to those of 17β-estradiol, particularly the phenolic ring and the distance between the 4′- and 7-hydroxy groups (**Figure 1**) (*1*). As such, genistein is an agonist for both the α isoform of the estrogen receptor (ER-α) and the β isoform (ER-β). The affinity of genistein for ER-β is greater than that for ER-α (*2*). In addition, genistein can inhibit tyrosine kinases and DNA topoisomerase (*3, 4*).

It is well established that genistein has many beneficial effects, such as the prevention of breast and prostate cancer and cardiovascular disease. Its favorable effects in the attenuation of osteoporosis and other postmenopausal symptoms as well as antioxidant properties are also known (*5*−*8*). A diet rich in genistein has been associated with lower incidences of both prostate and breast cancer, particularly for people in Southeast Asia, where soy and its constituents, such as genistein, are consumed at high levels. The nature of the beneficial effects of soy consumption may vary as a result of a number of factors, including age at exposure, exposure dose, the presence of other dietary components, and other yet unknown factors (*9*).

Although many studies have implicated a role of genistein in disease prevention, there are presently no guidelines for optimal dosages and a paucity of integrated data on its pharmacokinetic properties (*10*−*12*). Dietary intakes used in clinical studies have largely been empirically derived, and results remain controversial (*13*−*15*). Oral bioavailability is clearly a crucial factor influencing the efficacy of genistein. In general, naturally occurring genistein is found in plants in either aglycone or glycoside form. The aglycone is absorbed freely from the

* Author to whom correspondence should be addressed (e-mail qbmei@fmmu.edu.cn).
† Department of Pharmacology.
§ Department of Physiology.

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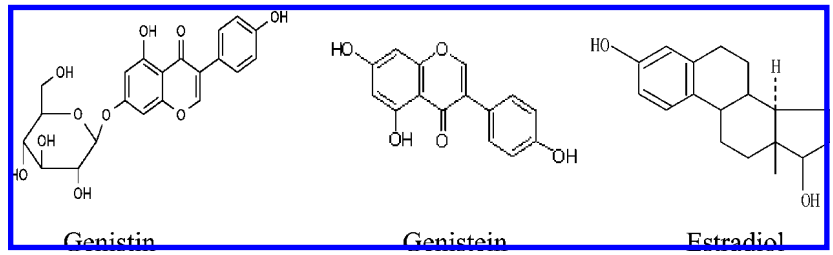

Figure 1. Chemical structures of genistin, genistein, and estradiol.

gut, whereas its glycoside is usually hydrolyzed to the corresponding aglycone molecule prior to gastrointestinal absorption. The toxicity of genistein is relatively low. Subchronic and chronic safety studies with genistein in dogs indicated that the daily oral administration of genistein by capsule at doses of 50, 150, or 500 mg·kg^{-1}·day^{-1} for 4 or 52 weeks was well tolerated and did not result in significant systemic toxicity in the dogs despite these very high doses (*16*). Although studies have shown that high doses of genistein are well tolerated, an understanding of the relationship between the bioavailability of genistein and its dose is essential.

To further elucidate the effects of genistein or to use it as dietary isoflavonoid markers of soy exposure in epidemiologic and clinical studies, its absorption, distribution, metabolism, and elimination need to be understood. The objectives of the present research were to study the distribution and dose-dependent absorption, metabolism, excretion, and oral bioavailability of genistein in rats.

MATERIALS AND METHODS

Materials. Genistein, dexamethasone, and glucuronidase were purchased from Sigma Chemical Co. Acetonitrile and methanol were of HPLC grade, and other chemicals used were of analytical reagent grade. Sprague−Dawley rats (200 ± 20 g) were purchased from the experimental animal research center, Fourth Military Medical University (Xi'an, China). The animals were provided ad libitum access to water and laboratory chow. A 12 h light−dark cycle was used. Rats were fasted for 12 h before all experiments. All animal procedures were performed in accordance with protocols approved by the University Animal Care and Use Committee.

Administration of Genistein. Genistein was suspended in 0.5% carboxymethylcellulose sodium solution. It was then administered to rats by gavage at doses of 6.25, 12.5, or 50 mg·kg^{-1} between 8 and 9 a.m. There were 72 rats in each dosage group. Genistein was solubilized in 10% dimethyl sulfoxide (DMSO)/25% polyethylene glycol (volume ratio was 1:1) and administered to rats through intravenous bolus injection by tail vein at a dose of 12.5 mg·kg^{-1} between 8 and 9 a.m.; 72 rats were used in this group.

Blood Sample Collection and Pretreatment. Rats were lightly anesthetized by diethyl ether, 2 mL blood samples were withdrawn from the abdominal aorta 5, 10, 20, 40, 120, 240, 360, 480, 660, 960, 1440, and 2160 min after dosing, and blood was heparinized. Six new rats were sampled for each sampling time. The plasma was separated by centrifugation (7 min, 4 °C, 2000*g*). To detect the free genistein, 0.5 mL of plasma and 200 μL of dexamethasone solution (internal standard, IS, 2 mg·L^{-1}) were added into the tubes. The samples were extracted twice with 2.0 mL of a mixture of methyl *tert*-butyl ether /pentane (8:2, v/v). The organic phase was transferred to a clean tube and evaporated to dryness at 25 °C under a gentle stream of nitrogen in a ventilation cabinet. The residue was dissolved in 50 μL of methanol, and 20 μL was injected into the HPLC system. To determine the glucuronidated genistein concentration, 100 μL of plasma was incubated with 150 μL of glucuronidase solution (2000 units·mL^{-1} in 0.17 mol·L^{-1} ammonium acetate, pH 4.6) in a water bath at 37 °C for 24 h. Preliminary studies showed that this protocol ensured the complete hydrolyzation of the glucuronidated genistein (*10*). After that, the sample was extracted, and the total genistein was detected as described above. The amount of glucuronidated genistein in plasma was calculated as the difference between total genistein and free genistein.

Tissue Sample Collection and Pretreatment. Following the collection of blood, tissues such as the heart, liver, kidney, spleen, brain, lung, muscle, stomach, intestine, fat, testicles, ovaries, and uterus were immediately removed and rinsed with normal saline. The tissue samples were homogenized with chilled (4 °C) physiological saline (1 g of tissue added to 5 mL of physiological saline). The genistein content of the different tissue samples was determined as described above.

Bile and Urine Sample Collection and Pretreatment. To investigate the biliary excretion of genistein, 18 rats were anesthetized with diethyl ether. The rats' body temperature was maintained at 37 °C after a midline incision. The bile duct was exposed and cannulated with PE tubing. The PE tubing was tied and secured with suture silk. The rats were allowed to recover from anesthesia, and genistein was administered to rats by gavage at doses of 6.25, 12.5, or 50 mg·kg^{-1}. There were six rats in each dosage group. Bile was collected through a biliary cannula at different time intervals throughout the experiment. Urine was also collected. The genistein and glucuronidated genistein in the bile and urine samples were extracted from the samples. The levels of genistein and glucuronidated genistein were then determined, as described above.

Analytical Method. Genistein was detected by using a Beckman high-performance liquid chromatography system (produced by Beckman Corp.) consisting of a 125 binary gradient pump, a 168 photodiode array detector, and gold software (*10*). The mobile phase consisted of 40% acetonitrile and 60% trisodium citrate buffer (50 mmol·L^{-1}, which were adjusted to pH 4.1 with phosphoric acid). A C_{18} analytical column (250 mm × 4.6 mm, 5 μm, Phenomenex) and a C_{18} guard column (50 mm × 0.6 mm, 5 μm, Tianhe Chromatography) were used. The column temperature was maintained at 25 °C. The flow rate was 1 mL·min^{-1} with detection wavelength at 260 nm. Injection volume was 20 μL. Under this HPLC condition, the genistein was clearly separated from interference. The retention time of genistein and dexamethasone (internal standard) were both less than 12.5 min. The precision of the analytical method, expressed as the intraday and interday relative standard deviations (RSDs), was below 5.1% for quality control samples. The accuracy, expressed as the relative error (RE), was within ±4.8% for all analytes. The recovery of genistein with this analytical method varied from 94 to 106%. The lower limit of quantification (LLOQ) was 0.01 μg·mL^{-1}.

Statistical Method. According to the concentration−time curve, the area under the curve (AUC) was calculated by linear trapezoidal rule from zero to the last plasma drug concentration. By semilogarithm graphic rules, terminal elimination rate constants (K_e) were estimated with least-squares regression of values in the terminal log-linear region of plasma concentration−time curves, and $t_{1/2}$ was calculated as 0.693/K_e. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/K_e$, where C_t is the last detectable plasma concentration and t is the time at which this concentration occurred. Peak concentration (C_{max}) was obtained from observed data. Statistical significance was determined with one-way analysis of variance (ANOVA).

RESULTS

Absorption of Genistein at Different Doses. The concentration−time curves for genistein in blood are shown in **Figure 2**.

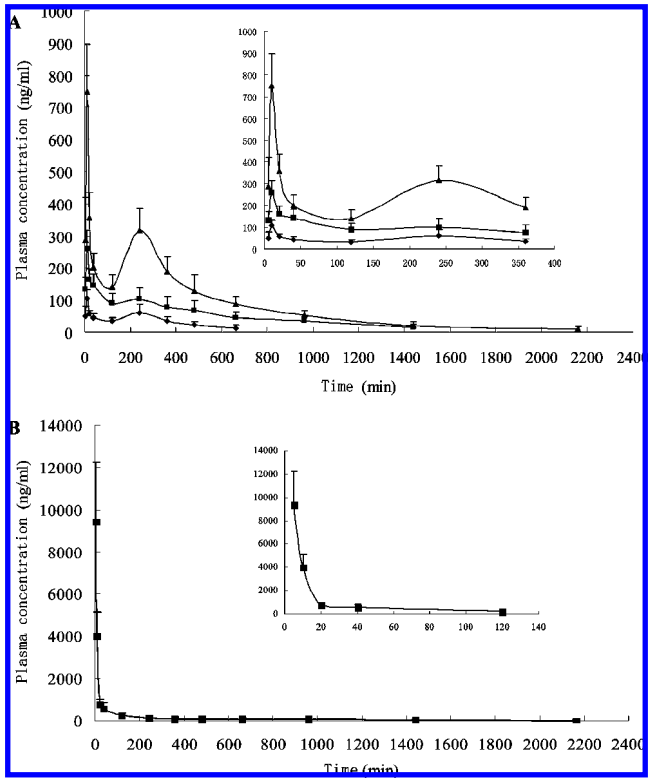

Figure 2. Concentration−time curves for genistein after oral or intravenous administration different doses: (**A**) (◆) 6.25 mg·kg^{-1}, (■) 12.5 mg·kg^{-1}, (▲) 50 mg·kg^{-1} oral (mean ± SD, n = 6); (**B**) (■) 12.5 mg·kg^{-1} intravenous (mean ± SD, n = 6).

Table 1. Main Pharmacokinetic Parameters of Genistein in Rats after Oral or Intravenous Administration of Genistein at the Different Doses[a]

	oral administration			intravenous administration
parameter	6.25 mg·kg^{-1}	12.5 mg·kg^{-1}	50 mg·kg^{-1}	12.5 mg·kg^{-1}
C_{max1} (ng·mL^{-1})	106	258	748	NA
C_{max2} (ng·mL^{-1})	60	102	317	NA
$t_{1/2}$ (min)	191.2	508.8	502.0	620
$AUC_{(0-t)}$ (mg·min·L^{-1})	23.5	80.9	177.9	234.0
$AUC_{(0-\infty)}$ (mg·min·L^{-1})	27.1	82.8	183.8	247.3
bioavailability (%)	21.9	33.5	19.0	NA
C_{max1}/dose	17.0	20.6	15.0*	NA
$AUC_{(0-t)}$/dose	3.8	6.5	3.6	18.7

[a] $t_{1/2}$, elimination half-life; C_{max}, peak concentration; AUC, area under curve; NA, not applicable. *, P < 0.05, vs 12.5 mg·kg^{-1}.

The results of the noncompartmental pharmacokinetic analysis are summarized in **Table 1**. Orally administered genistein was absorbed rapidly in rats. Genistein concentration in plasma reached its first peak (C_{max1}) at 10 min and its second peak (C_{max2}) at 240 min. C_{max2} was significantly lower than C_{max1}. When genistein was administrated intravenously, there was no second peak. As the oral dose increased from 6.25 to 50 mg·kg^{-1}, the peak concentration and AUC increased in a dose-dependent and nonlinear manner. It was evident that the bioavailability varied with the dose when the dose of genistein was increased from 6.25 to 50 mg·kg^{-1}. Interestingly, the bioavailability at 50 mg·kg^{-1} was markedly lower than that at 12.5 mg·kg^{-1}.

Biotransformation of Genistein at Different Doses. Genistein belongs to the family of polyphenol compounds; it can easily be metabolized into glucuronidated genistein. After genistein was orally or intravenously administered, it was rapidly conjugated; a large amount of glucuronidated genistein was found in plasma. The ratio of glucuronidated genistein to total genistein in plasma at different time points is shown in **Table 2**. The concentration−time curves for glucuronidated genistein are shown in **Figure 3**. The results of the noncompartmental pharmacokinetic analysis of glucuronidated genistein are summarized in **Table 3**. It is clear that the peak concentration of glucuronidated genistein increased in a dose-dependent and nonlinear manner when the dose was increased from 6.25 to 50 mg·kg^{-1}.

Compared with intravenous administration, more genistein was glucuronidated when genistein was administered orally. This indicates that a large amount of genistein can be glucuronidated in the gastrointestinal tract.

Table 2. Ratio of Glucuronidated Genistein to Total Genistein (Free + Conjugate) in Plasma after Oral or Intravenous Administration of the Drug to Rat at Different Doses[a]

	oral administration			intravenous administration
time (min)	6.25 mg·kg^{-1}	12.5 mg·kg^{-1}	50 mg·kg^{-1}	12.5 mg·kg^{-1}
5	93	93	93	59
10	97	95	92	70
20	97	93	94	83
40	95	93	96	75
120	89	82	94	77
240	79	86	79	90
360	80	84	81	89
480	78	77	90	91
660	66	80	88	85
960	NA	79	89	77
1440	NA	NA	93	72

[a] Ratio, glucuronidated genistein/total genistein; NA, not applicable.

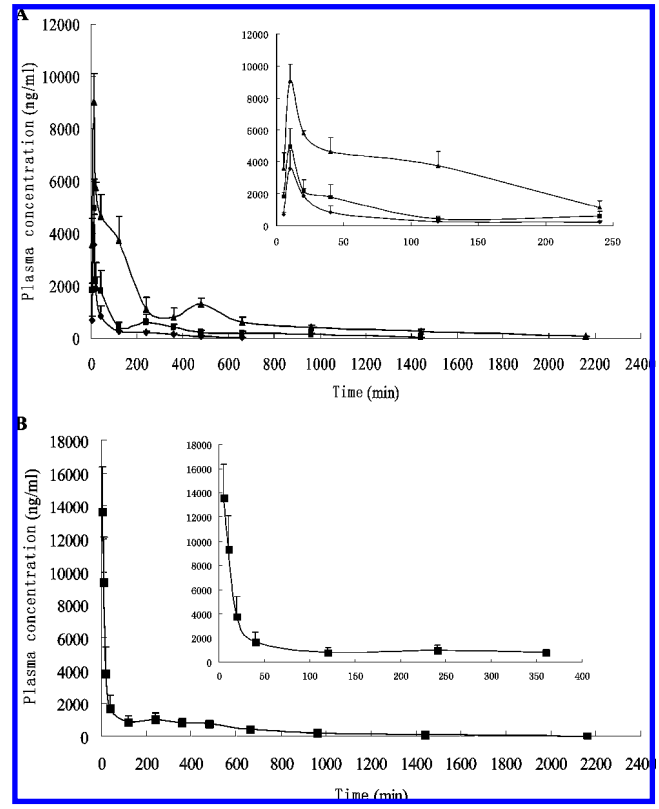

Figure 3. Concentration−time curves for glucuronidated genistein after oral or intravenous administration different doses: (**A**) (◆) 6.25 mg·kg^{-1}, (■) 12.5 mg·kg^{-1}, (▲) 50 mg·kg^{-1} oral (mean ± SD, n = 6); (**B**) (■) 12.5 mg·kg^{-1} intravenous (mean ± SD, n = 6).

Table 3. Main Pharmacokinetic Parameters of Glucuronidated Genistein in Rat after Oral or Intravenous Administration of Genistein at the Different Doses

	oral administration			intravenous administration
parameter	6.25 mg·kg^{-1}	12.5 mg·kg^{-1}	50 mg·kg^{-1}	12.5 mg·kg^{-1}
C_{max1} (ng·mL^{-1})	3569	4955	9050	NA
C_{max2} (ng·mL^{-1})	NA	615	1310	NA
$t_{1/2}$ (min)	130.7	376.6	429.9	395.0
$AUC_{(0-t)}$ (mg·min·L^{-1})	173.8	470.7	1721.2	647.4
$AUC_{(0-\infty)}$ (mg·min·L^{-1})	178.5	490.2	1759.9	661.7
C_{max1}/dose	571.0	396.4*	181.0**#	NA
$AUC_{(0-t)}$/dose	27.8	37.6	34.4	51.8

[a] $t_{1/2}$, elimination half-life; C_{max}, peak concentration; AUC, area under curve; NA, not applicable. *, $P < 0.05$, **, $P < 0.02$, vs 6.25 mg·kg^{-1}; #, $P < 0.05$, vs 12.5 mg·kg^{-1}.

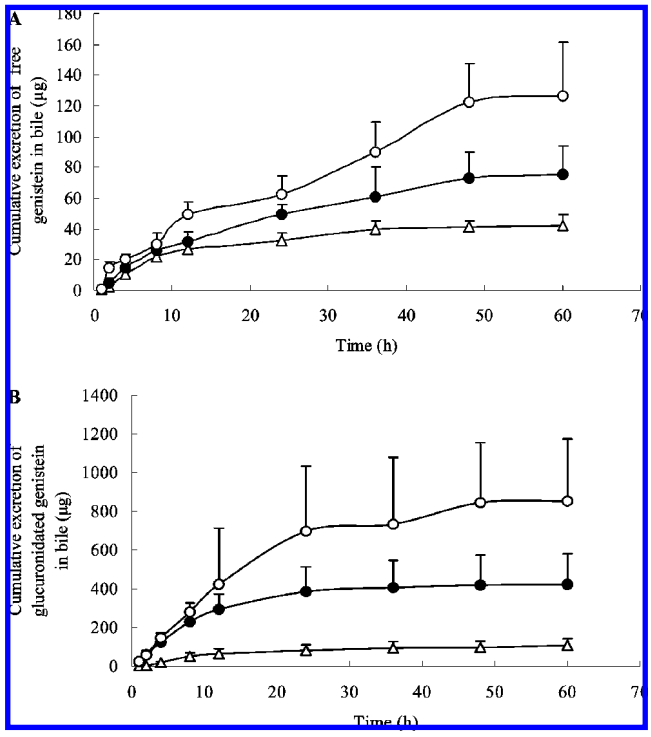

Figure 4. Cumulative excretions of genistein (**A**) and glucuronidated genistein (**B**) in rat bile after oral administration of genistein at different doses: (△) 6.25 mg·kg^{-1}; (●) 12.5 mg·kg^{-1}; (○) 50 mg·kg^{-1} (mean ± SD, $n = 6$).

Distribution of Genistein in Rats. After 12.5 mg·kg^{-1} genistein was orally administered, the parent compound was rapidly distributed across the whole body of the rat. In tissues, genistein reached its peak concentration in 80 min. The levels of genistein distributed in the stomach, intestine, liver, and kidney were elevated.

Biliary Excretion of Genistein. After oral administration of genistein, a high level of glucuronidated genistein was found in bile. Only a small amount of free genistein was slowly excreted in bile, indicating that in rat bile, genistein is excreted mainly in the form of glucuronidated genistein. Genistein and glucuronidated genistein were excreted in a nonlinear dose-dependent manner. The cumulative biliary excretion of free genistein was 42.6 ± 6.5, 75.2 ± 18.9, and 126.6 ± 34.8 μg within 60 h at doses of 6.25, 12.5, and 50 mg·kg^{-1}, respectively. The cumulative biliary excretion of glucuronidated genistein was 108.5 ± 35.2, 423.5 ± 158.3, and 853.7 ± 320.8 μg. The ratio of glucuronidated genistein to total genistein was 60.76, 82.25, and 85.17% at doses of 6.25, 12.5, and 50 mg·kg^{-1} genistein, respectively.

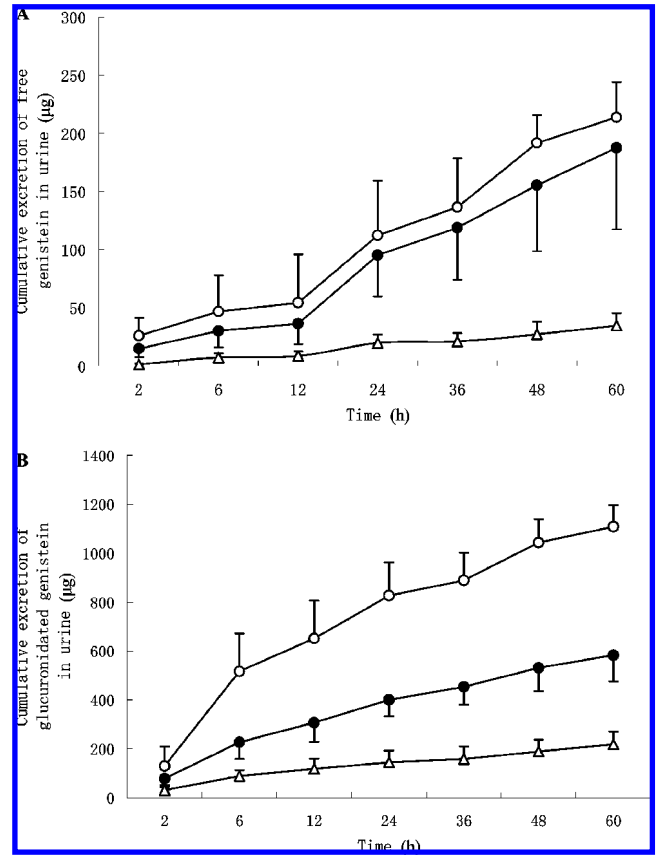

Figure 5. Cumulative excretions of genistein (**A**) and glucuronidated genistein (**B**) in rat urine after oral administration of genistein at different doses: (△) 6.25 mg·kg^{-1}; (●) 12.5 mg·kg^{-1}; (○) 50 mg·kg^{-1} (mean ± SD, $n = 6$).

Table 4. Tissue Distribution of Genistein in Rat after Oral Administration of 12.5 mg·kg^{-1} Genistein (Mean ± SD, $n = 6$)

	amount of genistein in tissue (μg/g of tissue) at		
organ	10 min	80 min	360 min
heart	0.265 ± 0.098	0.413 ± 0.097	0.229 ± 0.051
liver	1.150 ± 0.192	2.416 ± 0.495	1.126 ± 0.345
spleen	0.162 ± 0.063	0.346 ± 0.043	0.165 ± 0.037
lung	0.315 ± 0.156	0.379 ± 0.164	0.265 ± 0.030
kidney	0.610 ± 0.210	1.170 ± 0.226	0.408 ± 0.085
brain	0.147 ± 0.051	0.162 ± 0.083	0.097 ± 0.031
stomach	21.569 ± 9.159	7.989 ± 4.714	1.832 ± 0.410
intestine	8.131 ± 1.826	5.601 ± 1.994	1.503 ± 0.753
muscle	0.198 ± 0.028	0.526 ± 0.247	0.068 ± 0.027
fate	0.081 ± 0.012	0.355 ± 0.089	0.217 ± 0.040
uterus	0.185 ± 0.108	0.416 ± 0.117	0.088 ± 0.026
ovary	0.356 ± 0.147	0.564 ± 0.146	0.099 ± 0.056
testicle	0.039 ± 0.017	0.151 ± 0.079	0.124 ± 0.043

Urinary Excretion of Genistein. The pattern of urinary excretion of genistein and its metabolite was similar to that seen in biliary excretion. Genistein was excreted mainly in the form of glucuronidated genistein in rat urine. Only a small amount of free genistein was excreted in urine. Genistein and glucuronidated genistein were excreted in a nonlinear dose-dependent manner. The cumulative urinary excretion of free genistein was 34.8 ± 10.8, 187.3 ± 70.0, and 213.6 ± 30.6 μg within 60 h at doses of 6.25, 12.5, and 50 mg·kg^{-1}, respectively. The amount of glucuronidated genistein excreted from urine was 217.8 ± 52.1,

583.0 ± 106.9, and 1108.4 ± 88.1 μg. The ratio of glucuronidated genistein to total genistein was 84.03, 67.88, and 80.73% at doses of 6.25, 12.5, and 50 mg·kg^{-1}, respectively. When compared with biliary excretion, more genistein and glucuronidated genistein were excreted in urine.

DISCUSSION

Genistein is a phytochemical that occurs naturally in the diet and is found in a wide variety of plant-derived foods. The structure of genistein resembles that of 17β-estradiol. Genistein can bind to both α and β estrogen receptors, but with a much lower affinity than estradiol (about 100−1000-fold less than that of estradiol). Genistein can compete with estradiol for estrogen receptor binding sites. Once bound to the receptor, it can increase the expression of estrogen responsive genes (*17*). Because of its ability to exert estrogenic activity, genistein and related compounds are referred to as phytoestrogens (*18*). Although there has been interest in the potential risks and benefits of genistein, little research has been conducted examining the bioavailability, biotransformation, and excretion of genistein at different doses.

The results of this study show that genistein and glucuronidated genistein can be detected immediately in plasma within 5 min after oral administration. These results indicate that genistein can be quickly absorbed and metabolized in the gastrointestinal track. There are two distinct peaks in the plasma concentration−time curve, which indicate enterohepatic recirculation, as has already been suggested by several studies (*19, 20*). Furthermore, according to the time at which peaks 1 and 2 occur, the presence of distinct peaks 1 and 2 may also show a region-dependent absorption rate in the rat gastrointestinal tract (*21*).

Compared with the amount of glucuronidated genistein in plasma, the proportion of genistein in plasma was very small. The primary form of genistein in plasma appears to be glucuronidated genistein. As the dose of genistein was increased, the amount of genistein and glucuronidated genistein in plasma increased in a nonlinear manner, and the total plasma concentration of genistein + glucuronidated genistein approached saturation. The increase of AUC is less than that expected on the basis of a linear relationship, indicating the reduction of absorption or induction of metabolizing enzymes. Our observations suggest that the reduced absorption is more likely to account for the observed nonlinearity. As the $t_{1/2}$ was relatively unchanged from 12.5 to 50 mg·kg^{-1}, the amount of free genistein and its metabolite excreted from urine and bile tended to saturation. Therefore, the reduced bioavailability is most likely explained by a reduction in the absorption of genistein at high doses. In previous research using Caco-2 cells to model absorption and metabolism of genistein, Oitate et al. (*22*) found that genistein permeated Caco-2 monolayer cell membranes in a dose- and temperature-dependent manner and that the permeation of the monolayer could be saturated. Other flavones, such as rutin, and quercetin, can competitively inhibit transcellular transport of genistein (*22*). This indicates that absorption of genistein and other phytoestrogens occurs in the gastrointestinal tract by a mechanism other than simple diffusion. It is likely that an active transporter was involved in their transmembrane absorption of phytoestrogens. The cause of the low bioavailability at high doses needs to be further investigated.

Ng et al. reported that the bioavailability of natural flavones (aglycone or glycoside) was often lower than estimated (*23*). In vitro experiments have shown that soybean-derived isoflavones have anticancer activity. It is difficult to reach the plasma concentration needed for isoflavone to exert its pharmacological activity by dietary consumption of soybeans, so there is a large difference between the results of in vivo and in vitro studies. We now believe that the main reason for the low bioavailability of genistein is not only its poor absorption but also its significant first-pass metabolism (glucuronidation and sulfation) (*24*). At higher doses, a reduction in the absorption of genistein as well as extensive first-pass elimination might be responsible for the low toxicity of soybean-derived isoflavones. Glucuronidation is the primary metabolic route for genistein in rats. The majority of metabolite is genistein-7-*O*-β-D-glucuronide; other metabolites include genistein-4′-*O*-sulfate and genistein-4′-*O*-sulfate-7-*O*-β-D-glucuronide; however, they are formed in smaller amounts (*25, 26*). It has been reported that when rats were orally administered genistein, the glucuronidation often occurred in the intestine, but not in the liver (*11*). Some of the glucuronidated metabolite is absorbed into blood; the majority is excreted into the intestinal lumen by the intestinal epithelium (*27*). Because large quantities of the glucuronidatesd metabolites of genistein enter the intestinal lumen via bile and intestinal epithelial excretion, glucuronidated genistein can be deconjugated by glucuronidase in the intestine to release genistein. The released genistein can be absorbed, metabolized, and excreted for a second time, in the form of enteric recycling and enterohepatic circulation. Thus, the exposure time of the body to genistein is prolonged by enteric recycling and enterohepatic circulation, which may have important implications, requiring further study. In addition, the first-pass intestinal glucuronidation seems to be the most important factor for genistein disposition. The enteric recycling is likely to be more important than the enterohepatic recycling in determining the disposition of genistein (*28, 29*).

Our study showed that genistein was mainly excreted in the form of glucuronidated genistein by urine. The total genistein excretion rate in urine (expressed as a percentage of the administered dose) within 60 h was 17.42, 23.32, and 11.08% ($P < 0.05$ vs 6.25 mg·kg^{-1}; $P < 0.02$ vs 12.5 mg·kg^{-1}) when doses of 6.25, 12.5, and 50 mg·kg^{-1} of genistein were given, respectively. These values are consistent with the observed nonlinear increase in the AUC with increased dose. The rate of a drug excretion in urine is directly proportional to the systemically bioavailable fraction. When a xenobiotic shows linear pharmacokinetics over a dose range, this rate should remain unaltered. However, there was a decline in the fraction excreted in urine from the lowest to the highest level of genistein intake, indicating a trend toward nonlinear pharmacokinetics.

The excretion of genistein and its glucuronidated metabolite in bile was shown in a nonlinear dose-dependent manner. When we normalized the cumulative bile excretion of genistein or its glucuronidated metabolite to its dose, we found that excretion was significantly different at different doses. The total genistein excretion rate in bile (expressed as a percentage of the administered dose) within 60 h was 12.08, 19.58, and 9.80% ($P < 0.05$ vs 6.25 mg·kg^{-1}; $P < 0.02$ vs 12.5 mg·kg^{-1}) when 6.25, 12.5, or 50 mg·kg^{-1} of genistein was given, respectively. This also indicates that genistein has nonlinear pharmacokinetics.

The results of our study clearly indicate that the absorption, biotransformation, and excretion of genistein show a nonlinear dose-dependent relationship at high dose. The results of our pharmacokinetic studies raise important questions about the therapeutic significance of consuming large quantities of genistein, genistein analogues, or soy-based neutraceuticals.

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