Altered Expression and Glucocorticoid-Inducibility of Hepatic CYP3A and CYP2B Enzymes in Male Rats Fed Diets Containing Soy Protein Isolate

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ABSTRACT Hepatic CYP3A and CYP2B enzymes were studied in male Sprague-Dawley rats derived from 5–7 litters fed diets in which the protein source was either casein or soy protein isolate. At age 65 d, rats were gavaged with corn oil (vehicle) or 50 mg/kg dexamethasone. Hepatic expression of CYP3A and CYP2B1 mRNA, apoprotein and associated monooxygenase activities were measured. Consumption of soy diets significantly increased monooxygenase activity toward the following: the CYP3A substrates erythromycin and ethylmorphine N-demethylase; corticosterone and testosterone 6β-hydroxylase; and apoprotein and mRNA expression of CYP3A2 (P < 0.05). Dexamethasone significantly induced turnover of erythromycin and testosterone, expression of CYP3A apoprotein, and expression of CYP3A1 and CYP3A2 mRNA (P < 0.05). In addition, significant diet-inducer interactions were observed in the expression of CYP3A apoprotein and activities toward ethylmorphine, corticosterone and testosterone (P < 0.05). Significant diet-inducer interactions were also observed on CYP2B1-dependent pentoxyresorufin O-depentylase activity (P < 0.05). However, although dexamethasone significantly induced CYP2B1 expression at the apoprotein and mRNA level (P < 0.05), no significant diet effects were observed. These data suggest potential effects of soy consumption on the metabolism of a wide variety of CYP3A and CYP2B1 substrates, especially in situations involving coexposure to CYP inducers. J. Nutr. 129: 1958–1965, 1999.

KEY WORDS: soy protein isolate • cytochrome P450 • induction • rats

Consumption of diets rich in fruits and vegetables has been linked to significantly lowered cancer risk (Craig 1997, Graham 1980). Data from epidemiologic studies suggest that Asian populations consuming traditional diets containing high levels of soybean products have significantly lower incidence of breast, prostate and colon cancers (Persky and Van Horn 1995). In addition, the consumption of soy diets or of soy-associated isoflavones inhibits chemically induced breast cancer in experimental animals (Messina and Barnes 1991). In addition to the isoflavones, a number of other phytochemical components of soy have been suggested to contribute to this chemoprotective effect, including saponins, phytosterols, phytic acid, phenolic acids and coumarins (Messina and Barnes 1991).

Several potential chemoprotective mechanisms have been suggested including the following: interactions with estrogen receptors, antioxidant effects, inhibition of steroidogenic enzymes, tyrosine kinase inhibition and antiangiogenic effects (Adlercreutz et al. 1995, Barnes et al. 1996). An additional chemoprotective mechanism that has received less attention is the possible effect of soy consumption on procarcinogen activation and carcinogen metabolism. In general, increases in phase I combined with increases in phase II metabolism will result in faster clearance and reduced carcinogenicity of ingested procarcinogens such as polycyclic hydrocarbons (Pres- tera et al. 1993). This may occur systemically via alterations in enzyme activity in the liver or locally within the carcinogen-target tissue (Liu et al. 1994).

A number of in vitro and in vivo rodent studies have suggested that treatment with purified flavinoids and couma-rins found in soy may induce phase II enzymes such as quinone reductase, glutathione-S-transferases and UDP-glucuronyltransferases (Wang et al. 1998). In addition, a recent study in rats demonstrated that dietary consumption of soy flour or soy protein isolate resulted in significant increases in these phase II enzymes in liver, small intestine and colon in vivo (Appelt and Reicks 1997).

Fewer data exist on the effects of soy diet or soy-associated phytochemicals on phase I cytochrome P450 (CYP) expression and activity. In vitro studies with keratocyte and hepatocyte cell lines have demonstrated modulation of CYP1A2 inducibility by the soy-associated isoflavones genistin and daidzein (Backlund et al. 1997), and in vivo studies utilizing genistein injected intraperitoneally have demonstrated either no effects (Helsby et al. 1997) or inhibition (Helsby et al. 1998) of hepatic P450.
In addition to effects on cancer risk, dietary effects on P450 expression may also have considerable importance in terms of altering the clearance and efficacy of clinically utilized drugs as well as the risk of toxic side effects. In the U.S. and other Western countries, the effects of soy consumption in this regard may be of particular importance for pediatric medications because in these countries, ~20% of infants consume formula in which soy protein isolate is the sole protein source (Setchell et al. 1997).

In this study, we examined the effects of a soy-based diet, high in isoflavones, on hepatic P450 expression in a male rat model in comparison with soy-free, casein (CAS)-based diets. By providing soy protein isolate (SPI) as the sole protein source, average daily consumption of isoflavones was 19.3 mg/kg/day genistein and 9.2 mg/kg/day daidzein; total isoflavone concentrations in 24-h urine pools were in the range of 40 μmol/L (Ronis et al. 1999). We focused on expression and glucocorticoid-inducibility of members of the CYP3A family of cytochrome P450s (CYP3A1 and CYP3A18) because they represent the most abundant P450 enzymes found in human liver, are of great importance for the metabolism and clearance of a wide range of clinically utilized drugs including antibiotics, analgesics and steroids in addition to carcinogens; they have also been shown to be significantly inducible in humans in vivo (Ronis and Ingelman-Sundberg 1998).

The CYP3A family of enzymes is of special interest because they have also been shown to be significantly inducible in human liver, are of great importance for the metabolism and clearance of a wide range of clinically utilized drugs including antibiotics, analgesics and steroids in addition to carcinogens; they have also been shown to be significantly inducible in humans in vivo (Ronis and Ingelman-Sundberg 1998). Pentoxyresorufin and resorufin were obtained from Sigma Chemical (St. Louis, MO). Steroid metabolite standards were supplied by Steraloids (Wilton, NH). Pentoxyresorufin and resorufin were purchased from Pierce Chemical (Rockford, IL). [14C] testosterone was obtained from Amersham Life Science (Arlington Heights, IL). Micelles of a hot/cold steroid mixture were formed with the use of the nonionic detergent Brij 35 and incubated in a total volume of 1 mL. The rate of formaldehyde formation was determined spectrophotometrically at 412 nm using the Nash reagent.

**MATERIALS AND METHODS**

**Chemicals and reagents.** Dexamethasone, erythromycin, ethylmorphine, NADPH, and testosterone were obtained from Sigma Chemical (St. Louis, MO). Steroid metabolite standards were supplied by Steraloids (Wilton, NH). Pentoxyresorufin and resorufin were purchased from Pierce Chemical (Rockford, IL). [14C] testosterone (2.1 GBq/mmol) was purchased from DuPont NEN (Boston, MA). [1,2,6,7 H] corticosterone (2.6 GBq/mmol) was obtained from Amersham Life Science (Arlington Heights, IL). Lithocholic acid and [14C] lithocholic acid (1.9 GBq/mmol) were the gift of Dr. Anna Radomska-Pandya (UAMS, Little Rock, AR). Rabbit polyclonal antibodies against rat CYP3A1 (3A23) and CYP2B1 were the kind gift of Dr. Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden) (Ronis et al. 1998). Mouse monoclonal antibodies against human CYP3A4/5 were obtained from Gentest (Woburn, MA). [125I] goat anti-rabbit immunoglobulin G (IgG) and [125I] goat anti-mouse IgG were purchased from ICN Biomedicals (Costa Mesa, CA). LHP-KDF high performance normal phase silica TLC plates were obtained from Whatman International (Maidstone, Kent, UK).

**Oligonucleotide and cDNA probes.** Oligonucleotide probes were synthesized by Bio-Synthesis (Lewisville, TX) based on the +68 to +80 base-pair sequences of rat CYP3A1 (CYP3A23), CYP3A2 and CYP3A18 published by Wright et al. (1997). The oligonucleotide specific for CYP2B was designed using the CYP2B1 and CYP2B2 sequences described by Nakayama et al. (1993). The cDNA against rat CYP3A9 was the gift of Dr. Henry Strobel (University of Texas, Houston, TX) (Wang and Strobel 1997).

The experiment received prior approval from the Institutional Animal Care and Use Committee at UAMS. All animals were housed in an AAALAC-approved animal facility at ACHRI and all animal housing and husbandry conformed to USDA guidelines.

**Animals and diets.** Virus-free adult male and female Sprague-Dawley rats (~300 g) were purchased from Harlan Industries (Indianapolis, IN). Rats were housed separately in plastic cages, kept at constant temperature (22°C) and humidity with lights on between 0600 and 1800 h and were given free access to water. Diets were formulated exactly as described by Reeves et al. (1993) for AIN 93G except that soybean oil was replaced by corn oil. One diet contained casein (CAS) as the protein source and the other contained soy protein isolate (SPI) as the sole protein source. The diets were purchased from Charles River Laboratories (St. Louis, MO). After several weeks of consuming the purified diets, male and female rats were mated, and pregnant dams were fed their respective diets during gestation. At birth, pups were culled to five males and five females per litter; lactating dams continued to consume their respective diets until the pups were weaned. At weaning, male pups were given continued free access to their respective diets until the beginning of the induction experiment at age 65 d.

**Glucocorticoid induction experiment.** At 65 d of age, one male pup from each of 4–6 litters fed CAS or SPI diets were gavaged orally with either 2 mL of corn oil or 50 mg/kg dexamethasone (DEX) in 2 mL corn oil at 1600 h. At 0900 h the next morning, the animals were killed by decapitation, livers removed and microsomes prepared using the differential ultracentrifugation method of Chipman and Walker (1979).

**Monoxygenase activities**

**CYP3A substrates.** Because cytochrome P450 enzymes have overlapping substrate specificities and CYP3A enzymes are likely involved in the metabolism of a wide range of chemicals (Wilkinson 1996), we examined the effects of SPI consumption on hepatic microsomal metabolism of a number of different putative CYP3A substrates. In all cases, monoxygenase assays were standardized in the laboratory before the current study, and the incubation conditions described were within the linear range for incubation time and protein concentration. Erythromycin N-demethylation was measured according to the method of Werringloer (1978) with a 45-min incubation containing 12.5 mmol/L erythromycin in the presence of 0.5 mmol/L NADPH and 1 mg of microsomal protein in a total assay volume of 1 mL. The rate of formaldehyde formation was determined spectrophotometrically at 412 nm using the Nash reagent. Ethylmorphine N-dealkylation was also measured by following formaldehyde production using Nash reagent. In this case, 2 mmol/L ethylmorphine was incubated with 0.5 mmol/L NADPH and 1 mg of microsomal protein in a 1-mL final volume for 15 min. Testosterone 6β-hydroxylation was assayed as described previously (Ronis et al. 1991). Metabolism of [14C] lithocholic acid and [14C] corticosterone was measured by using a modification of the TLC methodology of Zimniak et al. (1991). Micelles of a hot/cold steroid mixture were formed with the use of the nonionic detergent Brij 35 and incubated in a total volume of 80 mL of 0.15 mol/L potassium phosphate, pH 7.4, 2 mmol/L NADPH, 0.1 mmol/L EDTA, 2 mmol/L lithocholesterol, 0.006% Brij 35 and 1.3 mmol/L of microsomal protein. The final concentration of lithocholic acid was 50 μmol/L; that of corticosterone was 1 μmol/L. Reactions were performed at 37°C for 20 and 10 min, respectively, and terminated by the addition of 20 μL of ethanol on ice. A proportion of the complete reaction mixture (60 μL) was immediately spotted onto the preabsorbent area of LHP-KDF high performance TLC plates. When completely dry, the TLC plates were run twice in the same dimension with a solvent system of 10:10:25 ethylacetate/isoctane/ethyl acetate/acetic acid. 6β-Hydroxylated products were identified by comigration with pure standards and quantified by phosphorimaging using a GS525 molecular imager (Bio-Rad Laboratories, Hercules, CA).

**CYP2B substrates.** Pentoxyresorufin O-depentylase (PROD) was measured by following the formation of resorufin spectrophotometrically at 536 nm (excitation) and 586 nm (emission) according to the method of Lubet et al. (1985) using an RF-5301PC scanning spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) under conditions of linearity for incubation time and protein.

**Western immunoblot analysis.** Western blotting was conducted on pools of liver microsomes derived from 4–6 rats at a concentration of 30 μg protein/well to illustrate differences in mean expression of CYP3A apoproteins in microsomes from vehicle-treated rats fed CAS or SPI diets. In addition, Western blots were performed on microsomes from individual vehicle-treated or DEX-treated rats at a loading of 10 or 50 μg protein/well. Varying lengths of autoradiographic
Effects of diet and dexamethasone treatment on body weight, liver weight and hepatic microsomal protein content in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight</th>
<th>Liver weight</th>
<th>Liver/Body weight</th>
<th>Microsomal protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>% of body wt</td>
<td>mg/g liver</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>352 ± 8.6b</td>
<td>14.0 ± 0.58a</td>
<td>3.96 ± 0.15a</td>
<td>12.2 ± 0.82a</td>
</tr>
<tr>
<td>CAS</td>
<td>339 ± 10.5a</td>
<td>12.8 ± 0.11a</td>
<td>4.21 ± 0.19a</td>
<td>12.7 ± 1.00a</td>
</tr>
<tr>
<td>SPI</td>
<td>345 ± 8.6b</td>
<td>17.2 ± 0.58b</td>
<td>4.99 ± 0.15b</td>
<td>13.9 ± 0.82a</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>330 ± 8.6a,b</td>
<td>15.7 ± 0.63b</td>
<td>4.94 ± 0.16b</td>
<td>10.5 ± 0.90a</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM for rats in 4–6 litters/treatment group. Means not sharing a common letter differ significantly, P < 0.05; a < b < c.

TABLE 2

Effects of diet and dexamethasone treatment on metabolism of CYP3A-dependent substrates in hepatic microsomes from male rats

<table>
<thead>
<tr>
<th>Monooxygenase activity</th>
<th>Control</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAS</td>
<td>SPI</td>
</tr>
<tr>
<td>Erythromycin N-demethylase³</td>
<td>0.18 ± 0.04a</td>
<td>0.25 ± 0.06a</td>
</tr>
<tr>
<td>Ethynilorphine N-demethylase³</td>
<td>3.92 ± 0.30a</td>
<td>3.25 ± 0.37a</td>
</tr>
<tr>
<td>Lithocholic Acid 6β-hydroxylase⁴</td>
<td>59.8 ± 6.89a</td>
<td>54.7 ± 8.43a</td>
</tr>
<tr>
<td>Corticosterone 6β-hydroxylase⁴</td>
<td>3.65 ± 0.53a</td>
<td>3.98 ± 0.65b</td>
</tr>
<tr>
<td>Testosterone 6β-hydroxylase³</td>
<td>6.13 ± 1.02a</td>
<td>7.80 ± 1.25a</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM for duplicate assays conducted on hepatic microsomes derived from rats in 4–6 litters/treatment group. Means not sharing a common letter differ significantly, P < 0.05; a < b < c.
2 See Table 1 for diet/treatment abbreviations.
³ nmol/(mg microsomal protein · min).
⁴ pmol/(mg microsomal protein · min).
detect differences among groups. An α-level of 0.05 was set to determine significance.

RESULTS

General. General variables including body weight, liver weight and yield of hepatic microsomal protein for rats fed the two different diets and treated with vehicle or DEX are presented in Table 1. Rats fed SPI-based diets throughout life had significantly smaller body weights (P < 0.05) than rats fed CAS-based diets, but liver weight, liver/body weight ratios or yield of liver microsomes were not significantly affected by diet. DEX treatment significantly increased liver weight and liver/body weight ratios in rats fed either diet (P < 0.05), but had no significant effects on body weight or yield of microsomal protein per gram of liver.

CYP3A-dependent activities. Data demonstrating the effects of the SPI-containing diet and DEX treatment on hepatic monoxygenase activities dependent on CYP3A enzymes are presented in Table 2. Two-way ANOVA demonstrated greater activities in rats fed the SPI diet compared with the CAS diet for the ethromycin and ethylmorphine N-demethylase and for corticosterone and testosterone 6β-hydroxylation (P < 0.05), but not for the CYP3A18-specific activity lithocholic acid 6β-hydroxylase. DEX treatment resulted in greater activity of both ethromycin N-demethylase and testosterone 6β-hydroxylase (P < 0.05). In addition, diet × DEX interactions (P < 0.05) were observed when ethylmorphine, corticosterone and testosterone were used as substrates. The mean activity toward all CYP3A substrates with the exception of lithocholic acid was higher in DEX-treated rats fed SPI diets than in DEX-treated rats fed CAS diets (P < 0.05).

CYP3A Western immunoblot analysis. Western immunoblot analysis with both the rabbit polyclonal anti-rat CYP3A1 (CYP3A23) and the mouse monoclonal anti-human CYP 3A4/5 antibody revealed a single apoprotein band in male rat liver microsomes, presumably with a contribution from several of the rat enzymes that generated identical results. This band has been labeled as CYP3A in Figure 1A,B, and illustrates differences in expression of CYP3A in rats fed the two diets after treatment with either corn oil vehicle or DEX. Immunoblot data from analysis of CYP3A expression in individual microsomes from 4–6 rats/treatment group are shown in Figure 1C. Two-way ANOVA revealed soy diet and DEX effects and a significant diet × DEX interaction on CYP3A apoprotein expression (P < 0.05).

Northern blot analysis of CYP3A. Northern analyses were conducted using total mRNA isolated from the liver. The data are presented in Figure 2 and Table 3. There was no diet-dependent effect on expression of CYP3A1 (CYP3A23) mRNA, but DEX induced steady-state concentrations 40– to 60-fold in rats fed diet (P < 0.05). CYP3A2 mRNA was present at a greater level in SPI-fed rats than in CAS-fed rats (P < 0.05) and DEX induced CYP3A2 mRNA (P < 0.05) four- to sevenfold in animals fed either diet. Neither CYP3A9 nor CYP3A18 mRNA levels were affected by diet or by DEX.

CYP2B1-dependent activity. CYP2B1-dependent monoxygenase activity was assessed using PROD as a prototypic substrate. PROD activity data for liver microsomes were followed spectrofluorimetrically and are presented in Figure 3. Two-way ANOVA revealed significantly greater activity in SPI-fed rats, DEX-treated rats and a significant diet × DEX interaction (P < 0.05). Microsomes from DEX-treated, SPI-fed rats had greater mean PROD activity than microsomes from DEX-treated, CAS-fed rats (P < 0.05).

CYP2B1 Western immunoblot analysis. A representative Western blot with a rabbit polyclonal antibody directed against rat CYP2B1 and immunquantitation of CYP2B1 apoprotein is presented in Figure 4. In addition to CYP2B1, a second constitutively expressed P450 enzyme of lower molecular weight, which is not phenobarbital-inducible, was recognized. CYP2B1 apoprotein expression was greater in both SPI-fed and CAS-fed rats after DEX treatment (P < 0.05).

Northern blot analysis of CYP2B1 mRNA. Northern blots were performed on total liver mRNA using an oligonucleotide designed to be specific for CYP2B1 (Fig. 5). CYP2B1 mRNA expression was greater in the livers of rats treated with DEX compared with those receiving corn oil vehicle (P < 0.05), but no significant effect of SPI-based diets on CYP2B1 expression was observed.
Epidemiologic studies suggest a lower risk for certain chronic diseases in countries with greater daily intake of soy-based foods (Adlercreutz et al. 1995, Persky and Van Horn 1995). Although it is recognized that there are many dietary factors other than soy intake that could account for these effects (e.g., rice, green tea or lower fat intake), data from our own laboratory using animal models of breast and colon cancer have demonstrated that factors associated with soy protein isolate significantly inhibit the development of chemically induced tumors (Badger et al. 1999). One potential mechanism underlying the chemoprotection afforded by soy consumption is an alteration in procarcinogen activation and carcinogen detoxication. Thus, we are interested in the effects of soy protein isolate on phase I xenobiotic metabolizing systems.

In this study, we demonstrated for the first time that dietary consumption of soy, in the form of the soy protein isolate used in the formulation of infant formula, results in significant effects on the expression and inducibility of hepatic cytochrome P450 enzymes in a rat model. Importantly, the CYP3A family, which is the major group of P450 enzymes expressed in human liver, appears to be affected by soy. Specifically, CYP3A2 mRNA expression was significantly enhanced by soy protein isolate consumption. These data are consistent with our observation of significant soy effects on testosterone 6β-hydroxylase, a known CYP3A2 substrate (Waxman et al. 1990).

In contrast to CYP3A2, no diet effects were observed on the mRNA expression of other CYP3A family members. The data for CYP3A18 are consistent with the lack of observed effects of diet or dexamethasone treatment on the hydroxylation of lithocholic acid at position 6β, an activity that has been ascribed to this enzymatic pathway.

### TABLE 3

<table>
<thead>
<tr>
<th>CYP3A mRNA2</th>
<th>CAS 100 ± 15a</th>
<th>SPI 123 ± 15a</th>
<th>CAS 4442 ± 438b</th>
<th>SPI 5547 ± 479b</th>
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<tbody>
<tr>
<td>CYP3A1 (CYP3A23)</td>
<td>100 ± 86a</td>
<td>363 ± 97a</td>
<td>769 ± 79b</td>
<td>1061 ± 88c</td>
</tr>
<tr>
<td>CYP3A2</td>
<td>100 ± 16a</td>
<td>128 ± 18a</td>
<td>151 ± 15a</td>
<td>155 ± 16a</td>
</tr>
<tr>
<td>CYP3A9</td>
<td>100 ± 13a</td>
<td>118 ± 14a</td>
<td>120 ± 12a</td>
<td>132 ± 13a</td>
</tr>
</tbody>
</table>

1 Data represent means ± SEM for densitometric analysis of Northern blots conducted using total hepatic mRNA derived from rats in 4–6 litters. Means not sharing a common letter differ significantly, *P < 0.05*; a < b < c.

2 Mean values are expressed as % of CAS control.

3 See Table 1 for diet/treatment abbreviations.
enzyme (Zimniak et al. 1991). The different patterns of diet and dexamethasone effects observed in the expression of CYP3A cross-reactive apoprotein and metabolism of the other CYP3A substrates examined probably reflect differing contributions from CYP3A1(3A23), CYP3A2, CYP3A9 and other as yet uncharacterized rat liver CYP3A enzymes because this family of enzymes displays extensive overlapping substrate specificity. For both immunoreactive CYP3A apoprotein and a number of CYP3A-dependent monooxygenase activities, i.e., ethylmorphine N-dealkylase, corticosterone and testosterone 6β-hydroxylase, a significant interaction was observed between the soy diet and effects of the glucocorticoid inducer dexamethasone. Small differences in CYP3A apoprotein expression and activities observed in rats fed CAS or SPI diets were significantly amplified after challenge with the inducer. Such a significant diet/inducer interaction was not observed at the mRNA level with any CYP3A enzyme (Zimniak et al. 1991). The mechanisms underlying the effects of soy consumption on expression of CYP3A2 are not yet known. These effects may result from the soy protein itself or from associated phytochemicals such as isoflavones, saponins, phytosterols or polyphenols. Expression of CYP3A enzymes appears to be under regulation by a number of endocrine systems including growth hormone, insulin and androgens (Ribeiro and Lechner 1992, Waxman et al. 1990, Woodcroft and Novak 1997). Glucocorticoid induction appears to be mediated via a newly described orphan receptor named PXR (Kliewer et al. 1998) and may also involve glucocorticoid receptor–mediated pathways (Huss and Kasper 1998). At present, the endocrine effects of SPI consumption have not been thoroughly examined. However, pure soy-associated isoflavones have been described as estrogenic (Hsieh et al. 1998, Whitten and Naftolin 1992). In this regard, it is interesting that no effects were observed on the expression of hepatic CYP3A9, an enzyme that has been described as female predominant and under positive estrogenic regulation in rat liver (Wang and Strobel 1997). This may be due to the relatively weak estrogen receptor agonist activity of soy-associated “phytoestrogens” compared with 17β-estradiol.

Although soy-associated isoflavones such as genistein and daidzein undergo metabolism primarily by direct glucuronidation and sulfation, it has been reported recently that CYP3A enzymes may also be involved in the metabolism of genistein and possibly other soy-associated isoflavones (Jager et al. 1998). In this study, rat hepatic microsomes were found to convert genistein to three NADPH-dependent metabolites, M1, M2 and M3, and this activity was found to be 10- to 20-fold inducible by treatment with 100 mg/kg of the CYP3A inducer dexamethasone. Moreover, a lower rate of formation of these metabolites was observed in a mutant TR− strain of rats, which expresses lower levels of CYP3A. These data suggest a significant CYP3A/genistein interaction and raise the possibility that genistein consumption as part of the soy diet, [19.3 mg(kg·d)] in this study), may stimulate its own oxidative metabolism, a common phenomenon observed after consumption of other xenobiotics such as phenobarbital and polycyclic aromatic hydrocarbons (Ruckpaul and Rein 1990).

In addition to effects on CYP3A-dependent monooxygenase activities, lifetime dietary consumption of SPI results in a significant enhancement of CYP2B1 expression (Wang and Strobel 1997). The mechanisms underlying the enhanced expression of CYP2B1 are not well understood. Diet and dexamethasone (DEX) effects on CYP2B1 apoprotein expression. Rats were fed casein (CAS) or soy protein isolate (SPI) diets. Panel A: representative blots containing 10 μg of hepatic microsomes were developed with rabbit anti-rat CYP2B1; hepatic microsomes were derived from individual CAS-fed, SPI-fed, dexamethasone (DEX)-treated CAS-fed and DEX-treated SPI-fed rats (n = 3) with 1 μg of microsomes from a CAS-fed, phenobarbital-induced rat included as a positive control for CYP2B1 expression. Panel B: immunoquantitation of CYP2B1 apoprotein expression in microsomes from individual rats in each treatment group. Data are presented as means ± SEM, n = 4–6. Means not sharing a common letter differ significantly, P < 0.05.
resulted in significantly greater CYP2B1-dependent PROD activity and significantly greater glucocorticoid induction of this activity. No significant dietary effects were observed on CYP2B1 apoprotein or mRNA expression. However, the effects may be too small to be demonstrable at the protein or mRNA level with the small number of animals in this study. Again, the mechanisms underlying the effect of dietary SPI on PROD activity remain obscure and may involve actions of the soy protein itself or associated phytochemicals. The mechanism by which glucocorticoids induce CYP2B1 is as yet unknown.

Thus, we have demonstrated significant effects of soy consumption on expression of hepatic CYP3A2 mRNA and CYP3A protein, and significant soy/glucocorticoid interactions on activity of a number of CYP3A and 2B substrates. Although the effects of diet alone were small, challenge with the CYP3A and CYP2B inducer DEX resulted in much greater CYP3A apoprotein expression and microsomal activities toward CYP3A and CYP2B substrates in SPI-fed vs. CAS-fed rats. These differences in vitro microsomal oxidation might be great enough to alter the clearance of these compounds in vivo. However, this remains to be determined empirically and will be the focus of future studies in our laboratory. The CYP3A enzymes are the major human hepatic phase I enzymes expressed; they are rate limiting for the clearance of a wide range of drugs (Wilkinson 1996). Therefore, soy consumption may contribute to the wide interindividual variability seen in expression and activity of this enzyme system. CYP3A enzymes are the major P450 enzymes found in the livers of fetuses and neonates (Schuetz et al. 1994). It remains to be seen whether maternal consumption of vegetarian, soy-based diets during pregnancy or consumption of soy protein isolate–based infant formulas by neonates alters the metabolism and clearance of pediatric medications. Moreover, because drugs are often consumed together, it remains to be seen whether differences in P450 inducibility observed after soy consumption contribute to interindividual variability in potentially toxic drug/drug interactions. In addition, such effects might contribute to the chemoprotective effects of soy consumption against various types of cancer via increased detoxication and clearance of dietary and environmental procarcinogens.

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LITERATURE CITED


