Intestinal Uptake and Biliary Excretion of the Isoflavone Genistein in Rats

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ABSTRACT The intestinal absorption, biliary excretion and metabolism of genistein, a potent and specific protein tyrosine kinase inhibitor that occurs naturally in soy foods, was examined in anesthetized, adult female rats fitted with indwelling biliary cannulas. 4-14C-Genistein, when infused into the duodenum, was rapidly absorbed from the intestine, taken up by the liver and excreted into the bile as its 7-O-β-glucuronide conjugate. Cumulative recovery of 14C-radioactivity in the bile over a 4-h period was 70–75% of the dose. When genistein was infused into the portal vein, it was also taken up efficiently by the liver, conjugated with glucuronic acid and transported into bile. However, portal blood collected after duodenal infusions of genistein contained mostly genistein 7-O-β-glucuronide, suggesting that in vivo glucuronidation occurred in the intestinal wall rather than the liver. This was confirmed using everted intestinal sac preparations. Reinfusion of genistein 7-O-β-glucuronide into the duodenum causes the reappearing in the bile, albeit more slowly than when genistein was infused. Over a 4-h collection period, the cumulative recovery of 14C-radioactivity in bile was 27 and 70–75% of the administered dose for duodenal and ileal infusions, respectively. These data indicate that genistein is highly bioavailable in rats and because of its enterohepatic circulation may accumulate within the gastrointestinal tract.


KEY WORDS: rats • biliary isoflavones • isoflavone uptake • portal blood • glucuronides

The soy isoflavone genistein (4',5,7-trihydroxyisoflavone) has recently emerged as an important dietary component associated with many health-related and clinical benefits (see March 1995 supplement to this journal). Much of this development occurred after the discovery that genistein is a potent and specific inhibitor of protein tyrosine kinases (Akiyama et al. 1987). As such, it is considered to be an important modulator of many mitogen-stimulated signal transduction events (Peterson and Barnes 1996).

Consumption of soy protein is associated with a reduction in the risk of several cancers (Messina et al. 1994) and causes a reduction in serum hypercholesterolemia in animals (Anthony et al. 1996, Carroll 1991) and in humans (Bakhit et al. 1994, Potter et al. 1993, Sitori et al. 1977). Evidence that the beneficial effects of soy are due to the isoflavones has been obtained from experiments in which the isoflavones were removed from the soy protein by alcohol extraction: the extracted protein no longer had its effect (Anthony et al. 1996, Barnes et al. 1994). As further evidence that this class of compounds has important biological effects, a synthetic isoflavone, ipriflavone (7-isopropoxyisoflavone), has been successfully used in the treatment of postmenopausal (Agrusdsen et al. 1992) and senile osteoporosis (Passeri et al. 1992). Genistein administered in the diet also prevents bone loss in ovariectomized female rats, a model of postmenopausal osteoporosis (Blair et al. 1996), as does isolated soybean protein (Arjmandi et al. 1996). Uckun et al. (1995) reported that genistein, conjugated to an antibody to the CD-19 receptor, was highly effective in treating leukemia in a nude mouse in a model of pre-B cell human leukemia.

et al. 1995) cause chemoprevention effects in in vivo animal models of cancer. These data have given support to the hypothesis that isoflavones (particularly genistein) are important contributors to the anticancer effect of soy (Adlercreutz et al. 1991, Barnes et al. 1990, Setchell et al. 1984).

Crucial issues for determining how much genistein, either in soy foods or as a supplement, is required for its beneficial effects are those which govern the efficacy of other xenobiotics, i.e., absorption, distribution, metabolism and excretion (Barnes et al. 1996). Renal excretion of genistein after its ingestion has been used to evaluate its bioavailability (Xu et al. 1994). On this basis, because 3–10% of the dose appears in the urine, genistein was claimed to be poorly bioavailable. However, this approach does not take into account genistein absorbed from the intestine and then secreted in the bile in a conjugated form (sulfate ester or glucuronide), which might not be reabsorbed but excreted eventually in the feces either as genistein or its metabolites. Therefore, renal excretion can be regarded only as an apparent measure of genistein’s absorption from the gastrointestinal tract. The pharmacokinetics of genistein in mice when administered by oral, intramuscular and intravenous routes revealed that genistein is rapidly eliminated from the blood compartment, with an apparent systemic availability of 12% (Supko and Malپeis 1995). Because of a prominent secondary peak in the plasma unconjugated genistein concentration 78 min after a bolus intravenous dose of genistein, these investigators suggested that genistein may undergo enterohepatic cycling.

In a preliminary study conducted in this laboratory in a bile duct–cannulated rat model (Armstrong, H. and Barnes, S., unpublished observations), 40–50% of a dose of genistein administered in the stomach appeared in bile over a 4-h period, consistent with genistein undergoing an enterohepatic circulation. However, our technologies at that time did not allow us to be certain of the identity of the chemical forms of genistein in bile, or from which part of the intestine genistein is absorbed. In the present study using the bile duct–cannulated rat model, we have administered 4-14C-genistein to determine the proportion of the dose recovered in the bile, the role of the sites of absorption and their effect on the first pass metabolism of genistein.

MATERIALS AND METHODS

Materials. Genistin, the 8-glucoside of genistein, was isolated from soy molasses, an aqueous alcohol extract of soy flour (Walter 1941). Genistin was prepared from genistin by hydrolysis in methanol/HCl (Walter 1941). These isoflavones, after recrystallization, were 98% pure (by reversed-phase HPLC), and had melting points, molecular weights (determined by atmospheric pressure chemical ionization mass spectrometry) and proton nuclear magnetic resonance (NMR) spectra consistent with genistin and genistein, as previously described (Coward et al. 1993 and 1996). Genistein 8-sulfate was synthesized chemically by a carbodiimide coupling procedure (Coward et al. 1996).

4-14C-Genistin and 4-14C-genistein were custom synthesized (Moravek Biochemicals, Brea, CA) and had a radiochemical purity of >98% by HPLC and a specific radioactivity of 38.7 GBq/mmol.

8-Glucuronidase and sulfatase were purchased from Sigma Chemical Co., St. Louis, MO. Acetonitrile and trifluoroacetic acid were sequencing grades and were obtained from Fisher Chemical, Norcross, GA, and Pierce Chemical, Milwaukee, WI, respectively. All other chemicals were of the highest grades obtainable. Sep-Pak C18 cartridges were purchased from Waters (Milford, MA).

Animals. Female Sprague-Dawley rats (225–275 g) were bred in the Department of Nutrition Sciences Animal Care Facility, University of Alabama at Birmingham. Both fathers and dams (purchased from Harlan Sprague Dawley, Indianapolis, IN) were fed an isolavone- and soy-free diet (AIN-76A, Harland Teklad, Madison, WI) before impregnation of the dams. The pregnant dams were maintained on this diet throughout pregnancy and weaning. The pups were also fed this diet after weaning and throughout the rest of the experiment. After weaning, each group of female adult rats, used in preliminary experiments, were fed a nonpurified Teklad 4% diet. The animal experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Rats used in these experiments were anesthetized with ketamine/xylazine (0.1 mL/100 g body weight); their body temperature was maintained at 38°C (monitored rectally) by placing them on a heating pad. After a midline incision, the bile duct was exposed and cannulated with PE-10 tubing, tied and secured with 5-0 silk (Ethicon, Somerville, NJ).

In Experiment 1, a section of the duodenum (~10 cm), proximal to the ligament of Trietz, was used to determine the rate of absorption from the small intestine. Each end of the intestinal segment was tied off around a piece of PE-50 polyethylene tubing. The perfusate consisted of 10 mmol/L sodium taurocholate, 10 mmol/L glucose, 154 mmol/L NaCl and 10 mmol/L KCl. Variable amounts of genistein were added to this basic perfusate.

After the initial surgery, the intestinal segment was infused with physiological saline for 20 min, followed by two 20-min periods with the basic perfusate, each at a flow rate of 70 μL/min. Test perfusates contained 4-14C-genistein (3.7 MBq/L) and 94, 188 or 376 μmol/L unlabeled genistein. The three genistein-containing perfusates were administered to each of four rats in random order from animal to animal. The intuses were warmed to 37°C and were administered for 1 h, followed by the basic perfusate for 20 min. Biles were collected over 20-min intervals throughout the experiment, as were perfusates that had passed through the intestinal segment. In a separate group of rats, portal blood (2 mL) was obtained after 1-h duodenal infusion of 4-14C-genistein.

In Experiment 2, to more accurately assess the total intestinal recovery, 0.37 MBq (630 nmol) of 4-14C-genistein was infused (70 μL/min) over a 1-h period into the duodenum of a bile duct–cannulated rat; then, the basic perfusate was infused for the remainder of the experimental period (4 h). Unlike in the first experiment, the perfusates were allowed to proceed down the intestine without collection. Biles were collected every 5 min. At the end of the experiment, the stomach, liver and large and small intestines were removed. The small intestines were further divided into six equal lengths. The intestinal contents were separated from the intestinal muscle and mucosa.

In Experiment 3, to determine whether the biliary genistein metabolites also underwent an enterohepatic circulation, the biles collected over the first 60–100 min from Experiment 2 in which 14C-genistein was infused into the duodenum were pooled and diluted using the basic perfusate. This mixture was infused into the duodenum or the proximal ileum for 60 min, followed by infusion of the basic perfusate for a further 4 h. Biles were collected through a biliary cannula at 20-min intervals throughout the experiment. Bile was removed and divided as described in Experiment 2.

The hepatic uptake and biliary excretion of genistein were examined by infusion of 4-14C-genistein into the portal vein in the bile duct–cannulated rat. The dose of genistein was varied from 0.77 to 8.8 nmol/min; genistein was dissolved in freshly prepared rat serum which was infused at 4.5 μL/min for 1 h. Biles were collected through a bile cannula at 5-min intervals throughout the experiment. Peripheral blood (2–3 mL) was collected by cardiac puncture at the end of the experiment.

Intestinal uptake of genistein was also examined using everted intestinal sac preparations. Sections (~10 cm) representing the proximal, mid and distal small intestine were rapidly removed, washed with physiological saline and everted on a glass rod. The everted intestinal segments were flushed with Tyrode solution (136 mmol/L NaCl, 2.7 mmol/L KCl, 1.4 mmol/L CaCl2, 1.0 mmol/L MgCl2, 1.2 mmol/L NaHCO3, 0.4 mmol/L NaH2PO4, and 16 mmol/L L-glutamic acid, pH 7.1), and the ends of the intestinal segments were tied off with...
5-O silk after filling the segments with Tyrode solution containing 27 μmol/L genistein. The everted intestinal segments were then placed in a 500-mL Erlenmeyer flask in Tyrode solution containing 27 μmol/L genistein and 3.7 MBq 14C-genistein/L. The incubation solution was perfused with 95% O2-5% CO2 throughout the experimental period. Incubations were conducted at 37°C for 3 h. Fluids inside the intestinal segments (the serosal side) were then removed and stored at −70°C until analyzed.

**Analysis of genistein in intestinal contents.** To recover the isoflavones therein, each sample was homogenized in three volumes of methanol and centrifuged at 2500 × g for 10 min to remove precipitated material. The precipitate was rehomogenized in 3 volumes of 80% aqueous methanol containing a few drops of ammonium hydroxide (sp gr 0.88) and recentrifuged. Aliquots of the supernatants were taken for determination of radioactivity by liquid scintillation counting. The remaining supernatants were combined and dried under a stream of nitrogen.

In an alternative extraction procedure, samples were frozen in liquid N2 and pulverized in a perchloric mortar cooled in liquid nitrogen. Powdered tissue (100 mg) was mixed with 1.2 mL of 10 mmol/L Tris-HCl buffer, pH 8.0, containing 100 mmol/L NaCl, 25 mmol/L EDTA, 17.3 mmol/L SDS and proteinase K (1.5 units), and incubated at 37°C overnight. Fats were removed by extraction (three times) with 4 volumes of n-hexane. The pH of the aqueous phase was reduced to 5 by addition of HCl and passed over a C18 Sep-Pak cartridge. The cartridge was washed with 10 mmol/L ammonium acetate, pH 5.0, and then bound material eluted with 2 × 2 mL methanol. The eluate was evaporated to dryness.

The dried residues from either extraction procedure were reconstituted in 80% aqueous methanol (100 μL/g of tissue processed) and analyzed by HPLC or HPLC mass spectrometry (HPLC-MS).

**HPLC analysis of isoflavones in bile, sera, serosal fluids and urines.** Biles, sera and urines were prediluted with 1 mL (bile) or 10 volumes (sera, serosal fluids and urines) of 50 mmol/L ammonium acetate buffer, pH 5.0, and the isoflavones extracted by passage over an activated Sep-Pak C18 cartridge equilibrated with 50 mmol/L ammonium acetate buffer, pH 5.0. The cartridge was washed with 3 × 1 mL 10 mmol/L ammonium acetate buffer, pH 5.0, and the isoflavones eluted with 2 × 2 mL methanol. The methanol was evaporated under a stream of nitrogen at room temperature and the residues reconstituted in 100 μL of methanol/water (4:1). Aliquots (1–10 μL) were used for HPLC analysis. Aliquot parts were added to scintillation vials for counting of total radioactivity (if 14C-labeled genistein they were adults). Three days before the experiment, the animals were fed a nonpurified diet (Teklad 4% mouse/rat diet) until they were adults. Three days before the experiment, they were switched to isoflavone-free AIN-76A diet. Nonethless, bile obtained before the administration of genistein contained a large peak detected by reversed-phase HPLC at 262 nm. Treatment with β-glucuronidase/sulfatase resulted in its eluting much more slowly (and with the same retention time as genistein), strongly suggesting that it was a genistein conjugate. This dietary source of genistein made it difficult to determine the rate of excretion of the administered genistein. Therefore, preliminary experiments were conducted on rats that had been fed a nonpurified diet (Teklad 4% mouse/rat diet) until they were adults. Three days before the experiment, they were switched to isoflavone-free AIN-76A diet. Nonetheless, bile obtained before the administration of genistein contained a large peak detected by reversed-phase HPLC at 262 nm. Treatment with β-glucuronidase/sulfatase resulted in its eluting much more slowly (and with the same retention time as genistein), strongly suggesting that it was a genistein conjugate. This dietary source of genistein made it difficult to determine the rate of excretion of the administered genistein. Therefore, all subsequent experiments were conducted with animals not previously exposed to genistein at any time during gestation, the neonatal period or after weaning. This result was the first to suggest that genistein is not rapidly cleared from the body.

When 14C-genistein was infused into the intestinal loop in the duodenum of an otherwise genistein-free, bile duct–cannulated rat, 14C-radioactivity appeared in the bile within 20 min, reaching equilibrium within 1 h (Fig. 1). The mass of genistein metabolites excreted into bile increased with the infusion rate of genistein into the intestinal loop. Compared with the rate of infusion of genistein into the intestinal loop, biliary output of genistein metabolites was 9.2 ± 1.1% for genistein infusion at 62 nmol/h, and declined to 7.7 ± 1.6% for infusion at 124 nmol/h and 6.7 ± 1.2% for infusion at 247 nmol/h. Genistein had no effect on the bile flow rate (data not shown).

When 14C-genistein was infused into the duodenum for 60 min and allowed to proceed down the small intestine (without collection as in the intestinal loop experiment), a concordant,
UPTAKE AND EXCRETION OF GENISTEIN IN RATS

FIGURE 1 Biliary excretion of 14C-radioactivity during administration of 4-14C-genistein over a 3-h period into a 10-cm section of the duodenum of anesthetized rats. The biliary excretion rate is expressed as the percentage of the duodenal infusion rate. Data are the means ± SEM for each 20-min collection period from bile samples obtained from three rats.

A large peak of radioactivity was observed in bile (Fig. 2A). Radioactivity continued to be secreted into bile in decreasing amounts for the remaining 4 h of the experiment; a total of 70–75% of the infused dose was recovered via the biliary route (Fig. 2B). When these bile were pooled and analyzed by reversed-phase HPLC, a single peak of radioactivity eluting at 11.5 min was observed. Direct injection of unextracted bile onto the HPLC column led to a similar profile, indicating that the Sep-Pak C18 cartridge recovered all of the hydrophilic genistein metabolites. In addition, there was full recovery of the radioactivity injected onto the HPLC column.

Treatment of the extracted bile with β-glucuronidase led to a shift in the elution of the major peak of radioactivity to 21 min, coincident with genistein (data not shown). On the other hand, treatment with sulfatase did not alter the chromatographic mobility of the peak (data not shown).

When the biliary extract was analyzed by HPLC-MS in the negative ion mode, several peaks were detected for ions with the m/z value of 445 (the expected [M-H]⁻ ion for a glucuronide conjugate of genistein). One of these peaks had a mass spectrum which had an M+2 isotope excess due to the 14C label. Daughter ion spectra of each of the m/z 445 ions (during HPLC analysis) revealed that only this peak gave rise to the expected m/z 269 ion.

Proton NMR spectroscopy of genistein and the genistein glucuronide isolated from rat bile showed that the C4 (9.63 δ) and C5 (12.95 δ) hydroxyl proton resonances were observed for the metabolite as for genistein, but the C7 hydroxyl proton resonance was absent. In addition, the chemical shifts for the C4, C5 proton resonances were unchanged (6.88 δ vs. 6.82 δ), whereas the chemical shifts for the C6 (6.47 δ) and C8 (6.73 δ) proton resonances were 0.25–0.3 δ downfield from those observed for genistein. As noted previously, these data are consistent with genistein 7-O-β-glucuronide as the biliary metabolite (Coward et al. 1996).

14C-Radioactivity analyzed by HPLC from portal blood collected after a 1-h duodenal infusion of genistein was predominantly genistein 7-O-β-glucuronide, rather than genistein (Fig. 3). This was independent of the dose rates of genistein administered in this study (data not shown).

Uptake of 14C-genistein 7-O-β-glucuronide from the small intestine. When the pooled bile from Experiment 2 was rein-

fused into the duodenum of rats for 1 h, 14C-radioactivity began to appear in bile immediately; however, when biliary radioactivity was plotted as a function of time, instead of there being a peak (as in Experiment 2), radioactivity continued to increase throughout the remainder of the 4-h collection period (data not shown). HPLC analysis of biliary radioactivity again showed that it gave rise to a single peak eluting at 11.5 min, i.e., genistein 7-O-β-glucuronide (data not shown).

Extraction of the luminal contents of the various parts of the intestines revealed that the radioactivity had reached two thirds of the way down the small intestine over the 4-h infusion period (Fig. 4A). HPLC analysis revealed that the small intestinal fractions contained a single peak of radioactivity eluting at 21 min, i.e., genistein (Fig. 4B). Radioactivity was also contained in the intestinal wall in the same fractions as it was found in the luminal contents (data not shown). When the pooled bile was infused more distally, into the mid small intestine, radioactivity appeared rapidly in bile, reaching a peak within 80 min and declining thereafter (Fig. 5A). Cumulative recovery of biliary radioactivity was 72% over the 4-h collection period (Fig. 5B). Again, the principal biliary metabolite...
Hepatic uptake and biliary excretion of 14C-genistein. When 14C-genistein was infused into the portal vein, 14C-radioactivity rapidly appeared in bile (Fig. 6). At the lowest dose (0.77 nmol/min), the rate of biliary excretion of 14C-radioactivity approached that of the rate of infusion of 14C-radioactivity. However, at higher dose rates, evidence of saturation of hepatobiliary transport was observed (Fig. 7). The estimated \( T_{\text{max}} \) was 10.2 nmol/min, and the half-maximal infusion rate was 11.7 nmol/min.

HPLC analysis of 14C-radioactivity in peripheral blood collected at the end of the 1-h infusion period revealed that, at the lower rates of portal vein infusion of 14C-genistein, only genistein 7-O-\( \beta \)-glucuronide was present in the peripheral circulation (Fig. 8A). At the highest infusion rate studied (8.8 nmol/min), \( \sim \) half of the 14C-radioactivity in peripheral blood was genistein (Fig. 8B).

Uptake of 14C-genistein by everted intestinal sacs. Over a 3-h period, 14C-radioactivity accumulated inside the everted sac preparation. The concentration of 14C-radioactivity (per mL) was three to four times higher than that in the bathing medium. This concentrative effect was observed in each of the small intestinal segments so long as they were removed from the animal one at a time and the everted sac immediately prepared.

HPLC analysis of the 14C-radioactivity contained in the inside of the everted sac revealed that it was a mixture of genistein and genistein 7-O-\( \beta \)-glucuronide (Fig. 9). The proportion of genistein 7-O-\( \beta \)-glucuronide increased in relation to the size of the gradient across the everted sac. The concentration of genistein inside the everted sac was the same as in the perfusing buffer.

Urinary excretion of 14C-radioactivity. Radioactive metabolites were excreted in the urine in each of the experimental designs used for small intestinal infusions. However, the fraction of the dose appearing in the urine was significantly higher over the 4-h time period after infusion of genistein 7-O-\( \beta \)-glucuronide (7.4 \pm 1.7\%, mean \pm SEM) than for genistein (2.4 \pm 0.5\%, mean \pm SEM).

DISCUSSION

The data from the present study demonstrate directly for the first time that, at least in rats, the isoflavone genistein undergoes an efficient enterohepatic circulation. Genistein and its principal metabolite, genistein 7-O-\( \beta \)-glucuronide, are not only well absorbed from the intestines, but are efficiently extracted from the portal blood into the liver and excreted into bile. Biliary excretion and enterohepatic circulation have been previously described for many flavonoids (Hackett 1986), although the extent to which genistein and its biliary metabolite are absorbed is substantially greater than those previously reported. The possibility of enterohepatic cycling of dietary phytoestrogens was originally proposed by Setchell et al. (1982) and of genistein by Supko and Malspeis (1995).

Data obtained using perfusion of a short intestinal segment in the duodenum suggested that the initial absorption of gen-
UPTAKE AND EXCRETION OF GENISTEIN IN RATS

Increasing biliary excretion of 14C-radioactivity in rats with an indwelling biliary catheter after portal vein infusion of various doses of 14C-labeled genistein. Data are the mean ± SEM values from 3 rats at each infusion rate. Bile samples were collected over 5-min intervals.

Genistein was excreted in bile as a 7-O-β-glucuronide conjugate. This was demonstrated in two ways: first, by its sensitivity to hydrolysis to genistein by β-glucuronidase, but not to sulfatase or to solvolysis; and second, by HPLC-electrospray ionization mass spectrometry which revealed that the molecular weight of the biliary metabolite was 446, consistent with the addition of a single β-glucuronide group (by the 176 increase in the molecular weight compared with genistein). There was no evidence that genistein was converted to its sulfate or sulfate/glucuronide conjugates as was recently reported for daidzein in rats (Yasuda et al. 1994). It should be noted that even in a simple physiological fluid such as bile, the detection of the [M-H]− ion of genistein 7-O-β-glucuronide (m/z 445) by HPLC-MS was interfered with by other substances giving rise to 445 m/z ions. It was essential to conduct HPLC-MS-MS to identify the peak that was due to genistein.

Biliary excretion of flavonoids as the percentage of the administered oral dose has been reported to range from 11.4% for naringin (Hackett and Griffiths 1979) to 56.5% for hesperetin (Honohan et al. 1976) over 24-h collection periods. The higher recovery of the dose of genistein in bile than these flavonoids may in part be due to its greater hydrophobic nature.

Genistein occurs by passive mechanisms; the rate of absorption from the duodenum increased over the entire range of genistein intake tested in this study [80–320 mg/(kg body wt · d)]. The slight nonlinearity of the biliary excretion rate vs. the rate of intestinal infusion of genistein suggested that one of the transport steps involved is saturable. This type of nonlinearity was also evident in experiments in which genistein was infused into the portal vein. The estimated maximum rate of transport of genistein into bile was 10.2 nmol/min, i.e., a daily rate of 14.7 mmol. On a body weight basis, this translates into 60 μmol/kg, which is ~20 times the intake of genistein by those who eat a soy-based diet.

The duodenal uptake data also suggested that the absorption of genistein would be essentially complete during normal intestinal passage. This was verified by the second set of experiments in which 70–75% of the dose administered into the duodenum was recovered in bile over a 4-h period. Biliary excretion of flavonoids as the percentage of the administered oral dose has been reported to range from 11.4% for naringin (Hackett and Griffiths 1979) to 56.5% for hesperetin (Honohan et al. 1976) over 24-h collection periods. The higher
the β-glucuronide and sulfate esters of genistein accounted for 95% of the genistein in peripheral blood. However, in rats fed higher oral doses [148 μmol/(kg body wt·d)], peripheral blood and urine of rats contain a substantial proportion of unconjugated genistein (Sfakianos, J., Coward, L., Kirk, M. and Barnes, S., unpublished observations), a reflection of saturation of Phase II conjugation.

Intestinal conjugation of genistein was confirmed in experiments using everted intestinal sacs, establishing a lumenal-to-serosal concentration gradient of 14C-radioactivity as a result of the glucuronidation of genistein during transit through the intestinal wall. The observed concentration gradient of genistein 7-O-β-glucuronide to the serosal side is due to its much slower rate of back diffusion (serosal side to luminal side) than genistein. Genistein is therefore taken up by the liver mostly as its 7-O-β-glucuronide rather than as genistein.

When the genistein 7-O-β-glucuronide metabolite was re-infused into the duodenum of rats, radioactivity quickly appeared in bile, with 27% of the infused dose reexcreted over a 4-h study period. It was apparent, however, in this model, that intestinal absorption and biliary recovery were underestimated because, after duodenal infusion, the radioactivity had

**FIGURE 8** The composition of genistein and its glucuronide metabolite in peripheral blood of rats depends on the infusion rate of genistein into portal blood. Composition of 14C-radioactivity in peripheral blood collected after infusion of 14C-genistein into the portal vein of anesthetized rats (3 rats at each infusion rate) for 1 h was determined by reversed-phase HPLC (as described in Fig. 3). A) A single peak was observed of genistein 7-O-glucuronide (GENGlcA) when the infusion rate of genistein was 0.77 nmol/min. B) Peaks corresponding to both genistein (GEN) and its glucuronide (GENGlcA) were observed when the infusion rate of genistein was 8.82 nmol/min.

**FIGURE 9** Genistein is converted to its glucuronide during passage through the rat small intestinal wall. Composition of 14C-radioactivity from the serosal side of closed, everted, rat intestinal loops (from each of 2 rats) after incubation (on the luminal side) with 14C-labeled genistein was determined by reversed-phase HPLC (as described in Fig. 3). Each radiochromatogram [(A) duodenum, (B) jejunum] shows the presence of peaks corresponding to both genistein (GEN) and its glucuronide (GENGlcA).
reached only the mid small intestine. In subsequent experiments, to better assess intestinal reabsorption, the metabolite was reintused into the mid small intestine. In this mode, intestinal absorption and reexcretion of the genistein metabolite into bile was extensive and reached 72% over a 4-h period. The slowed intestinal recovery can be explained by the larger concentrations of intestinal bacteria in the more distal parts of the small intestine that cause hydrolysis of genistein 7-O-β-glucuronide to genistein (and hence allow passive absorption of genistein to occur). This was observed in these experiments. The slower rate of excretion in bile, compared with infusion of genistein, is therefore a function of the extent of hydrolysis of the glucuronide within the intestine. No evidence was obtained of a specific transport system for genistein 7-O-β-glucuronide in the distal small intestine as has been described for transport of another class of organic anions secreted in bile (the bile acids), which is localized in this region of the small intestine (Lack and Weiner 1961).

The consequences of an efficient enterohepatic circulation of genistein and its metabolites are as follows: 1) genistein may accumulate within the enterohepatic circuit, and 2) it may be excreted with a long half-life. This slow rate of excretion may have been missed by investigators who are overwhelmed by the rapid excretion observed in the first 24 h after administration of genistein. In this respect, genistein may behave like the anticancer drug 5-fluorouracil; after initial rapid renal excretion of its metabolite, 2-fluoro-β-alanine (FBAL), this drug exhibits an extended half-life as a result of the conjugation of FBAL to bile acids (Sweeney et al. 1987), a consequence of the very efficient enterohepatic circulation of bile acid N-acetyl amidates (Zhang et al. 1991). The observation that humans fed purified isoflavones have a lower urinary output of genistein relative to daidzein has been interpreted to mean that genistein has a lower bioavailability than daidzein (Xu et al. 1994). However, the present data indicate that genistein is absorbed from the intestines very well and is excreted into bile with only a small proportion appearing in urine. Because daidzein is converted to sulfate and glucuronide conjugates in rats (Yasuda et al. 1994), it is likely to be eliminated more rapidly in the urine than genistein. The difference in the rates of elimination of genistein and daidzein would also explain why rats, which consumed the soy-containing nonpurified diets and were food-deprived overnight or consumed a soy-free diet for up to 3 d, had large amounts of genistein 7-O-β-glucuronide in their bile, but no measurable daidzein or its metabolites. Investigators should note that most animal diets contain soy, and hence genistein, but in unpredictable amounts.

An important factor that may alter the initial intestinal absorption and the enterohepatic recycling of genistein is bacterial metabolism. Xu et al. (1995) reported that two patients who had a renal excretion of 32–37% of the genistein dose (three times higher than five other subjects studied) also excreted large amounts of genistein in their feces. Studies are required to examine the effect of glycoside conjugation of genistein on its rate of absorption from soy foods although it has been anticipated (Barnes et al. 1996) that the absorption will be efficient, if somewhat delayed, as was observed for the β-glucuronide of genistein in the present study. In a recently reported study, it was shown that after administration of genistein in rats, the plasma concentration of genistein reached a peak 2 h later; in contrast, when genistein glycosidic conjugates (recovered from soy flour by ethanol extraction) were administered, the peak plasma concentration occurred after 8 h (King et al. 1996).

Although genistein, like many therapeutic drugs used in the treatment of cancer, could be used in the pill form for delivery as a chemopreventive agent, its delivery in soy foods would be far more economical. Such a food delivery mechanism is used by Southeast Asians who have the lowest breast and prostate cancer rates (Parker et al. 1996) and lowest cardiovascular disease risk among nations of the world.

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LITERATURE CITED


