JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pharmacokinetics of a Slow-Release Formulation of Soybean Isoflavones in Healthy Postmenopausal Women

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Pharmacokinetic studies of soybean isoflavones have shown that following oral ingestion, the two major isoflavones, daidzin and genistin, are hydrolyzed in the intestine, rapidly absorbed into the peripheral circulation, and eliminated from the body with a terminal half-life of 7−8 h. These characteristics make maintenance of steady-state plasma isoflavone concentrations difficult to attain unless there is repeated daily ingestion of foods or supplements containing isoflavones. In an attempt to sustain more constant plasma isoflavone concentrations, a new slow-release formulation of a soybean isoflavone extract was prepared by microencapsulation with a mixture of hydroxypropylcellulose and ethylcellulose to alter its dissolution characteristics. In vitro experiments confirmed slow aqueous dissolution of isoflavones from this formulation when compared with the conventional isoflavone extract. The pharmacokinetics of this slow-release isoflavone extract was studied in 10 healthy postmenopausal women after oral administration of a single capsule containing the equivalent of 22.3 mg of genistein and 7.47 mg of daidzein expressed as aglycons. A comparison of the key pharmacokinetic parameters obtained in this study with those established in extensive studies performed previously in this laboratory indicated that the mean residence time of genistein and daidzein increased 2-fold with microencapsulation. These findings are indicative of a decreased rate of absorption, consistent with the observed slow in vitro dissolution rate. These findings show that it is feasible to employ polymer matrices that slow the aqueous dissolution for preparing sustained-release formulations of soy isoflavones. Further studies to optimize such formulations are warranted.

KEYWORDS: Phytoestrogens; pharmacokinetics; humans; isoflavones; genistein; slow-release

INTRODUCTION

Isoflavones, a class of nonsteroidal estrogens found in relatively high concentrations in most soy foods and soy proteins (*1*−*5*), have become of immense interest to clinical nutritionists because of their potential to modulate and prevent numerous hormonal and non-hormonal-dependent conditions (*6*, *7*). These natural bioactive constituents have biological activities that range from their ability to act as partial estrogen agonists and antagonists to influencing cell biochemistry by their direct and indirect actions on enzymes, growth factors, and genes (*8*−*13*). Notwithstanding the nutritional health benefits of soy protein (*14*, *15*) and the recognition by the U.S. Food and Drug Administration (FDA) of its potential to reduce risk for cardiovascular disease (*16*), the resurgence of interest in soy foods is also driven by the fact that such foods offer a delivery system for attaining high plasma and urinary concentrations of isoflavones (*7*, *17*−*25*). As a consequence, many extracts of isoflavones have become widely available as over-the-counter supplements or as ingredients for the development of fortified foods (*26*, *27*). The range of conditions and diseases for which soy isoflavones have been investigated is diverse, and conclusions regarding the effectiveness of isoflavones in explaining the actions of soy are complicated by the variability in findings (*28*−*30*). Whether the variances among studies can be attributable to differences in the study designs, types of soy products used, doses of isoflavones consumed, timing of intakes, or metabolism is a matter of conjecture (*31*). The recently described

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10.1021/jf0488099 CCC: $30.25
Published on Web 02/18/2005

features of the pharmacokinetics of daidzein and genistein indicate that absorption is relatively rapid and that once peak plasma concentrations are attained the half-life of elimination is of the order of 8 h in most healthy adults (*19*, *20*, *22*−*24*, *26*, *27*, *32*−*34*). By analogy to many pharmaceuticals it has been suggested that once-a-day consumption of soy foods is likely to be less efficacious than repeated ingestion because plasma isoflavone levels will tend to fluctuate greatly throughout the day. To modify the pharmacokinetics, a slow-release formulation of a soybean isoflavone extract was developed with the goal of slowing the rate of dissolution and absorption from the gastrointestinal tract in a manner that will facilitate steady-state plasma concentrations. We report here on the in vitro characteristics and on the pharmacokinetics of this slow-release soy isoflavone formulation as determined by classical single-bolus oral administration to healthy postmenopausal women.

EXPERIMENTAL PROCEDURES

Subjects. Ten healthy postmenopausal women (aged 45−57 years, mean = 51.2 years) were recruited to the study from women attending the Women's Health Center at Hadassah Medical Center. Menopause was defined by at least 3 months of amenorrhea and elevated follicle-stimulating hormone (FSH) plasma levels (>25 mIU/mL). The subjects were not taking any over-the-counter (OTC) medications, antibiotics, or other medications likely to affect gastrointestinal, liver, or kidney function. They were instructed to abstain from taking phytoestrogen supplements or consuming foods containing soy protein for at least 1 week prior to the study. Vegetarians were not included in the study because this population commonly consumes soy protein foods. Women who were obese (body mass index > 30), who reported significant illness, or who had known allergy to soy proteins were excluded from the study. The participants' body weight ranged from 53 to 75 kg (mean = 66.2 kg).

Study Design. The study was carried out in the Clinical Research Center at the Hadassah Medical Center, in Ein-Kerem, Jerusalem, and the protocol was approved by the Institute's Human Investigation Review Board. Each individual was administered orally a single capsule of a slow-release formulated soy isoflavone extract (Solbar SR, Solbar, Israel), containing 50 mg of total isoflavones, given in the early morning, following an overnight fast.

Blood samples (5 mL) were obtained immediately before ingestion of the capsule (baseline) and then after 2, 4, 6, 8, 12, 16, 20, 24, 36, 48, and 72 h. Blood was obtained by venepuncture, via an indwelling catheter for the more frequent samplings, and/or by vacutainer for the later sample times subject to the choice of the individual. These sampling times were optimized on the basis of our previously obtained data on the pharmacokinetics of absorption of pure isoflavones (*26*, *34*). The samples were centrifuged immediately after the blood collection, and the plasma was kept frozen (−70° C) until analyzed.

Preparation of Slow-Release Form of Soy Isoflavone Extract. The slow-release formulation of soy isoflavones (Solgen SR) was prepared by BioDar Ltd., Yavne, Israel, using a patented process (Patent Application PCT WO 01/13890 A1). The starting material was a commercially available extract of soybean isoflavones (Solgen 40, Solbar, Israel) that was compacted with microcrystalline cellulose using a Bepex compactor to form particles of size 400−1000 μm. The compacted material was coated with a mixture of ethyl cellulose (Ethocel, Dow) and hydroxypropyl cellulose (Klucel, Hercules) using a fluidized bed coater (Glatt) equipped with a Wurster column. The coated material was filled in size 2 hard gelatin capsules. Each capsule contained 50 mg pf total isoflavones.

In Vitro Dissolution Studies of Isoflavones. The behavior of the slow-release preparation was checked by dissolution analysis in vitro. A 150 mg sample of Solgen SR was placed in each vessel of a standard USP dissolution apparatus type 2 paddles (Vankel). The vessels were filled with 900 mL of simulated intestinal fluid without pancreatin pH 6.8 (United States Pharmacopoeia, USP 24, p 2236), with the addition of 0.5 g of sodium dodecyl sulfate per vessel. The dissolution was performed for 12 h with the paddles rotating at 75 rpm, and the temperature of the fluid was maintained at 37 ± 0.5 °C. Samples were withdrawn at several timed intervals over the next 12 h and analyzed for isoflavone content by high-performance liquid chromatography (HPLC). The dissolution curve for the slow-release preparation was compared to the dissolution curves obtained for the soy isoflavone extract (Solgen 40) used as the starting material.

Analytical Methodology. *Analysis of Slow-Release Isoflavone Extract by Reverse-Phase HPLC.* The isoflavone compositions of Solgen SR and Solgen 40 were determined by reverse-phase HPLC essentially as described previously (*3*, *26*) and using gradient elution with a mobile phase of 0.1% acetic acid in acetonitrile. Isoflavones were detected by their absorbance at wavelength 254 nm. Pure samples of genistin, daidzin, glycitin, genistein, and daidzein were used to construct calibration curves for quantification of the individual isoflavone forms.

Determination of Isoflavones in Plasma by Gas Chromatography−Mass Spectrometry (GC-MS). The concentrations of daidzein, genistein, glycitein, and the intestinal bacterial metabolites equol and *O*-desmethylangolensin were measured by GC-MS using stable isotopically labeled internal standards that were added to the plasma samples prior to the extraction and purification steps. Total and individual isoflavones were determined after solid-phase extraction and enzymatic hydrolysis of the conjugates with a combined sulfatase and glucuronidase enzyme preparation. These methods have been used in previous studies of the pharmacokinetics of isoflavones (*26*, *34*, *35*). The plasma (0.50 mL) was equilibrated at 37 °C for 30 min with 50 ng of the internal standards [^{13}C]daidzein, [^{13}C]genistein, and [^{13}C]equol. The methods of synthesis of stable labeled isotopic internal standards have been reported previously (*34*, *36*). The plasma sample was diluted with 10 volumes of 0.5 M triethylamine sulfate (pH 5.0) and heated to 64 °C before passage through a prewetted solid-phase C_{18}-Bond Elut cartridge. The solid-phase cartridge was then washed with distilled water (10 mL), and isoflavones and their conjugates were then recovered by elution with methanol (5 mL). The methanol extract was evaporated to dryness under nitrogen, reconstituted in 2 mL of 0.5 M acetate buffer (pH 4.5), and hydrolyzed at 37 °C overnight with a solution of 2000 Fishman units of a mixed β-glucuronidase/sulfatase (*Helix pomatia*, Sigma Chemicals Inc.) that had been previously filtered through a cartridge of C_{18}-Bond Elut to remove naturally occurring isoflavones present in this enzyme preparation. After hydrolysis, isoflavones were isolated by solid-phase extraction on a C_{18}-Bond Elut cartridge as described above. Isoflavones were recovered by elution of the cartridge with methanol (3 mL), and the sample was taken to dryness under a stream of nitrogen gas; isoflavones were measured in plasma by GC-MS after conversion to the *tert*-butyldimethylsilyl (t-BDMS) ethers.

GC-MS Conditions. The t-BDMS ethers were prepared by addition of acetonitrile (100 μL) and *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide in 1% *tert*-butyldimethylchlorosilane (100 μL), and the sample was heated at 100 °C for 1 h. The reagents were removed by evaporation in a stream of nitrogen and the derivatives dissolved in hexane (100 μL). Isoflavone t-BDMS ethers were separated and quantified by GC-MS with selected ion monitoring. Chromatographic separation was achieved on a DB-1 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA) using helium as the carrier gas (flow rate ~ 2 mL/min) and with a temperature program from 260 to 310 °C with increments of 10 °C/min. Selected ion monitoring GC-MS of specific and characteristic ions in the electron ionization (70 eV) spectra of the t-BDMS ether derivatives of each isoflavone permitted highly sensitive and specific quantification. The following ions were monitored: *m*/*z* 425 (daidzein), *m*/*z* 426 ([^{13}C]daidzein), *m*/*z* 455 (glycitein) *m*/*z* 470 (equol), *m*/*z* 471 ([^{13}C]equol), *m*/*z* 543 (*O*-desmethylangolensin), *m*/*z* 555 (genistein), and *m*/*z* 556 ([^{13}C]genistein). The individual isoflavones were quantified by comparing the peak area in the specific ion channels at the correct retention time determined from authentic compounds with the peak area response for the stable labeled internal standards. This area ratio was then interpolated against calibration curves constructed for known amounts (0−200 ng) of the individual isoflavones. Concentrations were expressed as nanograms per milliliter for individual plasma isoflavones.

Determination of Plasma Isoflavone Pharmacokinetics. The plasma concentration−time profiles for each individual and mean concentration

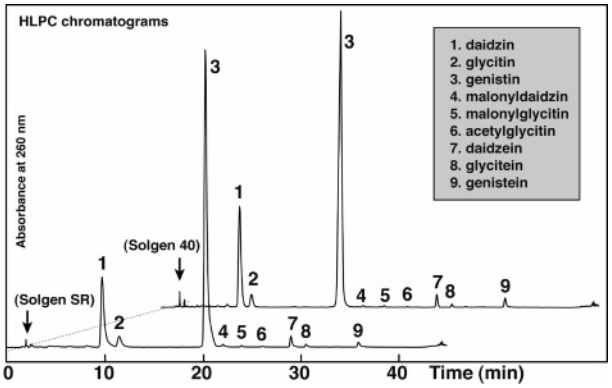

Figure 1. HPLC chromatograms comparing a slow-release form of isoflavones (Solgen SR) and the commercial soy isoflavone extract, Solgen 40, from which this was manufactured.

for each dose group for daidzein and genistein were determined by employing a noncompartmental approach. The WinNonlin 4.1 (Pharsight Corp., Cary, NC) computer program was employed for the analysis. The elimination rate constant (λ_z) was determined from the slope of the best-fitting regression line of the plasma samples in the terminal phase. At least four time points were included in the estimation of λ_z. The choice of the number of points included in the terminal phase of the plasma concentration−time curves was based on the weighted residual (difference between model predicted and observed concentrations) values, dispersion of the residual values, and regression coefficient. The terminal half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$. Mean residence time (MRT), which describes the average time for all of the molecules of an ingested substance to reside in the body, was determined as the ratio of AUMC to AUC. AUMC, or the area under the (first) moment curve, represents the area under a plot of the concentration × time (Ct) versus time. AUC (area under the curve) describes the area under the plasma concentration time curve. Both AUC and AUMC were calculated over the time limits of zero to infinity employing the trapezoidal rule (*37*) (the plasma concentration−time curve being described by a series of trapezoids determined by each concentration time point, with the area of the trapezoid being equal to half of the product of the sum of heights multiplied by the width according to the the following equation: area $= (1/2)(C_1 + C_2)(t_2 - t_1) + (1/2)(C_2 + C_3)(t_3 - t_2)$..., where C denotes isoflavone concentration and t denotes time (*38*).

Data Analysis. There have been no prior studies performed with a slow-release formulation of isoflavones, and therefore this study was observational; the number of subjects enrolled was based upon the feasibility of executing the complex analyses of the large number of plasma samples generated. The rates of absorption of the different species of isoflavones were calculated and compared from plots of the plasma concentrations versus time. The peak plasma concentration (C_{max}) and the time taken to reach peak concentrations (t_{max}) provide an indication of absorption characteristics, whereas the dose adjusted area under the curve (AUC/dose) of this plot provides an indication of the bioavailability. Comparisons of these data are made with historical studies of the pharmacokinetics of isoflavones given as pure compounds or as components of soy foods in healthy subjects (*19*, *22*, *23*, *26*, *27*, *32*−*34*) and from the later analysis of 10 plasma samples taken from postmenopausal women in an unrelated clinical trial of Solgen 40 (*39*), the conventional isoflavone extract used as the raw material to prepare the slow-release soy isoflavone formulation.

RESULTS

HPLC Analysis of Isoflavones. Figure 1 compares the reverse-phase HPLC chromatogram of the slow-release formulation and the original starting material (Solgen 40) from which it was prepared. No significant differences were evident with regard to the relative proportions of daidzein and genistein conjugates in the original starting material (Solgen 40) and the final slow-release formulation (Solgen SR). Approximately 90%

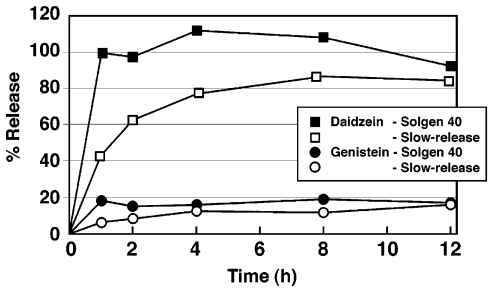

Figure 2. Comparison of the in vitro dissolution curves for the total amounts of daidzein and its conjugates and for genistein and its conjugates from a slow-release isoflavone preparation (Solgen SR) and the raw material (Solgen 40) used in its preparation.

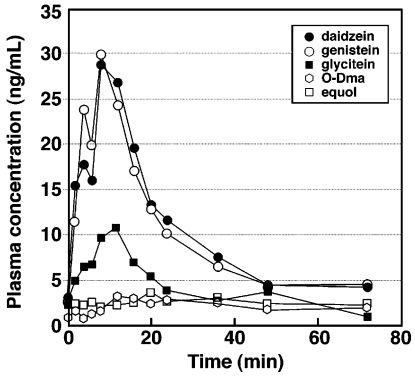

Figure 3. Typical plasma appearance and disappearance curves for soy isoflavones in a healthy postmenopausal woman after oral intake of a slow-release isoflavone preparation.

of the isoflavones were glucoside conjugates. Expressed on the basis of maximum bioavailable aglycon equivalents, the slow-release preparation provided a total of 22.23 mg of genistein and 7.47 mg of daidzein per 50 mg capsule.

In Vitro Characteristics of Isoflavones. Figure 2 compares the in vitro dissolution curves for total daidzein and genistein in the slow-release isoflavone preparation and the starting material, Solgen 40. The marked difference in the rates of dissolution of daidzein and genistein was striking, with the latter more hydrophobic isoflavone exhibiting slower and incomplete aqueous phase dissolution. The dissolution of daidzein and its conjugates from Solgen 40 was rapid and complete within 1 h, whereas no more than 20% of genistein and its conjugates dissolved even after 12 h. Microencapsulation of this material led to a much slower rate of dissolution of both forms of isoflavones, so that after 1 h only 40% of the daidzein and 7% of the genistein forms were in solution. The overall effect of microencapsulation on the dissolution characteristics of both daidzein and genistein and the glucoside conjugates was to slow the initial rate of dissolution when compared with the characteristics of the conventional isoflavone extract.

Plasma Pharmacokinetics of Slow-Release Isoflavone Formulation. A typical profile showing the plasma isoflavone concentrations in a healthy postmenopausal woman after a single oral administration of the slow-release isoflavone preparation is shown in **Figure 3**. The average peak plasma daidzein and genistein concentrations (C_{max}) were similar in these profiles even though the amount of genistein consumed was almost 3-fold higher than that of daidzein in the slow-release preparation (**Table 1**). Subsequent analysis of 10 plasma samples from a separate clinical study (*39*) of the isoflavone extract Solgen 40 (132 mg capsules, containing 19.5 mg of daidzein and 58 mg of genistein aglycon equivalents), used to prepare the slow-

Table 1. Individual Computed Plasma Isoflavone Pharmacokinetics in 10 Healthy Postmenopausal Women Given a Single Capsule of a Slow-Release Isoflavone Extract

subject	C_{max} (ng/mL)	t_{max} (h)	AUC (ng/mL·h)	Cl/F (L/h)	Vz/F (L)	$t_{1/2}$ (h)	AUC/dose (ng/mL·h)/mg	mean residence time (h)
Daidzein Pharmacokinetics: Slow-Release Preparation Containing 7.47 mg of Daidzein								
1	46	8	1461	5.11	243.6	37.2*	195	48.4
2	28	8	827	9.02	226.7	15.4	111	31.6
3	30	12	801	9.31	188.9	10.8	107	32.9
4	63	8	1192	6.26	213.7	17.7	159	25.9
5	63	12	1809	4.12	200.1	8.0	242	32.2
6	37	6	503	14.8	226.6	8.8	68	26.0
7	46	6	968	7.71	141.0	12.8	130	25.1
8	46	4	1345	5.55	128.7	12.6	180	22.6
9	38	6	1013	7.37	113.8	10.4	136	29.5
10	66	4	824	9.06	75.4	5.8	110	20.2
mean ± SEM	46 ± 4.4	7.4 ± 0.9	1074 ± 121	7.8 ± 1.0	175.8 ± 18.1	11.4 ± 1.2	143.1 ± 16.1	29.5 ± 2.4
Genistein Pharmacokinetics: Slow-Release Preparation Containing 22.3 mg of Genistein								
1	36	2	1000	22.3	342.2	10.6	44.4	37.2
2	30	8	790	28.3	504.1	12.4	35.4	30.3
3	38	12	1226	18.2	307.0	11.7	55.0	31.5
4	56	6	965	23.1	218.4	6.6	43.3	25.3
5	50	8	1668	13.3	278.1	14.4	74.8	33.6
6	53	6	990	22.5	323.9	10.0	44.4	28.3
7	39	6	722	30.9	578.6	13.0	32.4	29.5
8	26	12	645	34.6	386.5	7.7	28.9	21.9
9	42	6	1210	18.4	188.6	7.1	54.3	33.8
10	20	2	493	45.1	546.2	8.3	22.1	30.2
mean ± SEM	39 ± 3.7	6.7 ± 1.1	971 ± 107	37.6 ± 10.9	367.3 ± 42.6	10.2 ± 0.9	43.5 ± 4.8	30.1 ± 1.4
Glycitein Pharmacokinetics: Slow-Release Preparation Containing 2.03 mg of Genistein								
1	17	4	563	3.6	19.1	19.1	277	32.0
2	11	12	304	6.6	77.4	77.4	151	25.5
3[a]								
4	17	6	591	3.4	22.6	22.6	291	33.7
5	12	8	417	4.8	120.4	120.4	205	26.0
6	11	6	224	9.0	53.7	53.7	111	24.3
7	18	6	445	4.5	34.9	34.9	219	28.0
8	38	4	386	5.2	39.8	39.8	190	14.1
9	7	6	102	19.9	135.9	135.9	50	17.1
10	32	4	392	5.2	29.6	29.6	193	20.9
mean ± SEM	18 ± 1	6.2 ± 0.8	380 ± 51.3	6.9 ± 1.7	59.3 ± 14.3	59.3 ± 14.3	187 ± 25	24.6 ± 2.1

[a] Glycitein data for subject 3 not included due to difficulty in computing terminal elimination phase.

release formulation, revealed a mean (±SEM) peak genistein concentration of 127 ± 9 ng/mL compared 36 ± 3 ng/mL for daidzein (ratio of plasma genistein to daidzein of ~3.5). The much higher concentration of genistein relative to daidzein is consistent with previous data for the usual quantitative relationship between these two isoflavones in plasma and contrasts with that of the slow-release formulation (genistein/daidzein ratio of ~0.85).

The original soybean extract and the slow-release formulation contained small amounts of glycitin, and this is evident from the plasma appearance/disappearance curve (**Figure 3**) showing significant concentrations of glycitein. Low concentrations of the isoflavone metabolites, *O*-desmethylangolensin and equol, were consistently present in the plasma of all subjects (**Figure 3**).

Figure 4 plots the mean plasma isoflavone concentrations for all 10 postmenopausal women given the slow-release isoflavone extract, and **Table 1** summarizes the individual and group mean values of the pharmacokinetics computed from the plasma appearance/disappearance curves and expressed as apparent bioavailability measured as area under the curve to infinity (AUC_{inf}), dose-adjusted (AUC_{inf}/dose), terminal elimination half-life ($t_{1/2}$), and mean residence time (MRT). The corresponding data for glycitein are also shown in **Table 1**.

The mean (±SEM) C_{max} and AUC of daidzein were 46 ± 4.4 ng/mL and 1.074 ± 0.12 ng/mL·h, respectively. The daidzein half-life was 11.4 ± 1.2 h, and the MRT was determined to be 29.5 ± 2.4 h. Although the mean elimination half-life appears to be higher than that reported in earlier studies of isoflavones (*25*, *26*, *34*), there was significant interindividual variability (range = 5.8−15.4 h), and a comparison with these previously reported values indicated that there was

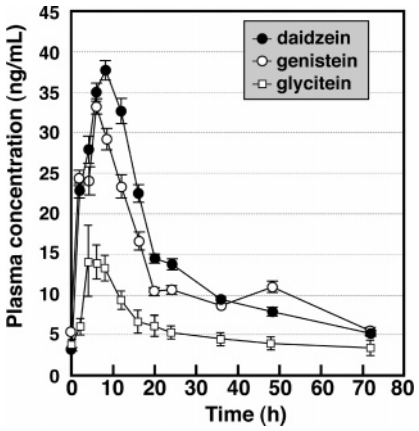

Figure 4. Plasma appearance and disappearance curves for soy isoflavones in 10 healthy postmenopausal women after oral intake of a slow-release isoflavone preparation. Graphs depict mean ± SD values for the plasma concentrations of daidzein and genistein at each time point after oral ingestion of a single capsule containing 22.3 mg of genistein and 7.47 mg of daidzein.

no significant change in the half-life from the sustained release formulation.

For genistein, the values for C_{max} and AUC_{inf} obtained following consumption of the slow-release isoflavone formulation were 39 ± 3.7 ng/mL and 971 ± 0.10 ng/mL·h, respectively. The mean elimination half-life was 10.2 ± 0.9 h, and the MRT was 30.1 ± 1.4 h. When compared to our previously reported studies of isoflavones, microencapsulation of the isoflavone extract did not alter the half-life or the dose-normalized AUC, but the C_{max} was significantly lower ($p < 0.05$). Similar to our observation with daidzein, the MRT of genistein was relatively long and ~2-fold higher than the MRT computed from data of earlier studies of isoflavones. This was also the case for glycitein (**Table 1**).

DISCUSSION

Numerous studies attest to the pharmacokinetics of soy isoflavones in animals and healthy humans, and in all cases data confirm that daidzein and genistein are absorbed relatively quickly from the gastrointestinal tract and appear rapidly in plasma (*20*, *23*−*27*, *32*−*35*, *40*). The biological half-life of daidzein and genistein as determined from their plasma elimination has been reported to range anywhere from 3.5 to 9.3 h in healthy adults (*19*, *22*, *23*, *25*−*27*, *32*−*34*). We have reported values of 7.5−10.5 h when pure isoflavones (*26*) or their stable labeled analogues (*34*) are administered orally under the study design identical to that used in this study of a new slow-release formulation. On the basis of these pharmacokinetic characteristics, maintenance of steady-state circulating concentrations of isoflavones is difficult to achieve unless isoflavones are ingested regularly and at multiple times throughout the day, a practice common to Asians consuming soy foods as a natural component of the traditional diet. In general, the rate of appearance in plasma and the shape of the plasma concentration curve of any drug or compound are influenced by many factors, not least of which are the route of administration and the formulation. For water-soluble compounds dissolution proceeds without significant delay in the intestinal fluids and so the absorption rate is not dependent on the dissolution rate. Conversely, for hydrophobic substances, the dissolution rate may be slower than the absorption rate, and as such the absorption is considered to be dissolution rate-limited. Ideally, the goal for most pharmaceuticals or bioactive agents is to achieve a slow and sustained absorption of the compound that has the effect of reducing the C_{max} value and increasing the MRT of the compound. Overall, this effectively minimizes the sharp peaks and troughs in plasma concentration seen with compounds that are rapidly absorbed and quickly eliminated.

For relatively hydrophilic substances, a useful approach to produce a prolonged release formulation is to encapsulate the bioactive substance in a polymer matrix. The rate of dissolution then depends on the rate of release from the matrix, which results in reduced rate of dissolution and, therefore, rate of absorption. The isoflavone extract from soybeans used in this study was compacted with microcrystalline cellulose and then coated with celluloses. This formulation resulted in slower in vitro dissolution characteristics when compared with the isoflavone extract used as the starting material (Solgen 40), and this was especially the case for daidzein and its conjugates (**Figure 1**). Genistein, by virtue of its hydroxyl at position C-5, is relatively hydrophobic due to its ability to form intramolecular hydrogen bonding between the C-5 hydroxyl and the oxygen at position C-1, thus decreasing its polarity (*41*). It is this physicochemical property that renders genistein poorly water-soluble, relative to daidzein, and undoubtedly accounts for the reported low fractional absorption of genistein observed from urinary excretion recoveries in many previous pharmacokinetic studies (*19*, *22*, *23*, *25*, *27*, *32*, *34*, *42*).

Overall, the effect of the microencapsulation process described was to promote a slower release or solubility of isoflavones in the intestine, leading to slower and more sustained absorption thereby increasing the MRT. Mean residence times for daidzein and genistein were similar and ~30 h (**Figure 4**). This is approximately double the MRT computed in our previous studies of isoflavones from soy nuts, which were consistently in the range of 13−16 h for daidzein and genistein (*25*). Assuming first-order absorption (absorption rate constant K_a), the MRT of a drug following oral administration (MRT_{oral}) is the sum of MRT following intravenous administration and $1/K_a$, that is, $MRT_{oral} = MRT_{iv} + 1/K_a$). MRT_{iv} is susceptible to changes only in the systemic elimination pathways. Intravenous pharmacokinetic data for isoflavones are unavailable; however, on the basis of the fact that the elimination half-life of the isoflavones observed was no different from those observed in prior studies employing pure compounds (*26*), ^{13}C-labeled isoflavones (*34*), or soy foods (*25*), it is reasonable to assume that increased MRT_{oral} was due to a decrease in K_a. Thus, microencapsulation effectively increases the MRT for both daidzein and genistein by controlling the release rate. This is in agreement with our findings from in vitro dissolution studies (**Figure 2**) and ultimately results in decreased absorption (*38*).

The effect of slower dissolution, particularly of daidzein, was to increase greatly the bioavailability of daidzein compared with genistein. The dose-adjusted AUC of daidzein was ~3-fold higher than for genistein (143.1 ±16.1 vs 43.5 ± 4.8 ng/mL·h/mg isoflavone ingested), whereas the C_{max} values were similar. Typically, genistein concentrations are much higher than daidzein concentrations in plasma, whereas the inverse relationship is observed for urine. Interestingly, analysis of serum samples from a study in which high doses of Solgen 40 had been administered orally to postmenopausal women (*39*) showed that genistein concentrations were ~3.5-fold higher than daidzein concentrations, as expected. Thus, microencapsulation altered the relative bioavailability of the two isoflavones. A change in the rate of systemic elimination is an unlikely explanation for the relatively higher plasma daidzein concentrations because the observed elimination half-life was similar to that reported in other isoflavone studies (*19*, *22*, *23*, *25*−*27*, *32*−*34*). It seems more likely that this difference is due to delayed and slow intraluminal release of daidzein, which may delay its metabolism and biotransformation. Isoflavones exist predominantly as glycoside conjugates in most soy foods (*1*−*5*). The first and most crucial step in their bioavailability involves hydrolysis of the glucoside residues (*35*). Recent studies using pure glycoside conjugates of daidzein and genistein and also soy milk, which contains mainly isoflavone glycosides, confirmed a lack of absorption of intact isoflavone glycosides in healthy adults, even though high plasma levels of isoflavones were quickly attained after oral administration (*35*). Hydrolysis of the glucosidic moiety is therefore an essential first step in making isoflavones bioavailable (*35*), and this occurs by the action of intestinal β-glucosidases that reside on the brush border membrane of the intestine and in bacteria (*43*). Whether coating isoflavones with celluloses impairs or reduces the rate of this hydrolytic reaction is uncertain, but such an effect might account for the higher bioavailability of daidzein relative to genistein, because degradation to other isoflavone metabolites would be limited by the protection afforded by microencapsulation. Regardless

of the mechanism, an enhancement in daidzein bioavailability observed with the slow-release formulation may have significant clinical value because it is the substrate for equol production by intestinal bacteria (*7*, *17*). Recent studies suggest that the presence of higher levels of equol may confer greater clinical efficacy of soy isoflavones (*31*).

The time to reach the maximum plasma concentration in these healthy postmenopausal women was variable but, on average, slightly longer than that reported in other studies in which conventional sources of isoflavones have been studied. Typically, isoflavones when given orally as pure compounds show peak plasma concentrations between 4 and 6 h in most healthy adults (*26*, *34*), and sustained plasma concentrations are difficult to attain due to the relatively rapid elimination half-life (*19*, *22*, *23*, *25*−*27*, *32*−*34*). Overall, the shapes of the plasma appearance/disappearance curves from the slow-release formulation differ from those previously observed for pure isoflavones (*26*, *34*), being characterized by lower dose-adjusted C_{max} values. The elimination half-life, which should be unaffected by slow dissolution, was 11.4 ± 1.2 h or 10.2 ± 0.9 h, respectively, for daidzein or genistein and was consistent with our previously reported values for these isoflavones from conventional soy sources (*25*). Very long terminal elimination half-lives are observed in patients with renal disease (*33*), and this is because the kidneys are the major site for elimination of isoflavones from the body. Kidney disease patients therefore maintain high steady-state plasma concentrations of daidzein and genistein due to reduced renal clearance, and half-lives of 39−53 and 30−99 h, respectively, have been reported for genistein and daidzein for three such patients (*33*). Our study shows that modifying the formulation of a soybean isoflavone extract to generate slow aqueous phase dissolution facilitates a more consistent plasma concentration over the day. Although this study did not directly compare the pharmacokinetics of the slow-release preparation with the starting material in the same subjects, the pharmacokinetic behavior of isoflavones in this formulation differed markedly from previously reported data for pure isoflavones using the same study design (*26*).

In summary, we have shown that soybean isoflavones can be formulated by a microencapsulation process to produce slow-release dissolution characteristics and that this formulation alters the characteristics of the plasma pharmacokinetics. The delayed in vitro release of isoflavones from this formulation leads to a slower absorption rate, lower C_{max} concentrations, and long mean residence times, which is likely to facilitate more sustained plasma isoflavone concentrations throughout the day when compared with single daily ingestion of pure isoflavones or those naturally present in soy foods. The effect is more pronounced with daidzein than with genistein due to marked differences in hydrophobicity of the two molecules. Whether this modification to the pharmacokinetics of isoflavones affects the overall efficacy remains to be established.

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Received for review July 16, 2004. Revised manuscript received December 15, 2004. Accepted December 29, 2004.

JF0488099