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Soy Food Frequency Questionnaire (FFQ) Does Not Correlate with Baseline Isoflavone Levels in Patients with Bladder Cancer

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Abstract

Background—The isoflavone genistein, a natural soy product with receptor tyrosine kinase inhibiting activity, as well as phytoestrogenic and other potential anticarcinogenic effects is being studied as an anticancer agent. Since isoflavones are commonly consumed in food products containing soy proteins, a method to control for baseline isoflavone consumption is needed.

Methods—HPLC was used to evaluate baseline plasma and urine concentrations of isoflavone in fifty-four participants with bladder cancer enrolled on a phase II chemoprevention study of G-2535. The soy food frequency questionnaire (Soy FFQ) was used to assess participant's baseline soy intake. The association between baseline isoflavone concentrations and intakes for genistein and daidzein was assessed by the Spearman's rank correlation coefficient.

Results—The majority of participants had no detectable genistein or daidzein in plasma at baseline. The median and range of values were 0 (0-1,480) nmol/L for genistein, and 0 (0-1,260) nmol/L for daidzein. In urine, the median and range of values were 91.0 (0-9,030) nmol/L for genistein and 623 (0-100,000) nmol/L for daidzein. The median and range of weekly estimated genistein intake was 0 (0-236) mg/wk; the median and range of weekly estimated daidzein intake was 0 (0-114) mg/wk. There was no relationship to soy intake as measured by the FFQ and baseline isoflavone levels in plasma or urine and the Spearman's rank correlation coefficients were not significant.

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Conclusion—The Soy FFQ did not correlate with plasma or urine concentrations of either isoflavone.

Impact—Alternative methods for controlling for soy consumption, including measuring plasma and urine concentrations, in isoflavone chemoprevention trials should be considered.

Keywords

Soy FFQ; isoflavones; pharmacokinetics; chemoprevention

Background

The isoflavone genistein, a natural soy product, has receptor tyrosine kinase inhibiting activity, as well as phytoestrogenic and other potential anticarcinogenic effects (1). Inhibition of epidermal growth factor receptor (EGFR) activity and EGF mediated responses such as proliferation and cell motility have been reported for genistein in bladder cancer (2,3).

G-2535 is an oral soy supplement comprised of the isoflavones genistein (62%), daidzein (33%), and glycitein (5%). We recently completed a trial assessing the impact of short-term oral ingestion of 300 or 600 mg genistein/day extract (in the form of the G-2535 supplement) on molecular pathways in bladder cancer and normal bladder epithelial specimens removed by transurethral resection (TURBT) or cystectomy (4) from patients with a bladder cancer detected by cystoscopy.

Plasma and urine concentrations of genistein and daidzein were evaluated in this trial. Given that dietary ingestion of foods containing soy proteins can generate detectable plasma levels of genistein and daidzein (5), the soy food frequency questionnaire (Soy FFQ) developed and validated by the Fred Hutchinson Cancer Research Center (FHCRC) was administered to survey study subjects to control for dietary intake. The Soy FFQ takes approximately 5 minutes to complete and contains questions about the consumption of 20 soy foods and supplements. In their validation FHCRC found daidzein and genistein intake calculated using the Soy FFQ correlated significantly with plasma daidzein and genistein concentrations, respectively ($P < 0.001$), in 96 postmenopausal women aged 50–79 y (5).

The main objective of the current study was to determine if there was a correlation between reported isoflavone intake as assessed by the Soy FFQ and the associated baseline levels of isoflavones, for genistein and daidzein.

Materials and Methods

Study Population

Patients found to have a bladder tumor through diagnostic office cystoscopy who were candidates for surgical removal by TURBT or cystectomy were recruited to the study. Participants were randomized in a 1:1:1 ratio to receive either 150 mg or 300 mg of genistein (study drug G-2535) or placebo twice daily (after stratification as ($T > 2$) invasive ($T > 2$ or noninvasive ($T < 2$) disease. Treatment was administered for 14–21 days before

surgery. A detailed report about trial design, patient inclusion criteria, study agent G-2535 and trial conclusions can be found in the published Phase 2 Chemoprevention trial (4).

Soy intake measurement

Baseline soy isoflavone intake was estimated using the Soy FFQ as previously described (5).

Pharmacokinetics

Prior to Day 1 of study agent dosing, 10 ml of whole blood was drawn into a green top tube and centrifuged for 10 minutes to separate the plasma. The plasma was drawn off and separated into 2 Nunc tubes for storage at -70°C until analyzed.

A clean catch urine sample was also collected prior to Day 1 of dosing. Collection time and total void volume were recorded as well as the time of the last previous void. The urine sample was refrigerated immediately at 4°C . Within 1-4 hours, 4 - 2 ml aliquots were drawn, placed in 3.6 ml Nunc tubes and stored at -70°C until analyzed.

Genistein and daidzein pharmacokinetics were evaluated in plasma and urine samples by a validated reversed phase HPLC with UV detection with a lower limit of quantitation of 14.4 nMol for genistein, and 12.2 nMol for daidzein, converted from previously described limits of 3.9 ng/mL for genistein, and 3.1 ng/mL for daidzein (4,6).

Statistical analyses

The primary objective was to examine the association between dietary soy intake and isoflavone levels, (i.e. genistein and daidzein) in plasma and urine. Soy isoflavone intakes were estimated using the soy FFQ (5). We examined the normality assumption prior to analysis for the dietary soy intake and isoflavone levels and found the data to be sufficiently non-normal to support log transformation. Log transformed values were plotted against respective log-transformed HPLC values of plasma and urine genistein and daidzein. As there were many zero values for the genistein and daidzein intake data, and many values of ND (not detected) for plasma genistein and daidzein, Spearman rank correlation coefficients were deemed more appropriate for this data and are presented here. For the following analyses, the ND values were considered to be 0.

Results

Descriptive statistics for plasma and urine concentrations of genistein and daidzein at baseline are shown in Table 1. The majority of patients had no detectable genistein (51, or 96%) or daidzein (46, or 87%) in plasma at baseline [the median and range of values were 0 (0-1,480) nmol/L for genistein, and 0 (0-1,260) nmol/L for daidzein]. In contrast very few patients had no detectable genistein (2, or 4%) and daidzein (1, or 2%) in urine at baseline [the median and range of values were 91.0 (0-9,030) nmol/L for genistein and 623 (0-100,000) nmol/L for daidzein].

Median and range for weekly dietary intake was 0 (0-236) mg/wk for genistein and 0 (0-114) for daidzein. Figures 1a-d gives the scatter plots of calculated isoflavone intakes (as estimated by the Soy FFQ) and the appropriate plasma and urine isoflavone concentrations.

There was no relationship to isoflavone intake as measured by the FFQ and baseline isoflavone levels in urine; the Spearman correlation coefficients were not significant (Figures 1b and 1d). It was not possible to assess the association between soy isoflavone intake and baseline isoflavone levels in plasma due to many values being not detected (Figure 1a and 1c).

Discussion

Dietary isoflavone intakes as assessed by the Soy FFQ in this study population were low and variable [median and range was 0 (0-236) mg/wk for genistein and 0 (0-114) mg/wk for daidzein] but consistent with the reported isoflavone intake in the population in which the Soy FFQ was validated (5). Plasma and urine concentrations were also consistent with those previously reported in individuals consuming a Western diet (7, 8). However, we failed to show a correlation between the soy isoflavones from the Soy FFQ and plasma and urine concentrations for genistein and daidzein.

A potential explanation for the differences between our study and the previous study was the study population (5). The Soy FFQ was validated for plasma levels in a postmenopausal female population. The majority of subjects enrolled in this study were male, (87%) and older, with a median age of 71 (range 46-97), consistent with individuals at risk for bladder cancer. Another potential explanation for the difference is that the Soy FFQ was validated for plasma concentrations and we also evaluated urine, although other Soy FFQs have been validated for urine concentrations (7, 8).

We anticipate that the most likely explanation is that the Soy FFQ failed to capture “hidden” isoflavanoid content in many foods not generally associated with soy. While the questionnaire asks specifically about soy powder, tempeh, miso and other soy supplemented foods, other readily available foods containing significant amounts of soy are not included (9). For comparison, soybeans contain approximately 150 mg of isoflavones per 100g/food. Tofu contains approximately 30mg of isoflavones per 100g/food and miso soup contains 1.5 mg per 100g/food. An example of foods not included in the Soy FFQ that contain comparable amounts of isoflavones include Kashi GoLean breakfast cereal with 17.3 mg isoflavones per 100g/food, beef containing 1.5 mg per 100g/food, donuts containing 5 mg per 100g/food, Subway meatball sandwich with 6 mg per 100g/food and Cliff Bar Crunchy Peanut Butter Flavor containing close to 30mg per 100g/food (9).

Conclusion

Few participants had measurable plasma genistein or daidzein concentrations at baseline, and as a result we were unable to adequately assess the association between soy intake as measured using the Soy FFQ with plasma concentrations of either compound. While urine genistein and daidzein concentrations were generally measurable, in our population they did not correlate with soy intake as measured using the Soy FFQ. This is most likely related to the number of commonly consumed soy containing foods that are not captured by the instrument. Alternative methods for controlling for soy consumption, including

measuring plasma and urine concentrations in isoflavone chemoprevention trials should be considered.

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Figure 1.
Urine and Plasma Level of Isoflavones vs Soy Intake

Table 1

Mean±Standard Deviation (SD), Median and Range of Plasma and Urine Genistein and Daidzein^{*} and estimated Soy Intake at Baseline

	N	mean±SD	median	range
Plasma Genistein (nmol/L)	53	28.4±203	0	(0 ¹ -1,480)
Plasma Daidzein (nmol/L)	53	34.7±177	0	(0 ² -1,260)
Urine Genistein (nmol/L)	55	385±1,230	91.0	(0 ³ -9,030)
Urine Daidzein (nmol/L)	54	3,390±14,000	623	(0 ⁴ -100,000)
Genistein Intake (mg/wk)	60	9.07±34.9	0	(0 ⁵ -236)
Daidzein Intake (mg/wk)	60	6.62±22.0	0	(0 ⁵ -114)

* As many observations are ND (not detected), and considered to be 0, mean ± SD as well as median (range) are presented to better characterize distributions

¹ 51 patients had values of ND

² 46 patients had values of ND

³ 2 patients had values of ND

⁴ 1 patient had value of ND

⁵ 44 calculated to be 0