Phase I Pharmacokinetic and Pharmacodynamic Analysis of Unconjugated Soy Isoflavones Administered to Individuals with Cancer

Abstract

Preclinical studies suggest that the isoflavone genistein may have prostate cancer chemopreventive activity. Genistein has been shown to alter cellular levels of protein-tyrosine phosphorylation and is present at high levels in soy. This study was designed to measure the pharmacokinetic parameters of two different preparations of unconjugated soy isoflavones, PTI G-2535 and PTI G-4660 (which contain 43% and 90% genistein, respectively), in human subjects with cancer, to evaluate toxicity and obtain pilot data on in vivo effects on protein-tyrosine phosphorylation. Cohorts of four patients were given single doses of each preparation; each dose was separated by 1 week. Sequential cohorts received genistein at 2, 4, or 8 mg/kg orally. Pharmacokinetic sampling was performed after each dose, and tyrosine phosphorylation was measured in proteins extracted from peripheral blood mononuclear cells. One of 13 patients treated developed a treatment-related rash. No other toxicities were observed. Maximal plasma concentrations (Cmax) ranged between 4.3 and 16.3 μM for total genistein and 0.066 and 0.17 μM for free genistein. For PTI G-2535 and PTI G-4660, half-life was 15.03 and 22.41 h, respectively, and volume of distribution was 189.9 and 653.8 liters, respectively, and there was a trend toward higher area under the concentration curve for PTI G-2535 (P = 0.07 at the 8 mg/kg dose). Treatment-related increases in tyrosine phosphorylation were observed in peripheral blood mononuclear cells. Oral administration of soy isoflavones gives plasma concentrations of genistein that have been associated with antimetastatic activity in vitro.

Introduction

Southeast Asians, who subsist on a soy bean-based diet, experience a lower incidence of metastatic prostate cancer than those in Western countries (1–3). However, some studies suggest that the incidence of primary, organ-confined, prostate cancer may be similar in Eastern and Western populations (3, 4). Within a few generations, migrants to the West experience an increase in prostate cancer, approaching rates seen in the West. Epidemiological studies, therefore, support the notion that the metastatic behavior of prostate cancer may be amenable to pharmacological manipulation, that there is a significant clinical benefit to doing so, and that dietary and/or lifestyle factors may be causal.

Through a series of investigations, driven by epidemiological evidence, we have shown that genistein increases adhesion of human prostate cells by increasing formation of focal adhesion complexes and that it does so in a time- and concentration-dependent fashion (5–7). This action by genistein functionally antagonizes the first step in metastasis formation. Importantly, we demonstrated efficacy at concentrations as low as 1–10 nM. This is significant because blood concentrations of free genistein (i.e., nonconjugated genistein) in those who subsist on a soy-based diet range between 3 and 19 nM; blood concentrations are 1–2 logs lower in non-soy consumers (8).

In addition to antimetastatic effects, a relatively large number of potential cancer chemopreventive mechanisms have been ascribed to genistein, with some of the more common mechanisms involving growth inhibition, induction of apoptosis, estrogenic activity, and antioxidant activity (7, 9–11). However, the spectrum of the pleiotropic effects of genistein is best illustrated by considering gene array-based investigations. Gene array technology can be used to identify potential drug targets and has recently been used to show that genistein does in fact modulate the expression of genes associated with a variety of cellular processes (12–14). However, most mechanistic studies have used concentrations of genistein that were in the high micromolar range and thus of unclear physiological significance.

Before evaluating the antimetastatic potential of genistein in humans, a greater understanding of its pharmacology and pharmacodynamics in humans is needed. Prospective and dietary animal studies (15, 16), as well as human dietary studies (8, 17, 18), have provided important information about the pharmacokinetics of genistein. Taken together, these studies show that increased oral consumption of soy-derived genistein is...
associated with higher blood concentrations of genistein, that this association may be linear, and that the majority of genistein is conjugated, presumably through a first pass effect in the liver. Prospective dose-escalation pharmacokinetic studies of genistein in normal volunteers, in both men and women, have been reported recently (19, 20). In parallel with those investigations, we sought to characterize the pharmacokinetics of genistein in an older cohort of men. Because cancer chemopreventive agents typically require administration for extended periods of time before realization of clinical benefits, it is important to evaluate the pharmacology of those agents in the target cohort of interest (21). In addition, we performed pilot investigations to evaluate the potential ability of genistein to alter protein-tyrosine phosphorylation in humans. Prior studies have shown that high genistein concentrations inhibit protein-tyrosine kinase activity, whereas at lower concentrations, inhibition of protein-tyrosine phosphatases appears to predominate (7, 22).

Materials and Methods

Patient Selection. Patients were entered onto an Institutional Review Board-approved protocol. Patients were eligible if they were at least 18 years old, had pathological evidence of cancer, had documented metastasis, had failed standard therapy, and were exhibiting disease progression at the time of protocol entry. Patients must have been off all forms of active therapy for at least 1 month before study entry. For patients with prostate cancer who were on a LHRH agonist, therapy with the LHRH agonist was continued to maintain castrate levels of testosterone, as is standard practice. Patients must have had an Eastern Cooperative Oncology Group performance status of 0 or 1, a life expectancy of >3 months, and intact liver (bilirubin < 2.0 mg/dl, transaminases < 3× the upper limit of normal), bone marrow (hemoglobin > 8.0 g/dl, platelets > 100,000/mm³, and absolute neutrophil count > 1000/mm³) and renal (creatinine < 2.0 mg/dl) function. Patients with a history of deep venous thrombosis within the past year (or on anticoagulation for such) were excluded, as were patients with known soy allergy, patients who were pregnant or breast feeding, patients with a history of breast cancer, or patients on estrogen therapy (including oral contraceptives).

Drug Administration and Toxicity Assessment. Two different preparations of unconjugated isoflavones were evaluated, PTI G-4660 and PTI G-2535. Both were manufactured under Good Manufacturing Practices guidelines by Protein Technology International (PTI, St. Louis, MO), and formulated into gelatin capsules by University Pharmaceuticals of Maryland Inc. (Baltimore, MD). Individual formulations were analyzed separately for composition by University Pharmaceuticals of Maryland, as well as by Sigma-Aldrich Inc. (St. Louis, MO). The composition of PTI G-2535 was 43% genistein, 21% daidzein, and 3% glycitein. The composition of PTI G-4660 was 90% genistein, 9% daidzein, and 1% glycitein. For each preparation, no residual protein was detected; individual capsules contained 150 mg of genistein. Preparations from a single manufacturing lot were used throughout the course of the study and provided by the National Cancer Institute.

All participants were counseled by a dietician, given a list of “forbidden foods” (i.e., high soy/genistein foods), and asked to refrain from consumption of soy/genistein supplements while on study. Participants were admitted to the General Clinical Research Center of Northwestern University or the Research Unit of the National Naval Medical Center for pharmacokinetic studies and fed a low-soy diet throughout the course of the study. All meals were given at specified times during the course of study. In a sequential fashion, patients were accrued in cohorts of four onto each of three dose levels of genistein: 2; 4; and 8 mg/kg. Each patient was given a single dose of each of two separate formulations of unconjugated isoflavones. There was a 1-week interval between doses to allow for washout. Within a given dose level, the order in which individual formulations were administered to an individual patient was determined by central randomization. Patients were required to fast for 2 h before drug dosing. Drug was administered orally along with 8 ounces of water.

After drug administration, patients were monitored for clinical toxicity during their 24 h of hospitalization and then monitored daily for 2 days in the outpatient clinic, and finally monitored at the 1-month post-drug treatment time point. Clinical chemistry profiles were assessed at baseline, 1 week after each dose, and 1 month after the last dose. Clinical disease response was not formally evaluated.

Pharmacokinetic Sampling. Venous blood samples for pharmacokinetic monitoring were collected into heparin-containing tubes at the following times: before treatment and after drug ingestion at 10, 20, 30, 45, 60, 90, and 120 min and at 3, 4, 5, 6, 8, 12, 15, 24, 32, 48 and 72 h after dosing. Blood samples were kept on ice until centrifugation at 4°C, and the resultant plasma samples were stored at −80°C until analysis.

Pharmacokinetic Analysis. Plasma concentrations of total and free genistein and daidzein were measured as previously described by Supko and Phillips (16), with modifications by Thomas et al. (23). Briefly, 10 μl of 1 mM 4-hydroxybenzophenone in DMSO internal standard solution were added to each ml of plasma and transferred to a glass test tube. Each sample was extracted with 6 ml of tert-butyl methyl ether and shaken vigorously on a rotating shaker for 30 min. After centrifugation for 10 min at 200 × g, the upper organic layer was transferred to a fresh glass tube and evaporated to dryness under a flow of compressed air while incubated at 50°C. Samples were redissolved in high-pressure liquid chromatography mobile phase consisting of 73:27 (v/v) 0.2 mM ammonium formate (pH 4.0)/acetonitrile.

Sample separation was performed on a Waters Alliance high-pressure liquid chromatography system (Waters Corp., Milford, MA), using a Nova-Pak C8 3.9 × 150-mm reverse-phase analytical column (Waters Corp.) and a Nova-Pak C8 guard column (Waters Corp.). The injection volume was 100 μl, and an isocratic mobile phase was used with detection based on UV absorbance at 260 nm using a Waters 996 diode-array detector. Analytical standards of free genistein and free daidzein were linear over a concentration range of 5000 nm, with a lower limit of quantitation of 20 nm. Under these conditions, the retention time was 8.8 ± 0.6 min for free genistein, 4.3 min for daidzein, and 12.9 min for the 4-hydroxybenzophenone internal standard.

Total plasma genistein and daidzein were measured by mixing 250 μl of plasma with 0.5 ml of a freshly prepared enzyme solution consisting of 0.2 mM ammonium acetate (pH 4.0), 85.2 mM ascorbic acid, and 500 μl of β-glucuronidase/sulfatase from Helix pomatia (Sigma Chemicals, St. Louis, MO). Samples were incubated overnight for 15–18 h at 37°C and cooled to room temperature. Then, 10 μl of internal standard solution were added, and samples were extracted in the same fashion as described for the free genistein and daidzein assay described above. Genistein and daidzein were obtained from Sigma Chemicals and used to prepare standard solutions.

Plasma pharmacokinetics were characterized using non-compartmental analytical methods as implemented in WinNon-
The density of individual bands on resultant phosphotyrosine Western blots was determined by scanning gels on a Molecular Dynamics Densitometer SI and using ImageQuant software (Molecular Dynamics) to obtain band density. Density of phosphotyrosine bands was then normalized for degree of protein loading. This was done by determining the density of a sample of 10 randomly selected protein bands on amido-black-stained blots. These 10 individual band densities were then summed, thus giving an average measure of protein loading that could be used for normalization purposes. Only samples run on the same gel were subjected to comparison.

### Results

**Patient Characteristics and Clinical Outcomes.** Between October 1999 and June 2000, 13 patients were accrued onto the study, and 12 completed it. Median performance status was 0, median age was 67 years (range, 40–86 years), 11 (85%) participants had prostate cancer, and 2 (15%) had colon cancer (Table 1). There were 13 men and no women, reflecting the large number of patients with prostate cancer; 38% of participants were African American, and 8% were Asian.

Of 13 patients accrued, all 13 (100%) received their first dose of drug; 12 patients (92%) received both doses. One patient, who had colon cancer, voluntarily withdrew from the study. Toxicity was observed in only one patient, and it consisted of a maculo-papular erythemic rash on the extremities and face, appearing after the first dose, worsening after the second dose, and resolving spontaneously thereafter.

**Pharmacokinetic Studies.** Pharmacokinetic samples were collected from 11 patients during cycles 1 and 2. One patient only received one cycle of genistein 90% preparation at 8 mg/kg, and no kinetic data were available for another patient treated at 8 mg/kg. One additional patient treated at 4 mg/kg had no total drug sample data due to insufficient amounts of plasma. Total plasma genistein and daidzein represent the sum of the free and conjugated drug forms circulating in plasma. Sufficient concentration 

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#### Table 1  Patient characteristics

<table>
<thead>
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<tr>
<td>Colon</td>
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</table>

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6 The abbreviations used are: MRT, mean residence time; PBMC, peripheral blood mononuclear cell.

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ECOG, Eastern Cooperative Oncology Group.
increase in \( C_{\text{max}} \) (Fig. 2C) or AUC (Fig. 2D) over this same dose range, consistent with non-dose-proportional drug kinetics. Although interpatient variability was high, the 90% preparation appeared to generate lower total drug plasma AUC values, particularly with higher genistein doses (Tables 2 and 3). At the 8 mg/kg dose level, AUC values were 221.2 ± 3.3 and 112.0 ± 96.6 \( \mu \text{g} \cdot \text{hr} \) for 43% and 90% preparations, respectively, and differences approached statistical significance \((P = 0.07)\). Interestingly, although participants were extensively counseled regarding avoidance of soy-containing foods, baseline concentrations of total genistein were above the level of detection in 5 of the 12 individuals tested (42%). The average mean ± SD total genistein concentration in these five individuals was 0.33 ± 0.16 \( \mu \text{M} \).

The estimated total genistein pharmacokinetic parameters are summarized by dose level in Tables 2 and 3 for the 43% and 90% preparations, respectively. The mean ± SD apparent clearance (CL/F) for total genistein was 8.86 ± 6.39 and 14.75 ± 17.38 liters/h for the 43% and 90% preparations, respectively, and the volume of distribution during the terminal elimination phase \((V_{z/F})\) was 189.9 ± 124.3 and 653.8 ± 830.8 liters for the 43% and 90% preparations, respectively. The median apparent time to maximum concentration \((T_{\text{max}})\) was 6.0 h (range, 3.0–24 h) and 4.5 h (range, 2.0–24.0 h), and the mean ± SD apparent half-life \((T_{1/2}; \text{harmonic mean ± pseudostandard deviation})\) was 15.03 ± 2.61 and 22.41 ± 10.40 h for the 43% and 90% preparations, respectively.

The pharmacokinetic parameters for daidzein are summarized in Tables 4 and 5. Total plasma daidzein kinetics were similar in general to genistein, with a trend toward higher total plasma drug concentrations with the 43% genistein preparation that approached statistical significance at the 8 mg/kg dose level \((P = 0.06)\). The concentrations of free genistein were considerably lower than that of total genistein (Table 6). For the 43% and 90% preparations, respectively, the median apparent \( T_{\text{max}} \) of free genistein was 3.0 h (2.0–24 h) and 3.0 h (0.5–10.0 h), and mean \( C_{\text{max}} \) values for the 2, 4, and 8 mg/kg dose levels ranged from 66.4 to 154.8 and 53.2 to 116.9 nM.

The impact of individual patient covariates on the clearance of total plasma genistein was examined after dosing with the 43% preparation. Covariates analyzed included age, weight, ethnicity, histological tumor type, history of prior chemother-

![Fig. 1. Structure of genistein.](image)

**Fig. 2.** Pharmacokinetic parameters associated with 43% and 90% preparations of genistein. In A and C, \( C_{\text{max}} \) (maximum concentration) values are depicted, and in B and D, AUC (area under the curve) values are depicted, as a function of genistein dose (2, 4, and 8 mg/kg). In A and B, the 43% preparation of genistein was administered; in C and D, the 90% preparation of genistein was administered.
aply, and history of prior radiation therapy. Interestingly, no correlation was observed between ideal body weight and drug clearance (coefficient of determination, $R^2 = 0.13$; Fig. 3).

**Protein Phosphotyrosine Analysis.** There were 13 different sets of samples available for analysis, from 9 different patients. A set of samples consists of blood cells collected from 10 of 13 sets of samples (77%) evaluated further. An increase in tyrosine phosphorylation was observed at the 6  h time point (i.e., at a time corresponding to high concentrations of plasma genistein) in 10 of 13 sets of samples (77%) or 7 of 9 patients (78%) evaluated.

Quantitation of changes in the 60-kDa band intensity (Table 7) demonstrated that, compared with relatively low baseline values, there was a 2.8 ± 0.9-fold (mean ± SE; $n = 12$; $P = 0.03$) increase in band intensity at the 6  h time point, decreasing to 1.1 ± 0.2-fold, compared with baseline, by 24  h ($P = 0.67$).

**Table 2** Total plasma genistein pharmacokinetic parameters after dosing with 43% genistein (means ± SD)

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Tablets taken</th>
<th>Dose level (mg/kg)</th>
<th>No. of patients</th>
<th>$T_{max} , ^{a,b}$ (h)</th>
<th>$C_{max}$ (µM)</th>
<th>AUC$_{0-24}$ (µM*h)</th>
<th>Apparent $T_{1/2}'$ (h)</th>
<th>CL/F (liters/h)</th>
<th>V$_Z$/F (liters)</th>
<th>MRT (h)</th>
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<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6.0 (4.5–6.0)</td>
<td>5.12 (1.91)</td>
<td>91.1 (20.3)</td>
<td>14.48 (2.76)</td>
<td>6.31 (1.35)</td>
<td>141.1 (64.4)</td>
<td>19.91 (2.53)</td>
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<td>3</td>
<td>3</td>
<td>4.5 (3–4.5)</td>
<td>14.12 (9.43)</td>
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<td>16.83 (3.46)</td>
<td>10.63 (11.66)</td>
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<td>23.17 (7.80)</td>
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<td>4</td>
<td>3</td>
<td>8.0 (4.5–24)</td>
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<td>221.2 (3.3)</td>
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<td>18.10 (0.98)</td>
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<td>8</td>
<td>6.0 (3–8)</td>
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<td>92.2 (32.3)</td>
<td>15.53 (2.37)</td>
<td>6.28 (2.00)</td>
<td>138.7 (31.0)</td>
<td>22.06 (2.32)</td>
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$^a$ $T_{max}$, time of maximal plasma concentration; $C_{max}$, maximal plasma concentration; AUC$_{0-24}$, area under the concentration-time curve extrapolated to infinity; CL/F, apparent clearance; $T_{1/2}'$, apparent terminal half-life of elimination; V$_Z$/F, apparent volume of distribution during the terminal elimination phase.

$^b$ Median and range.

$^d$ Harmonic means and pseudostandard deviations.

$^d$ Unable to estimate terminal elimination in one patient, $n = 2$.

**Table 3** Total plasma genistein pharmacokinetic parameters after dosing with 90% genistein (means ± SD)

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<tr>
<th>Subject no.</th>
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<th>Dose level (mg/kg)</th>
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<th>Apparent $T_{1/2}'$ (h)</th>
<th>CL/F (liters/h)</th>
<th>V$_Z$/F (liters)</th>
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$^a$ $T_{max}$, time of maximal plasma concentration; $C_{max}$, maximal plasma concentration; AUC$_{0-24}$, area under the concentration-time curve extrapolated to infinity; CL/F, apparent clearance; $T_{1/2}'$, apparent terminal half-life of elimination; V$_Z$/F, apparent volume of distribution during the terminal elimination phase.

$^b$ Median and range.

$^c$ Harmonic means and pseudostandard deviations.

**Discussion**

Accrual was heavily skewed toward prostate cancer, reflecting the patient population seen by the investigators. Toxicity was minimal and consisted of a self-limiting rash in one person that...
Phase I Study of Genistein

The study measured the pharmacokinetic parameters of genistein and daidzein after dosing with 43% and 90% genistein, respectively. The data is presented in Tables 4 and 5.

Table 4: Total plasma daidzein pharmacokinetic parameters after dosing with 43% genistein (means ± SD)

<table>
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<th>Genistein dose level (mg/kg)</th>
<th>Daidzein dose level (mg/kg)</th>
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<th>$C_{\text{max}}$ (µM)</th>
<th>AUC$_{0-\infty}$ (µM·h)</th>
<th>Apparent $T_{1/2}$ (h)</th>
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Table 5: Total plasma daidzein pharmacokinetic parameters after dosing with 90% genistein (means ± SD)

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<th>Daidzein dose level (mg/kg)</th>
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<th>AUC$_{0-\infty}$ (µM·h)</th>
<th>Apparent $T_{1/2}$ (h)</th>
<th>CL/F (liters/h)</th>
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</table>

likely represented an allergy to soy. However, because treatment time was short, additional toxicities will likely become apparent when long-term soy treatment is evaluated prospectively.

Estimates of average daily genistein consumption by soy consumers range from 0.3 to 1 mg/kg (29–41). Thus, participants in the current study were given 2–8× the maximum average dietary intake. Although participants subsisted on a red meat-based diet and were instructed regarding avoidance of soy products, 42% had baseline plasma levels of genistein that were above the level of detection. Within this group, the average baseline concentration of genistein was 0.33 µM. Interestingly, this corresponds closely to the average plasma level of genistein previously reported by Adlercreutz et al. (8) in normal Japanese men, which was 0.276 µM; in contrast, average levels in non-soy consumers (Finish men) were 0.006 µM. Whereas the Adlercreutz study evaluated normal volunteers, the current study evaluated subjects with cancer. Genistein is promoted through the mass media for its anticancer effects and is commercially available. Because rates of nutriceutical consumption by cancer patients are high (42, 43), it is likely that participants were taking soy supplements (either knowingly or unknowingly). Self-administration of experimental agents is an important consideration in cancer chemoprevention (44); pharmacokinetic monitoring of blood levels is thus an important part of chemoprevention studies (21). Because some putative chemopreventive dietary constituents have been shown to be harmful when studied prospectively (45), a cautionary note of the potential for harm is raised to eager investigators who overemphasize preliminary findings.

Both epidemiological (1–4) and preclinical mechanistic studies (5–7, 46) suggest that soy-associated genistein inhibits prostate cancer metastasis, and the lower limit of pharmacological efficacy seen in preclinical models was 1–10 nm (6, 7). These concentrations directly overlap with the 3–19 nm blood levels of free genistein measured in those who subsist on a soy-based diet, which in turn is 1–2 logs above the levels seen in non-soy consumers (8). The current study showed that after ingestion of twice dietary amounts of genistein, peak plasma levels of free genistein were 66–117 nm, thus supporting the

Table 6: Pharmacokinetic parameters for free plasma genistein

<table>
<thead>
<tr>
<th>Genistein dose level (mg/kg)</th>
<th>43% Genistein preparation</th>
<th>90% Genistein preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>$T_{\text{max},a}$ (h)</td>
<td>$C_{\text{max}}$ (µM)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>3.25 (2–24)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3.0 (2–4)</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>3.0 (2–3)</td>
</tr>
</tbody>
</table>

| No. of patients             | $T_{\text{max},a}$ (h)    | $C_{\text{max}}$ (µM)   |
| 2                           | 4                         | 3.0 (0.5–10)            | 116.9 (116.6)       |
| 4                           | 2                         | 6.0 (2–10)              | 108.7 (116.2)       |
| 8                           | 2                         | 3.25 (2–4.5)            | 53.2 (42.7)         |

a $T_{\text{max}}$, time of maximal plasma concentration; $C_{\text{max}}$, maximal plasma concentration.
b Median and range.
c Harmonic means and pseudostandard deviations.
d Unable to estimate terminal elimination in one patient, $n = 3$. 

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An important new pharmacokinetic observation was the apparent lack of association of apparent clearance of total plasma genistein with absolute body weight. This represents a preliminary finding that needs to be evaluated in expanded studies. Genistein doses were scaled to body weight, implicitly assuming greater clearance by larger individuals, a commonly adopted practice in oncology. This practice has recently come under criticism (47–49), and our data would further support such concerns. Our data suggest that absolute body weight does not predict increased clearance of total genistein, and thus, our dosing scheme may have actually inflated the interpatient variability in plasma drug concentrations. Further development of this agent should use fixed dose levels for all individuals.

Although we (7) and others (22) have shown that genistein can alter protein-tyrosine phosphorylation in cell culture systems, it was surprising to detect significant changes in vivo after only a single dose of drug. However, if in fact genistein were exerting significant biological effects on cancer, and because all doses significantly exceeded average dietary intake, it is not unreasonable to expect pharmacological effects throughout the dose range evaluated. Different patterns of tyrosine-phosphorylated proteins were seen in different patients, as were changes in phosphorylation with genistein treatment. This likely represents a sensitive measure of interindividual variability, including such factors as metabolizing enzymes, coadministration of other drugs and/or foods, and consumption of soy products. Interindividual variability is a common theme in pharmacotherapeutics, and its implications for cancer chemoprevention have recently been reviewed (21). In fact, significant interindividual variability in excretion of isoflavones and lignans after soy ingestion has been reported previously (50). Importantly, because tyrosine phosphorylation plays a central role in cell signaling, modulation of tyrosine phosphorylation status in vivo provides a measure of in vivo effects on intracellular signaling (46).

Whereas it is tempting to evaluate apparent differences in the intensity of baseline tyrosine phosphorylation between patients, the current study was not designed to measure such potential differences. Factors potentially responsible for observed differences relate to differences in the amount of cell-associated protein available for analysis from individual patients, as well as patient-specific differences in the number and type of circulating cells. The identity of the 60-kDa protein whose tyrosine phosphorylation is increased by genistein is
ically modulate predisposition to cancer, as a large body of
namely soy, given in dietary proportions, can modulate
crased if one is considering its use in women at risk for breast
evaluate the full spectrum of pleiotropic effects, and further investigation is required to eval-
of free genistein. It is important to note that genistein has
were observed
function of concentration. This notion is directly supported by
cells treated
albeit at concentrations of 100 μM and above (22). In contrast,
we have shown that at lower concentrations, genistein increases
the protein-tyrosine phosphorylation content of human prostate
cells treated in vitro, suggesting inhibition of phosphatase activity
(7). Taken together, data suggest differential effects as a
function of concentration. This notion is directly supported by
the current study, wherein increases in tyrosine phosphorylation
were observed in vivo, in the face of nanomolar concentrations
of free genistein. It is important to note that genistein has
pleiotropic effects, and further investigation is required to eval-
uate the full spectrum of in vitro effects. Because genistein
is known to have estrogenic activity, caution needs to be exer-
cised if one is considering its use in women at risk for breast
cancer.

The current study demonstrates that dietary components, namely soy, given in dietary proportions, can modulate in vitro
cell signaling pathways. If in fact dietary factors pharmacologi-
cally modulate predisposition to cancer, as a large body of
research suggests (51), it therefore stands to reason that dietary
components can modulate molecular events in vivo. This study
provides evidence of such. Currently, there is not enough
information to support the development of one particular prepa-
ration of isoflavones over another. The 90% preparation seems
attractive because of its greater “purity” and because many
preclinical studies point to genistein as having chemopreven-
tive activity. However, it is possible that clinical activity, if
present at all, is due to combinations of components found in
soy, which may or may not include genistein.

**Table 7** Intensity of phosphotyrosine bands*  

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genistein (mg/kg)</th>
<th>Preparation</th>
<th>Ratio of band intensity 6 h vs. 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>43</td>
<td>2.4</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>43</td>
<td>3.1</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>90</td>
<td>2.2</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>90</td>
<td>2.7</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>90</td>
<td>1.5</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>43</td>
<td>1.3</td>
</tr>
<tr>
<td>G</td>
<td>8</td>
<td>90</td>
<td>0.8</td>
</tr>
<tr>
<td>H</td>
<td>8</td>
<td>43</td>
<td>0.9</td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>90</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Changes in the intensity of 60-kDa phosphotyrosine bands at 6 and 24 h after genistein treatment are shown. Presented are the ratios of band intensity for the 6 and 24 h time points, as compared with baseline. Phosphotyrosine band intensities were normalized for the level of protein loading, as described in “Materials and Methods.”

References


Phase I Pharmacokinetic and Pharmacodynamic Analysis of Unconjugated Soy Isoflavones Administered to Individuals with Cancer

Chris H. Takimoto, Kira Glover, Xiaoke Huang, et al.


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