Herbal Composition PC-SPES for Management of Prostate Cancer: Identification of Active Principles

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**Background:** The herbal mixture PC-SPES, used to manage advanced prostate cancer, has proven thrombogenic and highly estrogenic in clinical trials. However, attempts to identify the active compounds in PC-SPES have yielded incongruous results. Moreover, warfarin was identified in the serum of a patient taking PC-SPES who experienced a bleeding disorder. To determine the active components in PC-SPES potentially responsible for these effects, we analyzed PC-SPES lots manufactured from 1996 through mid-2001.

**Methods:** Antineoplastic activity of PC-SPES and its individual component extracts was determined by colony-forming assays with several prostate cancer cell lines, and estrogenicity was determined by analyzing expression of an estrogen-responsive reporter gene in breast cancer cells. High-pressure liquid chromatography was used to isolate, identify, and quantify components of PC-SPES. Components were also identified by proton nuclear magnetic resonance, gas chromatography/mass spectrometry, and mass spectra analysis.

**Results:** PC-SPES lots manufactured from 1996 through mid-1999 contained the synthetic compounds indomethacin (range = 1.07–13.19 mg/g) and diethylstilbestrol (range = 107.28–159.27 µg/g) and were two to six times more antineoplastic and up to 50 times more estrogenic than lots manufactured after the spring of 1999. In lots manufactured after mid-1999, gradual declines in the concentrations of indomethacin (from 1.56 to 0.70 mg/g), diethylstilbestrol (from 46.36 to 0.00 µg/g), and total phytosterols (from 0.586 to 0.085 mg/g) were observed. Warfarin was identified for the first time in lots manufactured after July 1998 (range = 0.586–0.085 mg/g) and were two to six times more antineoplastic and up to 50 times more estrogenic than lots manufactured after the spring of 1999. In lots manufactured after mid-1999, gradual declines in the concentrations of indomethacin (from 1.56 to 0.70 mg/g), diethylstilbestrol (from 46.36 to 0.00 µg/g), and total phytosterols (from 0.586 to 0.085 mg/g) were observed. Warfarin was identified for the first time in lots manufactured after July 1998 (range = 0.586–0.085 mg/g). In the August 2001 lot, increases were found in concentrations of the natural products licorice and baicalin (from 27.6 to 289.2 µg/g) and baicaline (from 12.5 to 38.8 mg/g). The composition of PC-SPES varies by lot, and chemical analyses detected various amounts of the synthetic drugs diethylstilbestrol, indomethacin, and warfarin and several natural products. To qualify for clinical pharmacologic exploration, nutritional supplements including herbal mixtures should meet standards of quality control under the Good Manufacturing Practice system, and the manufacturers of such supplements should provide reliable analytical quality assurance. [J Natl Cancer Inst 2002;94:1275–81]

PC-SPES, an herbal mixture, has been widely used for the management of hormone-responsive and hormone-refractory prostate cancer. A prospective clinical study, conducted by Small et al. (1), found that a dose of nine 320-mg capsules per day reduced levels of prostate-specific antigen by 80% in hormone-responsive (median duration, 57 weeks) and by 54% in hormone-resistant (median duration, 16 weeks) patients. Loss of libido and sexual potency, gynecomastia, cardiovascular side effects, and thromboembolism were seen. Similar findings were reported in another study (2) that used a dose of three 320-mg PC-SPES capsules per day. DiPaola et al. (3) found thrombogenicity to be associated with a decline in serum levels of testosterone and prostate-specific antigen. The authors then showed that capsules of PC-SPES had estrogenic activity by use of transcriptional activation assays in yeast and in ovariectomized mice. They next derivatized the PC-SPES extract and used gas chromatography to analyze the extracts but obtained equivocal results, having detected an unknown organic substance that did not match the retention time of diethylstilbestrol, estrone, or estradiol.

The composition of PC-SPES is declared by the manufacturer to be an herbal mixture of *Ganoderma lucidum* Karst, *Dendranthema morifolium* Tzvel, *Glycyrrhiza glabra* L., *Isatis indigotica* Fort, *Panax pseudoginseng* Wall, *Rabdosia rubescens* Har., *Scutellaria baicalensis* Georgi, and *Serenoa repens* Small. Numerous investigations (4,5) have attempted to link the phytochemicals contained in these plants to the PC-SPES effects by establishing their bioactivity. A PC-SPES extract has been shown to disrupt the cell cycle of the human prostate cancer cell lines PC-3, LNCaP, and DU-145 and the breast cancer MCF-7 line and to have antiproliferative effects on MAT-LyLu tumors (a hormone-resistant prostate adenocarcinoma cell line) in Copenhagen rats (6). Several antiproliferative, antimutagenic, and other antineoplastic mechanisms have been proposed by de la Taille et al. (2). Two principle biologically active substances found in PC-SPES are baicalin (7), which inhibits prostate cancer cells via apoptosis, and licorice (8), an antitumor phytoestrogen with Bcl-2 protein-modulating properties. In these studies we noticed that concentrations of the substances tested were much higher than corresponding concentrations ob-
tained in the clinical trials of PC-SPES (nine 320-mg capsules per day) (9) and were thus unlikely to elicit the clinical effects reported (1,2). Substantial levels of warfarin also were found in serum of a patient taking PC-SPES who suffered a hemorrhagic diathesis (a bleeding disorder in which spontaneous or excessive bleeding occurs often from the gastrointestinal tract or mucosal surfaces and that is associated with deficiencies in clotting factors) (10). These incongruities prompted us to analyze the chemical composition of PC-SPES and to reassess its antineoplastic activity and estrogenicity.

**MATERIALS AND METHODS**

PC-SPES lots 5436285, 5438126, 5438196, 5438362, 5438763, 5430125, 5431106, and 5431219, manufactured from 1996 through mid-2001, were purchased from the manufacturer (BotanicLab, Brea, CA) in sealed bottles as part of ongoing analytical studies and were stored and handled according to Good Laboratory Practices (the approximate manufacturing dates of these lots preceded the label expiration dates by 2 years). Reference standards of diethylstilbestrol (product D-4628, 99% purity), indomethacin (product I-7378, 99% purity), β-sitosterol (product S-9889), and stigmasterol (product S-2424) were obtained from Sigma (St. Louis, MO); baicalin (product 37,575–6) and warfarin (product 25,801–6) were obtained from Aldrich (St. Louis, MO). We synthesized licochalcone A as described (11). All solvents were high-pressure liquid chromatography (HPLC) grade. Individual herbal components of PC-SPES were bought from various commercial sources, extracted, and chromatographed individually and as a mixture.

**Extract Preparation**

Water, ethanol (100%), ethanol (70%), ethanol (50%), toluene, and tetrahydrofuran were explored as extraction solvents for HPLC analysis and preparative isolation. We selected 70% aqueous ethanol or, for extraction of highly hydrophobic substances, toluene. PC-SPES (1.5 g) was sonicated in 10 mL of solvent for 2 hours at 50 °C (50/60 Hz, Bransonic® 221; Bronson Cleaning Equipment Co., Shelton, CT). The centrifuged liquid material was detected at a wavelength of 245 nm. Alternatively, a Labio (Prague, Czech Republic) PS1 120 C18 preparative column (7-μm particle size, 25-mm internal diameter × 250-mm length) was used on an LCP3102 pump/LCD2563 UV detector at 254 nm with acetonitrile/0.001 M formic acid, 1:1 (vol/vol), at a rate of 30 mL/minute.

**HPLC**

For preparative HPLC isolation, we used a Spectra System P2000 pump, a Spectra Physics SP8450 UV/Vis detector (Thermo Separation Products, San Jose, CA), and an Alltima C18 semi-preparative column (10-μm particle size, 10-mm internal diameter × 250-mm length; Alltech, Deerfield, IL) with an acetonitrile/water, 1:1 (vol/vol), mobile phase containing 100 parts per million of acetic acid at a rate of 4 mL/minute. Eluted material was detected at a wavelength of 245 nm. Alternatively, a Labio (Prague, Czech Republic) PS1 120 C18 preparative column (7-μm particle size, 25-mm internal diameter × 250-mm length) was used on an LCP3102 pump/LCD2563 UV detector at 254 nm with acetonitrile/0.001 M formic acid, 1:1 (vol/vol), at a rate of 30 mL/minute.

**Phytosterol Determination**

PC-SPES was initially extracted with toluene, chloroform/methanol (2:1 [vol/vol]), or diethyl ether to determine phytosterol extraction efficiency. With all extraction efficiencies being equal, diethyl ether was chosen and used according to the method described by the International Union of Pure and Applied Chemistry (13). After evaporation of the solvent, residual material was dissolved in 0.5 mL of ethanol containing 1.28 g/mL aqueous potassium hydroxide, heated to 80 °C for 30 minutes, and cooled to 20 °C. Evaporated samples were derivatized with a mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane as described (12) and were analyzed by capillary gas chromatography on an HP-6890 with flame ionization detector (FID; Hewlett Packard) and Supelco (Bellefonte, PA) SAC-5 capillary column (30-m length × 25-mm internal diameter, 0.25-μm particle size) with an internal standard (5α-cholestan) and external calibration.

**Colony-Forming Assay**

For the colony-forming assay (CFA), the content of PC-SPES capsules was extracted in 70% ethanol (250 mg/mL) at room temperature overnight on a roller. The solid phase was pelleted by centrifugation, and the soluble ethanolic extract was removed.
Statistical Analysis

response. defined as the concentration eliciting a 50% maximum observed effective concentration (LOEC). Biologic equivalence was confidence limits did not overlap with those of the control (low-

the lowest effective concentration of PC-SPES at which the 95% Concentration–response curves were plotted (Statistica; Stat-

istics, Tulsa, OK), and the estrogenic potency was expressed as stilbestrol and/or indomethacin content in individual PC-SPES

software, version 1.4; San Diego, CA). All statistical tests

lots was determined by a Pearson correlation test (GraphPad

stilbestrol or indomethacin, are shown. Various PC-SPES lots have shown assorted new peaks of considerable variability and magnitude, as exemplified in Fig. 2. Concentrations of selected natural and synthetic components of PC-SPES in lots manufactured from 1996 through 2001 are shown in Table 2. Lots manufactured from 1996 through mid-1999 contained various quantities of indomethacin (1.07–13.19 mg/g) and diethylstilbestrol (107.28–159.27 μg/g). For those lots manufactured after mid-1999, we observed a gradual decline in the concentrations of these two synthetic compounds, from 1.56 to 0.70 mg/g and from 46.36 to 0.00 μg/g, respectively, and for lots manufactured after July 1998 for the first time, we detected the man-made anticoagulant warfarin (341–560 μg/g). The content of the naturally derived compounds baicalin and licochalcone A varied among these lots. Baicalin concentrations reached a maximum of 38.8 mg/g by the middle of 2001, and a substantial increase in licochalcone A content was observed in lots manufactured from April 2001 through August 2001 (27.6 to 289.2 μg/g). The total phytoestrogen content of PC-SPES was 0.586 mg/g in the older lot 5438763 and 0.085 mg/g in the more recent lot 5431106, a sevenfold decrease. Principle phytoestro-

gens, β-sitosterol and stigmasterol, were present in trace amounts, 0.355 mg/g and 0.026 mg/g, respectively, in lot 5438763 and declined to 0.058 mg/g and 0.007 mg/g, respectively, in lot 5431106. The structure of the synthetic compounds indomethacin, diethylstilbestrol, and warfarin isolated by preparative HPLC from PC-SPES were confirmed by comparison with the mass and proton nuclear magnetic resonance spectra of purchased reference standards and spectral libraries (12), as shown in Fig. 3.

DISCUSSION

PC-SPES is a mixture of seven medicinal herbs used in Chinese and Ayurvedic medicine with added Saw Palmetto ex-

and frozen at –20°C until thawed for use. For CFAs, the PC-SPES Ethanolic extract was reconstituted with cell growth medium (RPMI 1640) with 2 mM glutamine, sodium bicarbon-

ate, HEPES, penicillin [100 IU/mL], streptomycin [100 μg/mL], and 10% fetal calf serum) to a concentration of 1 mg/mL. Various

concentrations of PC-SPES extract were added to cells, and cells were cultured in an atmosphere of 5% CO₂/95% air at 37 °C for 10–14 days until colonies appeared (14). Cells were

fixed and stained with crystal violet, and dose–response curves and the concentration of PC-SPES inhibiting growth by 50% (IC₅₀) were obtained. We used prostate cancer cell lines DU-145 and PC-3 containing mutated androgen receptors (ARs) or no ARs and the prostate cancer cell line LNCaP containing ARs.

Estrogenicity of PC-SPES Extracts

PC-SPES Ethanolic extract from individual lots was prepared as described above. The MVLN cell line was established from estrogen receptor α (ERα) wild-type MCF-7 cells by stable transfection with the luciferase gene under the control of an ERα–responsive element from the Xenopus vitellogenin A2 gene (15). MVLN cells were cultured with PC-SPES extracts, and luciferase expression was measured by chemiluminescence to quantify light emissions as a function of hormone activity, which directly correlates with gene expression. This technique has been applied previously to determine steroid activity in plant extracts (16). The luciferase activity reflects the level of ERe-

mediated activation (15). 17β-Estradiol was used as a reference. Concentration–response curves were plotted (Statistica; Stat-

Soft, Tulsa, OK), and the estrogenic potency was expressed as the lowest effective concentration of PC-SPES at which the 95% confidence limits did not overlap with those of the control (low-

est observed effective concentration). Biologic equivalence was defined as the concentration eliciting a 50% maximum response.

Statistical Analysis

The relationship between the biologic activity and diethyl-

stilbestrol and/or indomethacin content in individual PC-SPES lots was determined by a Pearson correlation test (GraphPad Prism software, version 1.4; San Diego, CA). All statistical tests were two-sided.

RESULTS

The antineoplastic potency of PC-SPES manufactured from June 1996 through August 2001 (Table 1) decreased twofold to sixfold, as measured by the IC₅₀ of PC-SPES ethanol extracts, depending on the cell line. The estrogenicity, expressed by the lowest observed effective concentration of PC-SPES and the biologic equivalence (Table 1), decreased by up to 50-fold (47.5 to 0.9 μg/mL), paralleling a decrease of diethylstilbestrol (154.00 to 0.00 μg/g) and indomethacin (12.24 to 0.70 mg/g) (Table 2) content. Correlation analysis (Fig. 1) identified a highly statistically significant association of CFA activity, estrogenicity, and concentration of diethylstilbestrol in PC-SPES lots (correlation coefficients are listed in Fig. 1). We also found a marginally statistically significant association between CFA and the concentration of indomethacin (P<0.01), which seems to result from the fact that indomethacin values are closely linked to the concentration of diethylstilbestrol in the PC-SPES mixture (P<0.001) (Fig. 1).

Chromatograms of combined individual herbal components did not qualitatively match that of PC-SPES (data not shown). Various PC-SPES lots have shown assorted new peaks of considerable variability and magnitude, as exemplified in Fig. 2. Concentrations of selected natural and synthetic components of PC-SPES in lots manufactured from 1996 through 2001 are shown in Table 2. Lots manufactured from 1996 through mid-

1999 contained various quantities of indomethacin (1.07–13.19 mg/g) and diethylstilbestrol (107.28–159.27 μg/g). For those lots manufactured after mid-1999, we observed a gradual decline in the concentrations of these two synthetic compounds, from 1.56 to 0.70 mg/g and from 46.36 to 0.00 μg/g, respectively, and for lots manufactured after July 1998 for the first time, we detected the man-made anticoagulant warfarin (341–560 μg/g). The content of the naturally derived compounds baicalin and licochalcone A varied among these lots. Baicalin concentrations reached a maximum of 38.8 mg/g by the middle of 2001, and a substantial increase in licochalcone A content was observed in lots manufactured from April 2001 through August 2001 (27.6 to 289.2 μg/g). The total phytoestrogen content of PC-SPES was 0.586 mg/g in the older lot 5438763 and 0.085 mg/g in the more recent lot 5431106, a sevenfold decrease. Principle phytoestro-

gens, β-sitosterol and stigmasterol, were present in trace amounts, 0.355 mg/g and 0.026 mg/g, respectively, in lot 5438763 and declined to 0.058 mg/g and 0.007 mg/g, respectively, in lot 5431106.

The structure of the synthetic compounds indomethacin, di-

ethylstilbestrol, and warfarin isolated by preparative HPLC from PC-SPES were confirmed by comparison with the mass and proton nuclear magnetic resonance spectra of purchased reference standards and spectral libraries (12), as shown in Fig. 3.

Table 1. Antineoplastic effects and estrogenicity of different PC-SPES lots*

<table>
<thead>
<tr>
<th>PC-SPES lot No. (manufacture date)</th>
<th>CFA</th>
<th>Estrogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LNCaP</td>
<td>PC-3</td>
</tr>
<tr>
<td>5438763 (06/1998)</td>
<td>90 (58 to 122)</td>
<td>74 (49 to 99)</td>
</tr>
<tr>
<td>5438362 (03/1999)</td>
<td>105 (70 to 140)</td>
<td>88 (65 to 113)</td>
</tr>
<tr>
<td>5431106 (04/2001)</td>
<td>351 (164 to 537)</td>
<td>490 (445 to 534)</td>
</tr>
<tr>
<td>5431219 (08/2001)</td>
<td>408 (373 to 443)</td>
<td>498 (488 to 508)</td>
</tr>
</tbody>
</table>

*Data are the mean (95% confidence interval). Data for the colony-forming assay (CFA) are expressed as the concentration of PC-SPES (μg/mL) inhibiting CFA by 50% (IC₅₀). For estrogenicity, the concentration of PC-SPES (μg/mL) giving the lowest observed effective concentration (LOEC) and the biologic equivalence (BE), defined as the concentration inducing the same level of luciferase as 17β-estradiol, are shown.
tract that is a widely used food supplement for the management of prostate cancer. Clinical efficacy of PC-SPES in both hormone-resistant and hormone-responsive prostate cancer has been reported (17) but not yet pharmacologically explained.

Our serial lot analyses found that PC-SPES lots manufactured from 1996 through the middle of 1999 contained indomethacin and diethylstilbestrol and that lots manufactured from the middle of 1999 through August 2001 contained progressively lower amounts of both substances and also had correspondingly decreased antineoplastic activity and estrogenicity. Whether the estrogenicity of PC-SPES results from diethylstilbestrol, as suggested by the correlation of its content with in vitro assays, or from the principle phytosterols (β-sitosterol and stigmasterol), baicalin, or licochalcone A requires further study (although only small amounts of these substances are present in PC-SPES). Diethylstilbestrol therapy is associated with gynecomastia, loss of libido, and thrombotic sequelae (18–23). The frequency of these side effects in PC-SPES trials (1,2) is similar to the frequency of those in the earlier diethylstilbestrol trials (22). DiPaola et al. (3) justifiably suspected the presence of a synthetic estrogen in PC-SPES and obtained spectra but, because of the absence of the signature parent ion, did not identify diethylstilbestrol or its derivatized analog. The variability in the content of diethylstilbestrol, which we observed, could explain the results of DiPaola et al. (3), if they tested a PC-SPES lot.

<table>
<thead>
<tr>
<th>Lot No. (manufacture date)</th>
<th>IN, mg/g</th>
<th>DES, μg/g</th>
<th>WA, μg/g</th>
<th>LA, μg/g</th>
<th>B, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>5436285 (10/1996)</td>
<td>1.07 (1.05 to 1.09)</td>
<td>122.35 (121.53 to 123.17)</td>
<td>n/d</td>
<td>48.5 (47.3 to 49.7)</td>
<td>n/d</td>
</tr>
<tr>
<td>5438126 (06/1998)</td>
<td>13.19 (11.95 to 14.43)</td>
<td>114.74 (113.92 to 115.56)</td>
<td>n/d</td>
<td>12.8 (11.8 to 13.3)</td>
<td>21.2 (20.9 to 21.4)</td>
</tr>
<tr>
<td>5438763 (06/1998)</td>
<td>12.24 (11.87 to 12.61)</td>
<td>154.00 (150.12 to 157.88)</td>
<td>n/d</td>
<td>10 (9.5 to 11.0)</td>
<td>7.1 (6.8 to 7.3)</td>
</tr>
<tr>
<td>5438196 (07/1998)</td>
<td>12.81 (11.17 to 14.45)</td>
<td>159.27 (155.99 to 162.55)</td>
<td>560 (555 to 565)</td>
<td>19.0 (18.0 to 20.0)</td>
<td>7.5 (7.2 to 7.8)</td>
</tr>
<tr>
<td>5438362 (03/1999)</td>
<td>3.44 (3.39 to 3.49)</td>
<td>107.28 (105.22 to 109.34)</td>
<td>341 (321 to 361)</td>
<td>3.8 (2.8 to 4.8)</td>
<td>15.0 (14.8 to 15.2)</td>
</tr>
<tr>
<td>5430125 (06/2000)</td>
<td>1.56 (0.74 to 2.38)</td>
<td>46.36 (43.08 to 49.64)</td>
<td>527 (510 to 544)</td>
<td>14.1 (13.6 to 14.6)</td>
<td>12.5 (12.0 to 13.0)</td>
</tr>
<tr>
<td>5431106 (04/2001)</td>
<td>0.70 (0.63 to 0.77)</td>
<td>11.92 (11.10 to 12.74)</td>
<td>398 (378 to 418)</td>
<td>27.6 (26.1 to 29.1)</td>
<td>28.8 (28.5 to 29.1)</td>
</tr>
<tr>
<td>5431219 (08/2001)</td>
<td>0.89 (0.84 to 0.94)</td>
<td>n/d</td>
<td>483 (446 to 520)</td>
<td>289.2 (276.6 to 301.4)</td>
<td>38.8 (36.8 to 40.8)</td>
</tr>
</tbody>
</table>

*Data are the mean (95% confidence intervals). The approximate manufacturing dates of the lots were found to precede the label expiration dates by 2 years. n/d = not detectable.

![Fig. 1. Correlation of antineoplastic potency and estrogenic activity in vitro of different PC-SPES lots. A) A statistically significant inverse correlation between diethylstilbestrol (DES) content and results of colony-forming assays (CFAs) in three cell lines and four PC-SPES lots was found. B) A statistically significant correlation of antineoplasticity, estrogenicity (lowest observed effective concentration [LOEC] and biologic equivalence [BE]), and the concentrations of DES and indomethacin (IN) in different PC-SPES lots was found. All statistical tests were two-sided. r = Pearson correlation coefficient; n = number of analyses in aggregate, representing all cell lines combined (results of estrogenicity versus CFAs of four PC-SPES lots analyzed on three prostate cancer cell lines). P = level of statistical significance.](image1)

![Fig. 2. PC-SPES lots 5438362 (left) and 5431219 (right). High-pressure liquid chromatograms (254 nm) of toluene extracts of PC-SPES show peaks corresponding to the retention times (minutes) of indomethacin, diethylstilbestrol, licochalcone A, and warfarin, in various amounts. The retention time of baicalin is 23.4 minutes, and that of its aglycon, baicalein, is 28.3 minutes (region not shown).](image2)

bestrol, as suggested by the correlation of its content with in vitro assays, or from the principle phytosterols (β-sitosterol and stigmasterol), baicalin, or licochalcone A requires further study (although only small amounts of these substances are present in PC-SPES). Diethylstilbestrol therapy is associated with gynecomastia, loss of libido, and thrombotic sequelae (18–23). The frequency of these side effects in PC-SPES trials (1,2) is similar to the frequency of those in the earlier diethylstilbestrol trials (22). DiPaola et al. (3) justifiably suspected the presence of a synthetic estrogen in PC-SPES and obtained spectra but, because of the absence of the signature parent ion, did not identify diethylstilbestrol or its derivatized analog. The variability in the content of diethylstilbestrol, which we observed, could explain the results of DiPaola et al. (3), if they tested a PC-SPES...
lot containing a low concentration of diethylstilbestrol. Alternatively, the choice of mass spectroscopy settings or analysis of derivatized extracts of PC-SPES rather than the direct analysis of isolated components, as we have done in this study, may have contributed to this discrepancy.

Diethylstilbestrol is effective against both hormone-resistant and hormone-responsive prostate cancer. In trials conducted in the 1970s (22), diethylstilbestrol at a dose of 5 mg/day caused cardiovascular, mainly thromboembolic, side effects and was replaced by luteinizing hormone-releasing hormone agonists and oral androgens. However, diethylstilbestrol at 1 mg/day (23) and even 0.1 mg/day (24) was both efficacious and tolerable. The nine capsules of PC-SPES (2880 mg) per day used clinically (1,2) would have delivered about 0.5 mg of diethylstilbestrol and 30 mg of indomethacin each day. When results of the earlier diethylstilbestrol trials (23) were compared with the results reported for PC-SPES trials (1,2), the times to progression and mean survival times for patients with advanced disease were quite similar. However, in a preliminary report of a head-to-head study of PC-SPES versus diethylstilbestrol in patients with advanced androgen-independent prostate cancer, patients treated with PC-SPES had a greater reduction in prostate-specific antigen levels (25).

Synthetic and natural estrogens induce apoptosis (26). Diethylstilbestrol suppresses serum levels of testosterone better than castration because it reduces the level of serum dehydroepiandrosterone (27). Diethylstilbestrol and indomethacin also inhibit prostaglandin synthesis (28), an event related to apoptosis. Like other nonsteroidal anti-inflammatory drugs, indomethacin has antineoplastic properties (29,30). As a nonspecific inhibitor of cyclooxygenase 2, indomethacin induced apoptosis in athymic murine xenografts of gastric cancer cells (29). In VX2 tumors in rabbits, indomethacin reduced osteoclast proliferation, and indomethacin at 4 mg/kg inhibited bone destruction, indicating...
utility in patients prone to skeletal metastases (30). Indomethacin has also been shown to enhance chemotherapeutic drug efficacy (31).

Warfarin is a drug that requires careful supervision by a physician because individuals have different responses to it. The amounts of warfarin detected in PC-SPES lots manufactured from July 1998 through August 2001 were, on average, 400–500 μg/g, which would amount to about 1.5 mg/day in nine PC-SPES capsules. Warfarin dosing schedules are determined according to individual patients’ responses as measured by prothrombin time testing. Effective doses are usually in the range of 1–7.5 mg/day.

Licochalcone A, a licorice flavonoid, appears to have both antineoplastic and estrogenic activities and has been shown to modulate the expression of Bcl-2 protein in human cell lines derived from patients with acute leukemia, breast cancer, and prostate cancer (8), but its concentration in PC-SPES until recently was too low to be of pharmacologic significance. Whether the 10-fold increase in licochalcone A in the PC-SPES lot manufactured in August 2001 could have any clinical effects is unknown.

Baicalin, a flavonoid from Scutellaria baicalensis, has been a major component of PC-SPES, but its concentration nevertheless increased considerably in the lot manufactured in August 2001. Baicalin was reported to have activity in prostate and myeloblastic leukemia cell lines and to promote the expression of the cyclin-dependent kinase inhibitor protein, p27kip1 (7).

The origin of the three potent synthetic drugs in PC-SPES is puzzling. Diethylstilbestrol had been used in the livestock industry, but the consistently high concentrations of indomethacin and diethylstilbestrol and the presence of warfarin in PC-SPES make an agricultural source improbable, and thus far, the in situ synthesis of these compounds has not been observed in plants. The diethylstilbestrol extracted from PC-SPES is of synthetic origin because it was composed predominantly of the biologically active trans isomer when compared with the proton nuclear magnetic resonance spectra of the reference standard and spectra library (12). As for indomethacin, the condensation of indoles by plants has been described, but the incorporation of a chlorobenzene moiety in plants is not plausible. Warfarin is a synthetic drug. Although licochalcone A is contained in licorice and baicalin is present in Scutellaria baicalensis, their concentrations are too low in these plants (9) to account for the high levels that we found in recent lots of PC-SPES.

Overall, our findings show that diethylstilbestrol and indomethacin were present in PC-SPES produced before 1999 and that the composition of PC-SPES is not consistent. When we compared recent lots with older lots, we detected progressively lower levels of diethylstilbestrol and indomethacin, variable levels of phytoestrogens and warfarin, and higher concentrations of baicalin and licochalcone A. Consequently, we recommend that the efficacy and side effects of PC-SPES be monitored and compared with earlier lots and that physicians and patients taking PC-SPES should be watchful for potential thrombosis and/or bleeding.

In addition, we recommend that the utility of phytoestrogens and possibly other phytochemicals be investigated for the management of prostate cancer. To qualify for clinical pharmacologic exploration, nutritional supplements including herbal mixtures should meet standards of quality control under the Good Manufacturing Practice system, and the manufacturers of such supplements should provide reliable analytical quality assurance.

References

(25) Small EJ, Kantoff PW, Weinberg VK, Nguyen S, Smith M, Bubley


**NOTES**

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