In Vivo Effect of PC-SPES on Prostate Growth and Hepatic CYP3A Expression in Rats

TERI WADSWORTH, HATAYA POONYAGARIYAGORN, ELINORE SULLIVAN, DENNIS KOOP, and CHARLES E. ROSELLI

Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, Oregon

Received January 6, 2003; accepted April 1, 2003

ABSTRACT

PC-SPES, a proprietary mixture composed of eight different herbs, is used worldwide as an alternative treatment by prostate cancer patients. It has been suggested that the clinical and in vitro antitumor activity exhibited by PC-SPES may be due to estrogenic activity, which in turn may be mediated by the presence of undeclared prescription drug contaminants. Here, we evaluated the in vivo effects of two different commercial lots of PC-SPES in male and female rats. Our high-pressure liquid chromatography analysis coupled with gas chromatography/mass spectrometry analysis by an independent laboratory suggested that PC-SPES lot 5430125 was contaminated with diethylstilbestrol (DES), whereas lot 5431249 was not. Treatment of male rats with PC-SPES lot 5430125 or DES alone reduced the weight of androgen target organs and decreased circulating levels of sex steroids and luteinizing hormone, whereas lot 5431249 was without effect. In addition, lot 5430125 and DES, but not lot 5431249 increased uterine weight in female rats. These results suggest that the inhibitory effects on androgen targets are mediated through suppression of the hypothalamic-pituitary axis and this suppression is probably due to DES contamination. We assessed the effects of both lots of PC-SPES and DES on hepatic cytochrome P450 expression and activity. Both lots of PC-SPES and DES reduced CYP3A activity and protein levels. Because the response of CYP3A to PC-SPES was not dependent on whether it contained DES, a phytochemical component of PC-SPES is most likely responsible for this effect. Inhibition of CYP3A has important implications for potential herbal-drug interactions.

In recent years, the use of complementary or alternative medicine by cancer patients has increased dramatically, with herbal medicines being the most common form of alternative treatment (Eisenberg et al., 1998). Rather than replacing traditional therapy, most herbal treatment is used in conjunction with it. Herbal preparations are sold over the counter as dietary supplements, thus prospective regulation by the Food and Drug Administration is not required (Talalay and Talalay, 2001). Therefore, very little scientific information is available regarding effectiveness, mechanism of action, side effects, and the potential for interactions with other medications.

Many components of herbal remedies are potential substrates for cytochrome P450 (P450), a family of enzymes that catalyze the phase I detoxification of both endo- and xenobiotics. Several studies illustrate that constituents from plants modulate P450 enzyme activity. An example is the inhibition of intestinal CYP3A by grapefruit juice, resulting in increased oral availability of drugs such as cyclosporine and felodipine (Bailey et al., 1998). In contrast, St. John’s wort, an herbal remedy commonly used for depression, contains a potent inducer of CYP3A. Repeated administration of St. John’s wort decreases cyclosporin concentrations in transplant patients and reduces plasma concentrations of digoxin and human immunodeficiency virus-1 protease inhibitors in healthy volunteers (John et al., 1999; Mai et al., 2000; Piscitelli et al., 2000). In a screening of 21 commercial ethanolic extracts of commonly used herbal remedies, approximately 75% of the products inhibited CYP3A metabolite formation in vitro (Budzinski et al., 2000). According to a recent review, four of the seven top-selling herbal medicines in this country interfere with the metabolism or efficacy of approved synthetic drugs (Izzo and Ernst, 2001). Together, these findings demonstrate the potential for drug interactions with herbs that are administered concomitantly with other medications and illustrate the need for studies evaluating the effects of herbal medicines on drug metabolizing enzymes.

PC-SPES is a proprietary herbal mixture used as an alter-
native form of treatment by prostate cancer patients. It is composed of the extracts of eight different herbs: *Dendranthera morifolium* Tzvel, *Ganoderma lucidum* Karst, *Glycyrrhiza glabra* L., *Isatis indigotica* Fort, *Panax pseudoginseng* Wall, *Rabdosia rubescens* Hara, *Scutellaria baicalensis* Georgi, and *Serenoa repens* Small. PC-SPES is considered a form of combination therapy, in which individual herbal components are thought to act synergistically to exert antitumor actions (Darzynkiewicz et al., 2000). Individual components of PC-SPES include flavonoids, glycans, phytosterogens, polysaccharides, sterols, fatty acids, and saponins. Several of these compounds exhibit antiproliferative, antitumor, antimutagenic, analgesic, and/or differentiation-inducing activity (Chen, 2001). PC-SPES has been shown to mediate antiproliferative effects on prostate cancer cells in vivo and in vitro (Darzynkiewicz et al., 2000) and to reduce prostate-specific antigen levels in some individuals with prostate cancer (Small et al., 2000; Oh et al., 2001). Although the mechanism of action of PC-SPES has not been fully elucidated, it has been suggested that the clinical and in vitro effects may be attributed to its estrogenic activity. Side effects include nipple tenderness, gynecomastia, and decreased libido (DiPaola et al., 1998). Molecular activities include in vitro down-regulation of the antiapoptotic protein bcl-2, estrogen receptor-α, and androgen receptor (Hsieh et al., 1997; Kubota et al., 2000; Hsieh and Wu, 2002). Although phytosterrogenic components may be responsible for some of these effects, it is also likely that effects may be mediated by the presence of undeclared prescription drug contaminants. In 2002, the Food and Drug Administration warned consumers to stop taking PC-SPES because it contains undeclared prescription drug ingredients, including the synthetic estrogen diethylstilbestrol (DES). The manufacturer voluntarily recalled PC-SPES nationwide (California Department of Health, 2002).

In these studies, we evaluated the in vivo effects of two different commercial lots of PC-SPES in male and female rats. Gas chromatography/mass spectroscopy (GC/MS) analysis of lot 5430125 by an independent laboratory indicates that this lot contains 25 μg/capsule DES (California Department of Health, 2002). Lot 5431249 does not contain this impurity. We evaluated the effects of oral administration of both lots of PC-SPES as well as DES on the weights of the prostate and other androgen target organs in male rats. We measured serum concentrations of steroid and gonadotropin hormones to determine whether PC-SPES acts by suppressing the hypothalamic-anterior pituitary-testicular axis and compared the effect of treatment with PC-SPES and DES on uterine weights to evaluate whether the effects of PC-SPES could be attributed entirely to its estrogenic activity. We also assessed the effects of PC-SPES and DES on hepatic P450 expression and activity to identify potential drug interactions in patients with prostate cancer that use PC-SPES concomitantly with other medications.

**Materials and Methods**

11-α-Hydroxyprogesterone was purchased from Sigma-Aldrich (St. Louis, MO). PC-SPES (lot 5430125 and lot 5431249) was obtained from BotanicLab (Brea, CA). Bio-Rad protein assay and Molecular Analyst software were obtained from Bio-Rad (Hercules, CA). [γ-32P]ATP and GeneScreenJ hybridization transfer membranes were obtained from PerkinElmer Life Sciences (Boston, MA). TRizol® reagent, oligonucleotide probes, and T4 kinase were obtained from Invitrogen (Carlsbad, CA). Supersignal Ultra chemiluminescent substrate was obtained from Pierce Chemical (Rockford, IL). G-25Quick Spin columns were purchased from Roche Diagnostics (Indianapolis, IN). Nitrocellulose membrane was obtained from Schleicher & Schuell (Keene, NH). DES, Denhardt’s reagent, EDTA, and NADP (grade III) were obtained from Sigma-Aldrich. Testosterone (T), dihydrotestosterone (DHT), estradiol (E), 6β-hydroxytestoster-
tone (T-6β-OH), 7α-hydroxytestosterone (T-7α-OH), 16α-hydroxytestosterone (T-16α-OH), and 16β-hydroxytestosterone (T-16β-OH) were purchased from Steraloids (Wilton, NH). Rabbit anti-rat CYP2B1 and CYP3A2 were generously provided by Dr. James Halpert (University of Texas Medical Branch, Galveston, TX). Goat anti-rat CYP4A1 was a generous gift from Dr. Gordon Gibson (University of Surrey Guildford, Surrey, England). Rabbit anti-rat CYP2C11 was provided by Dr. Edward Morgan (Emory University, Atlanta, GA) and rabbit anti-mouse 2A5 was provided by Dr. Xin Xin Ding (Wadsworth Center, Albany, NY).

**Animals.** Forty adult Male Sprague-Dawley rats (200–220 g) and 40 juvenile female (120–140 g) Sprague-Dawley rats, purchased from Simonsen Laboratories (Gilroy, CA), were used in the study. They were maintained on 12-h light and 12-dark cycles and had free access to food and water at all times. Rats were allowed to acclimatize in the facilities for at least 7 days before use.

**Treatments.** Treatments were given orally by gavage in 1.5% carboxymethylcellulose with 0.2% Tween 20 and were performed daily between 8:00 AM and 10:00 AM for 10 days. Rats were weighed and examined daily for general health (morbidity and mortality) and checked for signs of fluid aspiration. All procedures were approved by the Institutional Animal Care and Use Committee of Oregon Health and Science University.

**Effect of PC-SPES on Ventral Prostate Growth and Hormone Secretion.** Male rats were randomized to one of the following treatment groups: 1) vehicle (n = 13), 2) PC-SPES lot 5430125, 50 mg/kg/day (n = 7), 3) PC-SPES lot 5430125, 250 mg/kg/day (n = 7), 4) PC-SPES lot 5431249, 250 mg/kg/day (n = 6), or 5) DES, 37.5 μg/kg/day (n = 7). Rats were exsanguinated by decapitation the morning after the last treatment and trunk blood collected for steroid hormone and gonadotropin determinations. The ventral prostate, liver, seminal vesicles, levator ani, anterior pituitary, adrenal, kidney, and spleen were collected, weighed, and immediately frozen and stored at −80°C.

**Effect of PC-SPES on Uterine Weight (Estrogen Bioassay).** Female rats were ovariec-tomized and allowed to recover for 2 weeks. At the beginning of the 3rd week, female rats were placed on a phytoestrogen-free diet (PMI, St. Louis, MO) and randomized to one of the following treatment groups: 1) control (n = 13), 2) PC-SPES lot 5430125, 250 mg/kg/day (n = 8), 3) PC-SPES lot 5431249, 250 mg/kg/day (n = 6), or 4) DES, 37.5 μg/kg/day (n = 13). The morning after the last treatment, rats were exsanguinated by decapitation and the uterus was dissected and weighed after all fluid was expressed. The degree of uterine growth is proportional to the dose of estrogen exposure (Zarrow et al., 1964).

**Hormone Measurements.** T, DHT, and LH were quantified in serum by radioimmunoassay after extraction with ethyl ether and chromatography on Sephadex LH-20 columns as described previously (Hess and Resko, 1973). Assay extraction efficiency was for 66% for T and 75% for DHT. Intra-assay coefficients of variation were 3.4% for T and 9.9% for DHT. The levels of sensitivity were 2 pg/tube for T and 1.8 pg/tube for DHT. Concentrations of LH were determined by radioimmunoassay using materials supplied by the National Institute of Diabetes and Digestive and Kidney Disease. Reagents were provided by Dr. A. F. Parlow (National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Disease). The standard used in this assay was LH RP-3, and the level of sensitivity was 10 pg/tube. The intra-assay coefficient of variation was 4.7%.
High-Pressure Liquid Chromatography (HPLC) Analysis of PC-SPES. HPLC was used to characterize the composition of the two lots of PC-SPES used in these experiments. Fresh ethanolic extracts were prepared by suspending 300 mg of PC-SPES in 1 ml of 70% ethanol. The suspension was shaken at room temperature for 1 h, centrifuged at 1000g for 10 min, and the supernatant filtered through 0.20-μm nylon membranes. Aliquots (10 μl) of the clarified extracts were injected on an HPLC system (Waters, Milford, MA) consisting of a model 717 plus autosampler, dual 510 solvent pumps, and a model 486 absorbance detector. Components were resolved at room temperature on a 150 × 4.6 mm C18 column (Supelco, Bellefonte, PA) preceded by a 10 × 4.3 mm C18 guard column (Upchurch Scientific, Oak Harbor, WA). A linear gradient from 90% solvent A (H2O with 0.25% glacial acetic acid) to 100% solvent B (acetonitrile with 0.25% acetic acid) was delivered over 60 min at 0.5 ml/min. Absorbance of the eluate was monitored at 254 nm. In addition, 10-μl aliquots of pure samples of DES (40 μg/ml), warfarin (60 μg/ml), and PC-SPES spiked with DES or warfarin were also analyzed with this system. For all HPLC analysis, the Millennium32 software system (Waters) was used for instrument control, data acquisition, and processing.

The stability of the PC-SPES suspensions used for gavage treatments was assessed by extracting 1-ml aliquots of the suspension with 2 volumes of ethyl acetate at the beginning and end of treatment. The organic layer was removed and dried under reduced pressure. The dried extract was dissolved in 100 μl of 70% ethanol, filtered through 0.2-μm nylon filters, and analyzed by HPLC as described above.

Microsome Isolation. Microsomes were isolated from rat livers by differential centrifugation as described previously (Koop et al., 1997). Briefly, liver tissue (250–300 mg) was homogenized in 3.5 volumes of ice-cold homogenization buffer (10 mM Tris-hydrochloride buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA). Samples were then centrifuged in a Beckman TL-100 ultracentrifuge at 10,000g for 20 min at 4°C. The supernatant was collected and centrifuged at 100,000g for 1 h at 4°C. The supernatant was discarded and the pellet resuspended in 1 volume of microsome storage buffer (10 mM Tris-hydrochloride buffer, pH 7.5 containing 20% glycerol). Protein concentrations were determined using the Bio-Rad protein assay, and microsomes were stored at -80°C until assayed.

P450 Activity. T hydroxylation was assayed by HPLC as described previously (Brunner et al., 1998). In brief, reaction mixtures containing 0.1 M potassium phosphate, pH 7.4, 0.3 μg of microsomal protein, 250 μM T (dissolved in methanol, final methanol concentration did not exceed 0.7% v/v) and an NADPH regeneration system consisting of 0.5 mM NADP, 10 mM glucose 6-phosphate, and 10 mM magnesium chloride. The total reaction volume was 1 ml. Mixtures were preincubated at 37°C for 3 min and the reactions initiated by the addition of 5 U of glucose-6-phosphate dehydrogenase. Incubations proceeded for 15 min and were quenched by the addition of 5 ml of dichloromethane. The internal standard (3.6 nmol of 11α-hydroxyprogesterone) was added, the samples vortexed for 30 s, and then centrifuged at 2000g for 5 min at 4°C. The organic layer was removed and dried under reduced pressure. Dried extracts were dissolved in 20 μl of T solvent A (methanol/water/acetonitrile, 39:60:1) and analyzed by HPLC. Extracts (50 μl) were injected on the HPLC system (Waters) described above. Metabolites were resolved at room temperature. A concave gradient (curve 6) from 90% T solvent A to 85% T solvent B (methanol/water/acetonitrile, 80:18:2) was delivered over 22 min at a flow rate of 0.5 ml/min. An 8-min washout of 90% T solvent A preceded each analysis. Absorbance was monitored at 238 nm. T metabolites were quantitated by comparison of peak area ratios (metabolite/internal standard) with those generated with authentic standards. Rates were determined under conditions that were linear with protein and time (data not shown). Chlorzoxazone hydroxylation was analyzed as previously described by Barmada et al. (1995).

Northern Blot Analysis. Total RNA was isolated from rat liver with TRIzol reagent as specified by the manufacturer. RNA was quantified spectrophotometrically (A260), and 30 μg was subjected to Northern blot analysis. Briefly, total RNA was electrophoresed in 1% agarose/15% formaldehyde gels, transferred overnight to GeneScreen membranes, cross-linked to the membrane by UV irradiation, and baked at 80°C for 1 h. Membranes were prehybridized for 1 h at 55°C in hybridization buffer (5× standard saline citrate, 1% SDS, 1× Denhardt’s reagent and 0.5 M sodium phosphate buffer, pH 6.5) and then hybridized overnight in the same buffer containing 1 × 10⁶ cpm/ml [32P]ATP-labeled oligonucleotide probe (Table 4). After hybridization, filters were washed four times, 15 min/wash, in 5× standard saline citrate, 0.1% SDS at 55°C. The presence of comparable amounts of total RNA in each lane was verified by hybridization of membranes with an oligonucleotide probe for 18S rRNA. Radioactivity was detected with a GS-563 Bio-Rad molecular imager with a BI imaging screen and signal intensity quantified with Bio-Rad molecular analyst software.

Western Blot Analysis. Western blot analysis of microsomal fractions was used to screen for the presence of distinct P450 isozymes. Proteins (20 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis using 9% SDS-polyacrylamide gels and transferred to nitrocellulose. Blots were blocked 1 h in blocking buffer (5% nonfat dry milk dissolved in 10 mM Tris-HCl, pH 7.4, 200 mM sodium chloride, and 0.1% Tween 20; TBST). Blocked nitrocellulose blots were incubated 1 h with primary antibody in 1% bovine serum albumin and TBST, washed in TBST, and then incubated 30 min with a 1:10,000 dilution of the appropriate secondary antibody coupled with horseradish peroxidase in blocking buffer. After washing, the immune complexes were detected by enhanced chemiluminescence using Supersignal Ultra chemiluminescent substrate. The following dilutions of the primary antibodies were used: CYP2A1, 1:1,000; CYP3A, 1:10,000; CYP4A1, 1:2,500; CYP2B1, 1:5,000; CYP2C11, 1:600; and CYP2E1, 1:5,000.

Statistics. Data were analyzed using GB-STAT statistical software, version 7.0 (Dynamic Microsystems, Inc., Silver Spring, MD). Tissue weights and hormone concentrations were analyzed by either t tests (comparisons between two groups) or one-way analysis of variance followed by Fisher’s least-square difference post hoc test (comparisons between multiple groups). All Western blot and Northern blot statistics were expressed as percentage of control and analyzed by t tests (two-tailed).

Results

HPLC Analysis of PC-SPES. HPLC was used to characterize the composition of 70% ethanolic extracts of PC-SPES that was administered in these studies (Fig. 1). Lot 5430125 and lot 5431249 exhibited significantly different HPLC profiles. Although the overall pattern of UV-detectable peaks was similar, very striking differences were noted when comparing area ratios for different peaks. Although our analysis did not identify the compound(s) eluting with each peak, the differences we observed suggest that PC-SPES constituents in each lot are present in very different ratios. In addition, lot 5430125 exhibited a unique peak at a retention time of 39 min, which coeluted with DES (DES standard profile not shown). In addition, when the ethanolic extract from this lot was spiked with 40 μg/ml DES, the peak was increased with no evident shoulder. DES contamination in this lot was confirmed by GC/MS analysis by the California Health Department, and the level was estimated to be 25 μg/capsule (California Department of Health, 2002). Another peak at 38 min, present in both lots of PC-SPES, was found to coelute with a pure sample of warfarin (warfarin standard profile not shown). The presence of warfarin in both lots was also con-
Effect of PC-SPES Treatment on Body and Organ Weights. As shown in Table 1, animals treated with 250 mg/kg PC-SPES lot 5430125 or DES exhibited a significant decrease in the ventral prostate and seminal vesicle relative weights compared with control treatment. DES and 250 mg/kg PC-SPES lot 5430125 treatment significantly increased relative kidney weights. In contrast, PC-SPES lot 5431249 had no effect on the relative weights of the ventral prostate or other androgen target organs, yet decreased body weight and relative spleen weight. The weights of the levator ani, anterior pituitary, and adrenal gland were not affected by DES or either lot of PC-SPES.

Effect of PC-SPES on Gonadal Steroids and Gonadotropin Hormone Levels. As shown in Table 2, PC-SPES lot 5430125 at a dose of 250 mg/kg significantly decreased circulating concentrations of LH and showed a trend toward a decrease in T and DHT concentrations compared with control. PC-SPES lot 5431249 had no effect on these hormones. After DES treatment, there was a significant decrease in concentrations of T, a trend toward decreased LH concentrations, but surprisingly, an increase in concentrations of DHT.

Effect of PC-SPES Treatment on Uterine Weight. As shown in Table 3, PC-SPES lot 5430125 but not lot 5431249 exerted significant estrogenic action in the rat uterine weight bioassay. Rats treated with PC-SPES lot 5430125 or DES exhibited a significant increase in uterine weight compared with control treatment, whereas PC-SPES lot 5431249 had no effect. Both lots of PC-SPES and DES decreased body weight compared with control.

Effect of PC-SPES and DES Treatment on Hepatic P450 Activity. The effect of two different doses of PC-SPES lot 5430125, PC-SPES lot 5431249, and DES treatment on microsomal testosterone metabolism is shown in Fig. 2A. Both lots of PC-SPES at a dose of 250 mg/kg and DES at a dose of 37.5 μg/kg significantly decreased the production of T-6β-OH, a functional marker of CYP3A activity. As shown in Fig. 2B, DES significantly inhibited the production of T-16α-OH, an activity catalyzed predominantly by CYP2C11. Neither lot of PC-SPES affected CYP2C11 catalyzed T-16α-hydroxylase activity. No significant treatment effects were observed on the formation of T-16β-OH or T-7α-OH, indicative of no change in the activities of CYP2B or CYP2A1 (data not shown). PC-SPES treatment did not significantly affect chloroxazone hydroxylation, a functional marker of CYP2E1 activity (data not shown).

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>b.wt.</th>
<th>V. Pro</th>
<th>SV</th>
<th>LA</th>
<th>Adrenal</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>253 ± 14</td>
<td>1.16 ± 0.08b</td>
<td>1.38 ± 0.10bc</td>
<td>0.52 ± 0.04</td>
<td>0.037 ± 0.003</td>
<td>0.24 ± 0.02</td>
<td>3.75 ± 0.16a</td>
</tr>
<tr>
<td>5430125 (50 mg/kg, n = 7)</td>
<td>257 ± 11</td>
<td>1.01 ± 0.06a</td>
<td>1.18 ± 0.14cd</td>
<td>0.53 ± 0.03</td>
<td>0.040 ± 0.004</td>
<td>0.22 ± 0.03</td>
<td>4.09 ± 0.06ab</td>
</tr>
<tr>
<td>5430125 (250 mg/kg, n = 7)</td>
<td>249 ± 4</td>
<td>0.83 ± 0.04a</td>
<td>0.96 ± 0.06a</td>
<td>0.40 ± 0.05</td>
<td>0.039 ± 0.003</td>
<td>0.21 ± 0.02</td>
<td>4.18 ± 0.08ab</td>
</tr>
<tr>
<td>DES (37.5 μg/kg, n = 7)</td>
<td>282 ± 13</td>
<td>0.50 ± 0.04a</td>
<td>0.64 ± 0.05a</td>
<td>0.41 ± 0.02</td>
<td>0.038 ± 0.001</td>
<td>0.22 ± 0.01</td>
<td>4.38 ± 0.16ab</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>314 ± 7a</td>
<td>1.09 ± 0.08a</td>
<td>1.41 ± 0.10a</td>
<td>0.65 ± 0.05</td>
<td>0.030 ± 0.001</td>
<td>0.16 ± 0.01</td>
<td>7.45 ± 0.49a</td>
</tr>
<tr>
<td>5431249 (250 mg/kg, n = 7)</td>
<td>270 ± 6b</td>
<td>1.02 ± 0.05a</td>
<td>1.32 ± 0.06b</td>
<td>0.64 ± 0.02</td>
<td>0.031 ± 0.005</td>
<td>0.17 ± 0.01</td>
<td>8.42 ± 0.12a</td>
</tr>
</tbody>
</table>

Apt, anterior pituitary; LA, levator ani; SV, seminal vesicle; V. Pro, ventral prostate.

---

**Fig. 1.** Comparison of HPLC profiles of PC-SPES. HPLC was used to characterize the composition of 70% ethanolic extracts of PC-SPES lot 5430125 (A) and lot 5431249 (B). Although the retention times of most absorbance peaks are similar, each lot exhibits a unique HPLC profile and different constituent ratios. Arrows point to a peak at 38 min that coelutes with warfarin and to a unique peak at 39 min in lot 5430125 that coelutes with DES.

---

**Fig. 2.** Comparison of HPLC profiles of PC-SPES. HPLC was used to characterize the composition of 70% ethanolic extracts of PC-SPES lot 5430125 (A) and lot 5431249 (B). Although the retention times of most absorbance peaks are similar, each lot exhibits a unique HPLC profile and different constituent ratios. Arrows point to a peak at 38 min that coelutes with warfarin and to a unique peak at 39 min in lot 5430125 that coelutes with DES.
TABLE 2
Effect of PC-SPES and DES on gonadal steroids and gonadotropin hormones in male rats (mean ± S.E.)
Data were analyzed by either \( t \) tests (comparisons between two groups) or by one-way ANOVA followed by Fisher’s least-square difference (comparisons between multiple groups). Means with different superscript letters are significantly different, \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hormone</th>
<th>T (ng/ml)</th>
<th>DHT (ng/ml)</th>
<th>LH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td></td>
<td>4.0 ± 1.2a</td>
<td>0.6 ± 0.2a</td>
<td>0.8 ± 0.2a</td>
</tr>
<tr>
<td>5430125 (50 mg/kg, n = 7)</td>
<td></td>
<td>2.5 ± 0.9a</td>
<td>0.3 ± 0.1a</td>
<td>0.5 ± 0.1b</td>
</tr>
<tr>
<td>5430125 (250 mg/kg, n = 7)</td>
<td></td>
<td>1.4 ± 0.4b</td>
<td>0.2 ± 0.1b</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>DES (37.5 μg/kg, n = 7)</td>
<td></td>
<td>0.7 ± 0.4b</td>
<td>0.9 ± 0.03b</td>
<td>0.5 ± 0.06b</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td></td>
<td>4.2 ± 0.9</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>5431249 (250 mg/kg, n = 6)</td>
<td></td>
<td>3.3 ± 0.7</td>
<td>1.1 ± 0.4</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

TABLE 3
Effect of PC-SPES and DES on uterine weights (mean ± S.E.)
Data were analyzed by one-way ANOVA followed by Fisher’s least-square difference. Means with different superscript letters are significantly different, \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>b.wt.</th>
<th>Uterine Weight/b.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>233.5 ± 10.4a</td>
<td>0.37 ± 0.09a</td>
</tr>
<tr>
<td>5430125 (250 mg/kg, n = 5)</td>
<td>178.0 ± 6.2b</td>
<td>1.1 ± 0.1b</td>
</tr>
<tr>
<td>DES (37.5 μg/kg, n = 5)</td>
<td>200.0 ± 5.3b</td>
<td>1.1 ± 0.06a</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>253.0 ± 1.7a</td>
<td>0.52 ± 0.03a</td>
</tr>
<tr>
<td>5431249 (250 mg/kg, n = 6)</td>
<td>196.0 ± 9.8b</td>
<td>0.69 ± 0.07a</td>
</tr>
<tr>
<td>DES (37.5 μg/kg, n = 6)</td>
<td>198.0 ± 2.8b</td>
<td>1.7 ± 0.12a</td>
</tr>
</tbody>
</table>

**Effect of PC-SPES Treatment on P450 Protein.** Western blot analysis of hepatic microsomes was used to monitor the effect of two doses of PC-SPES lot 5430125 (Fig. 3A), PC-SPES lot 5431249 (Fig. 3B), and DES (Fig. 3C) treatment of male rats on CYP3A and CYP2C11 protein levels. Quantification of Western blots is consistent with results from T hydroxylatation. A significant decrease in CYP3A protein was observed in microsomes from rats treated with the higher dose of PC-SPES lot 5430125, PC-SPES lot 5431249, and DES that is consistent with the decrease in T-6α-hydroxylase activity. As predicted from the T-16α-hydroxylase results, PC-SPES did not affect CYP2C11 protein levels. Although not statistically significant, there was a trend toward decreased CYP2C11 protein levels after DES treatment. This loss was more evident in the significant suppression of T-16α-hydroxylase activity. None of the treatments affected levels of CYP2E1 or CYP4A1 protein (data not shown).

**Effects of PC-SPES and DES Treatment on Expression of CYP3A mRNA.** Northern blot analysis using oligonucleotide probes (Table 4) was used to evaluate CYP3A2 (Fig. 4A) and CYP3A18 (Fig. 4B) mRNA expression in the livers of male rats treated with vehicle, PC-SPES lot 5430125, PC-SPES lot 5431249, or DES. The intensities of the 21-kilobase CYP3A mRNA transcripts were normalized to 18S rRNA. Although there was a trend toward inhibition of CYP3A2 expression with all treatments, the decrease was not statistically significant. Neither lot of PC-SPES affected CYP3A18 mRNA levels compared with control. CYP3A9 mRNA was not detected in any of the samples (data not shown).

**Discussion**
In the present study, we evaluated the in vivo effects of two different commercial lots of PC-SPES. Our results demonstrate that PC-SPES lot 5430125 exhibited significant estrogenic activity, but lot 5431249 did not. HPLC analysis identified a peak that coeluted with DES, a potent synthetic estrogen that was present in lot 5430125 only. In vivo treatment with PC-SPES lot 5430125 or DES, but not lot 5431249, stimulated uterine wet growth in male rats. The effect exerted on prostate growth seemed to be mediated through inhibition of the hypothalamic-pituitary-gonadal axis because treatment with PC-SPES lot 5430125 or DES also suppressed serum concentrations of T and LH. Conversely, both lots of PC-SPES used in this study as well as DES significantly decreased CYP3A protein levels and activity, but only DES suppressed CYP3C11 activity. Because the response of CYP3A to PC-SPES was not dependent on whether it contained DES, we conclude that a phytochemical component of PC-SPES other than DES was responsible for this effect.

Previous in vivo and in vitro studies have shown that PC-SPES exhibits estrogenic activity. Clinical studies have noted significant estrogen-like effects when three to nine 320-mg cap-
sules are administered per day (de la Taille et al., 2000; Small et al., 2000). These effects include loss of libido and sexual potency, gynecomastia, and thromboembolism. Di Paola et al. (1998) showed that PC-SPES had estrogenic activity by using transcription activation assays in yeast and uterotropic assays in ovariectomized mice. Our present results, suggesting that the estrogenic activity of PC-SPES is variable and dependent on the particular lot examined, agrees with the recent report by Sovak et al. (2002), which showed that the composition of PC-SPES varies by lot. Chemical analyses detected various amounts of synthetic drugs, including DES as well as several natural products, including licochalcone A.

One explanation for the estrogenic activity of PC-SPES that is consistent with our results is the presence of DES contamination. However, it has also been suggested that this activity is chemically and functionally distinct from DES. For instance, DiPaola et al. (1998) detected an unknown organic substance that coeluted with DES on HPLC, but did not match the retention time of DES, estrone, or estradiol when fractionated with GC/MS. Phytochemical components of PC-SPES, such as licorice root and its derivative licochalcone A, exhibit phytoestrogenic activity (Rafi et al., 2000). LNCaP cells, an androgen-responsive human prostate cancer cell line, exhibits a differential proliferative response to PC-SPES compared with estradiol and different gene expression profile compared with diethylstilbestrol (Bonham et al., 2002; Hsieh et al., 2002). Although our HPLC is in agreement with GC/MS and NMR data from other laboratories (California Department of Health, 2002; Sovak et al., 2002), suggesting that PC-SPES lot 5430125 contains DES, whereas lot 5431249 does not, it is possible that other phytochemical components, acting alone or in synergy with DES, contribute

**Table 4**

<table>
<thead>
<tr>
<th>Target</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A2</td>
<td>5'-CATCTCCTTGAGTCTTCCAC 3'</td>
</tr>
<tr>
<td>CYP3A18</td>
<td>5'-AGCCACGAGCCCTTCAGTGA 3'</td>
</tr>
<tr>
<td>CYP3A9</td>
<td>5'-CTTGCACAGGAGCTGAGAA 3'</td>
</tr>
<tr>
<td>18S</td>
<td>5'-CACCTCTAGCCGCGCAATAC 3'</td>
</tr>
</tbody>
</table>

Effects of both lots of PC-SPES and DES on CYP3A and CYP2C11 protein levels. Hepatic microsomes were examined for levels of CYP3A and CYP2C11 protein by Western blot analysis as described under Materials and Methods. Quantitation of western blots and representative images are shown for A, rats treated with vehicle (n = 7); PC-SPES lot 5430125 (50 mg/kg, n = 7) or PC-SPES lot 5430125 (250 mg/kg, n = 7). B, rats treated with vehicle (n = 6) or PC-SPES lot 5431249 (250 mg/kg, n = 6). C, rats treated with vehicle (n = 7) or DES (37.5 µg/kg, n = 7). Graphs show percentage of control P450 protein expression (bars, S.E., *P < 0.05). Both lots of PC-SPES at a dose of 250 mg/kg and DES at a dose of 37.5 µg/kg significantly decreased CYP3A protein levels. None of the treatments significantly altered levels of CYP2C11 protein.
In Vivo Effects of PC-SPES 193

Fig. 4. Effects of PC-SPES and DES on hepatic CYP3A gene expression. Total RNA was extracted from rat livers and Northern blotting was performed as described under Materials and Methods. A, relative CYP3A2 gene expression in male rats treated with vehicle (n = 7) or PC-SPES lot 5430125 (250 mg/kg, n = 6); and vehicle (n = 7) or DES (37.5 μg/kg, n = 7). B, relative CYP3A18 gene expression in male rats treated with vehicle (n = 7) or PC-SPES lot 5430125 (250 mg/kg, n = 7); and vehicle (n = 6) or PC-SPES lot 5431249 (250 mg/kg, n = 6). Graphs represent quantitation (bars, S.E.) of relative CYP3A mRNA levels normalized to 18S rRNA levels. All treatments showed a trend toward inhibition of CYP3A2 gene expression compared with vehicle alone, but the effects were not statistically significant.

to the profile of estrogenic activity that was observed. Furthermore, the chemical and functional differences that we observed between lots of PC-SPES highlight the problems that arise due to variation in concentration of active ingredients when an herbal preparation is not rigorously standardized and its production is insufficiently monitored.

The statistically significant difference in T levels and relative weights of the ventral prostate and seminal vesicles in male rats treated with DES compared with rats treated with PC-SPES lot 5430125 may be due to a difference in the dose. Although animals treated with DES were given a dose of 37.5 μg/kg, animals treated with 250 mg/kg PC-SPES lot 5430125 theoretically received 20 μg/kg DES (based on an estimated contamination of 25 μg/320-mg capsule). If in addition to DES, lot 5430125 contains higher levels of phytoestrogen, the differential effects could also be attributed to differences in potency between this phytoestrogen and DES. DES treatment did not have a greater effect on relative uterine weight than PC-SPES lot 5430125.

Doses of PC-SPES used in our studies were calculated using an allometric exponent of 0.75 (based on whole body oxygen consumption) (Porter, 2001) and agree with the previous work of Kubota et al. (2000). PC-SPES doses of 50 and 250 mg/kg/day in a 200-mg rat translates to approximately three and 13 capsules of PC-SPES per day, respectively, for a 70-kg human. A rat dose of 37.5 μg/kg DES is equivalent to half the therapeutic dose of DES prescribed for human prostate cancer and reflects a dose of approximately 24 capsules of PC-SPES lot 5430125. These doses of PC-SPES, although higher than those used in clinical trials, are relevant given the fact that PC-SPES is not purchased with a prescription and dose and duration of treatment could therefore be extremely variable.

Although the two lots of PC-SPES exhibited different estrogenic activities, both lots had similar effects on hepatic P450 enzymes. Both lots of PC-SPES and DES reduced total hepatic CYP3A2 activity and protein levels. Although it seemed that there was a trend toward inhibition of CYP3A2 mRNA by both lots of PC-SPES, the effect was not statistically significant. We are therefore not able to confirm whether the inhibition of CYP3A protein by PC-SPES and DES occurred at a transcriptional or post-transcriptional level. To the best of our knowledge, this is the first time that inhibition of CYP3A2 activity by DES has been reported. Although neither lot of PC-SPES or DES alone significantly affected protein levels of CYP2C11, DES significantly inhibited CYP2C11-dependent T hydroxylation.

The inhibitory effect of DES on CYP3A2 and CYP2C11 is consistent with an alteration of the hypothalamic-pituitary axis. Both enzymes are expressed in a male-specific manner, and the sexually dimorphic pattern of growth hormone secretion is thought to play a key role in the regulation of these enzymes (Morgan et al., 1985; Waxman et al., 1995). Both androgens and estrogens can influence growth hormone secretion (Hull and Harvey, 2002). Although GC/MS data suggests that lot 5730125 contains DES, this lot did not exert the same effects on CYP2C11 as DES alone. This apparent inconsistency may be due to the lower dose of DES in PC-SPES lot 5430125 compared with the dose of DES received by rats treated with DES alone.

Because the response of CYP3A to PC-SPES was not dependent on whether it contained DES, a phytochemical component of PC-SPES other than DES is most likely responsible for the inhibitory effect on CYP3A2. PC-SPES is a complex mixture containing the concentrated extracts from eight different herbs. It is comprised of perhaps hundreds of distinct compounds, several of which may contribute to CYP3A2 inhibition. Baicalein and wogonin, major chemical constituents of Scutellaria baicalensis are flavone derivatives that have been shown to decrease hepatic CYP3A protein and activity in vivo (Ueng et al., 2000). Panax ginseng contains saponin constituents (panaxosides and ginsenosides) (Darzynekiewicz et al., 2000). Purified ginsenosides display weak inhibitory activity against recombinant human CYP3A4 (Henderson et al., 1999). Glycyrrhiza contains the phytoestrogens glycyrol, glycyrrin, and glycycomarin (Tanaka et al., 2001). Studies suggest that coumarin and furanocoumarins exhibit inhibitory effects on the in vitro activity of CYP3A in human microsomes (Bailey et al., 1998; Guo et al., 2000). Although many phytochemicals can
CYP3A is the largest subfamily of P450 enzymes expressed in the human liver and gastrointestinal tract. It is involved in the metabolism of endogenous substrates such as sex steroids, bile acids, and retinoids as well as clinically important drugs such as the anticancer drug Taxol, the 5α-reductase inhibitor finasteride, and the antianxiety agents diazepam and triazolam. Inhibition of CYP3A therefore has important implications for potential herb-drug interactions (Thummel and Wilkinson, 1998). Thus, if common regulatory mechanisms exist in humans, PC-SPEs consumption by prostate cancer patients could potentially cause increased bioavailability of coadministered medications. This could be readily examined in vivo using noninvasive assessments of CYP3A4 activity such as the erythromycin breath test (Watkins, 1994) or midazolam clearance (Lin et al., 2001).

In summary, although herbal dietary supplements are often considered by patients to be safe and free from side effects, these studies demonstrate that in addition to the potential risk of interaction between herbal medicines and conventional drugs, the possibility for inconsistent composition, contamination or product adulteration with prescription drugs is possible. Herbal preparations such as PC-SPEs need to undergo rigorous scientific evaluation to assess therapeutic value, safety, purity, and potential for herb-drug interactions.

Acknowledgments

We thank Lisa Bleyle and Henry Stadelman for expert technical assistance.

References