Effects of the Herbal Extract PC-SPES on Microtubule Dynamics and Paclitaxel-Mediated Prostate Tumor Growth Inhibition

Michael J. Bonham, Anna Galkin, Bruce Montgomery, William L. Stahl, David Agus, Peter S. Nelson

Background: PC-SPES is a botanical preparation shown to have efficacy in patients with androgen-dependent and androgen-independent prostate carcinoma. Several herbal constituents in PC-SPES inhibit tumor growth through cell cycle arrest and apoptosis, although the mechanisms of these activities are poorly defined. We sought to identify PC-SPES-induced changes in gene expression, specifically in those genes encoding cytoskeletal proteins that could be associated with PC-SPES-induced cytotoxicity. Methods: LNCAp prostate carcinoma cells were treated with PC-SPES, and changes in gene expression were determined by complementary DNA (cDNA) microarray hybridization and northern blot analyses. PC-SPES and paclitaxel, a microtubule-stabilizing drug, effects on microtubules were assessed by immunofluorescence of treated cells and by in vitro tubulin polymerization assays. In vivo effects of PC-SPES and paclitaxel were assessed using CWR22R androgen-independent prostate cancer xenografts. All statistical tests were two-sided. Results: PC-SPES treatment of LNCAp cells for 24 hours altered the expression of 17 cytoskeletal genes. mRNA levels of α-tubulin decreased sevenfold. Although paclitaxel stabilized and PC-SPES treatment disrupted microtubule architecture in LNCAp cells, the combination of both agents had an intermediate effect. PC-SPES inhibited tubulin polymerization in vitro, even in the presence of paclitaxel. Compared with tumors in control mice (mean tumor volume = 2983 mm³, 95% confidence interval [CI] = 2380 to 3586 mm³), tumors were statistically significantly smaller in mice that received PC-SPES (mean tumor volume = 2018 mm³, 95% CI = 1450 to 2568 mm³; P = .028), paclitaxel (mean tumor volume = 1340 mm³, 95% CI = 697 to 1983 mm³; P < .001), or the combination of PC-SPES and paclitaxel (mean tumor volume = 1955 mm³, 95% CI = 1260 to 2650 mm³; P = .034). Conclusion: PC-SPES may interfere with microtubule polymerization. This activity has implications for the clinical management of patients with advanced prostate cancer who may be taking PC-SPES concurrently with microtubule-modulating chemotherapeutic agents, such as paclitaxel. [J Natl Cancer Inst 2002;94:1641–7]
Cell Culture and Treatments

This study used the LNCaP, DU-145, and PC-3 human prostate cancer cell lines, the T47D and MCF-7 human breast cancer cell lines, the HepG2 human hepatocarcinoma cell line, and the U-87 human malignant glioma cell line. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained according to the supplier’s instructions.

For complementary DNA (cDNA) microarray studies and northern blot analyses, all cell lines were plated at a concentration of $10^6$ cells in 75-cm² flasks. After growing to approximately 50% confluence, the cells were washed and treated with complete medium containing 5 μL/mL PC-SPES or ethanol, which was used as the vehicle control. After various time points, the cells were harvested, and total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s directions. For some experiments, LNCaP cells were treated for 24 hours with 100 nM vinblastine (Sigma Chemical Co., St. Louis, MO), 10 μM doxorubicin (Sigma Chemical Co.), 10 μM DES (Sigma Chemical Co.), 100 nM paclitaxel (Taxol; Bristol-Myers Squibb, Princeton, NJ), or the combination of 5 μL/mL PC-SPES and 100 nM paclitaxel before RNA was extracted.

cDNA Microarray Analysis

cDNA microarrays were constructed as previously described (11). A set of 3000 distinct prostate-derived cDNA clones was identified from the Prostate Expression DataBase (PEDB), a sequence repository of human prostate expressed sequence tag (EST) data available to the public (www.pedb.org) (12). The inserts of individual cDNA clones were amplified by polymerase chain reaction (PCR), purified, and spotted in duplicate onto glass microscope slides (Amersham, Buckinghamshire, U.K.) with a robotic spotting tool (Molecular Dynamics, Sunnyvale, CA).

Probes labeled with Cy3 and Cy5 fluorescent dyes were synthesized by using 30 μg of total RNA from PC-SPES-treated and vehicle-treated cells, respectively, in a reaction volume of 20 μL containing 1 μL of anchored oligo-dT primer (Amersham), 0.05 mM Cy3-dCTP or Cy5-dCTP (Amersham), 0.05 mM dCTP, 0.1 mM each of dGTP, dATP, dTTP, and 200 U Superscript II reverse transcriptase (Invitrogen). Reactions were incubated at 42°C for 120 minutes. After RNA hydrolysis, the labeled cDNA probes were purified, hybridized to the microarray slides, and placed in a humid chamber at 42°C for 16 hours. After the incubation, any unbound probe was removed by washing the slides sequentially with 1x saline sodium citrate (SSC)/0.2% sodium dodecyl sulfate (SDS), 0.1x SSC/0.2% SDS, 0.1x SSC, and water.

For each time point, Cy3-labeled cDNA generated from PC-SPES-treated cells was directly compared with Cy5-labeled cDNA generated from vehicle-treated cells. To control for differential dye incorporation and fluorescence emissions, an additional experiment was performed for each time point in which the cDNA generated from PC-SPES-treated cells was labeled with Cy5 and the cDNA generated from vehicle-treated cells was labeled with Cy3.

The fluorescence intensities, reflecting the labeled cDNA hybridized to the immobilized cDNA clones, were measured with a Gen II slide scanner (Molecular Dynamics). Quantitative data were extracted using the SpotFinder, version 2.4, program (software provided by R. Bumgarner, University of Washington). Local background hybridization signals were measured and subtracted from foreground signals before calculating expression ratios. For each experiment, each cDNA clone was represented twice on each slide, and the experiments were performed in duplicate producing four data points per cDNA clone per hybridization probe. Intensity ratios were calculated for each cDNA clone hybridized with probes generated from PC-SPES-treated and vehicle-treated cells. Gene expression levels were considered to be differentially expressed between the two treatment conditions (PC-SPES versus vehicle) if the intensity ratios from all four replicate data points for a given cDNA had a ratio that was consistently more than 1.5 or less than –1.5 and if the average fluorescence signal was greater than 800 arbitrary intensity units. Selected genes were subjected to hierarchical cluster analysis based on an average linkage-clustering algorithm using Cluster software (13). The graphic display of clustered genes was generated by Treeview software (13).

Northern Blot Analysis

Ten micrograms of total RNA from cells treated as described above (cell culture and treatments) were separated on 1.2% agarose denaturing gels, transferred to nylon membranes by capillary action, and hybridized with a DNA probe for α-tubulin. The probe was labeled with α-32P-dCTP by random priming using the Rediprime II random primer labeling system (Amersham), according to the manufacturer’s recommended protocol. After extensive washings to remove any unbound probe, the filters were imaged and the bound radioactivity was quantified in arbitrary intensity units by using a phosphor-capture screen and ImageQuant software (Molecular Dynamics). To provide loading comparisons between samples, all filters were also stained with methylene blue to identify 28S and 18S ribosomal RNAs. DNA manipulations including transformations, plasmid preparations, gel electrophoresis, and probe labeling were performed according to standard procedures (14).

Immunocytochemistry for the Detection of Microtubules

LNCaP cells, grown on Superfrost+ slides (Fisher Scientific, Pittsburgh, PA) to 50% confluence, were treated with 5 μL/mL PC-SPES, 100 nM paclitaxel, 100 nM vinblastine, or 10 μM doxorubicin for various time periods, washed in cold phosphate-buffered saline (PBS) three times, and then treated with microtubule fixative (0.1 M PIPES [1,4-piperazinediethanesulfonic acid], 4% PEG [polyethylene glycol]-8000, 1 mM EGTA [ethylene glycol bis(β-aminoethyl ether)-N, N', N', N'-tetraacetic acid], 1 mM MgCl₂, 1% Triton X-100, 2% paraformaldehyde) [pH 6.8] for 20 minutes at 37°C. The fixed cells were washed with PBS for 10 minutes at room temperature, washed with PBS for 10 minutes at 4°C, extracted with 100% methanol for 5 minutes at –20°C, and then washed with PBS for 10 minutes at 4°C. The cells were blocked with 5% normal goat serum in PBS for 30 minutes at room temperature and then incubated with the primary monoclonal anti-α-tubulin antibody (clone DM 1A; Sigma Chemical Co.) diluted 1:5000 in 0.1% bovine serum albumin in PBS at 4°C for 16 hours. After extensive washings in cold PBS, the cells were incubated with the secondary antibody solution containing 10 μg/mL goat anti-mouse antibody conjugated with Alexa Fluor® 488 (Molecular Probes, Eugene, OR) and 1 μg/mL TOTO-3 (Molecular Probes), a fluorescently
labeled DNA binding dye, for 1 hour at room temperature. The cells were washed in PBS and mounted with aqueous mounting medium (M01; Biomeka, Foster City, CA) using glass coverslips. Cells were studied by confocal microscopy. The fluorochrome Alexa Fluor® 488 was excited at 488 nm by using an argon laser, and TOTO-3 was excited at 633 nm by using a helium–neon laser.

In Vitro Tubulin Polymerization Assay

Purified tubulin (10 μM) from bovine brain (Molecular Probes) was incubated with 0.8 M glutamate, 4% dimethyl sulfoxide (DMSO), and 1% PC-SPES, 1% paclitaxel, or an equivalent volume of ethanol for 15 minutes at 30°C, in accordance with a previously published method (15). After cooling on ice, 0.4 mM guanine triphosphate (GTP; Sigma) was added to the mixture, and the reaction was transferred to a spectrophotometer cuvette at 4°C. After establishing a baseline reading at 4°C, the temperature was increased to 30°C over 60 seconds and then followed by a 20- to 30-minute incubation at 30°C. Absorbance measurements at 350 nm were acquired at 1-minute intervals during the incubation. Because polymerized tubulin increases the absorbance of the solution, the total amount of polymerized tubulin can be estimated for each assay by plotting the absorbance versus time and calculating the area under the curve (AUC). The experiment was performed in triplicate.

Animal Studies

For experiments involving animals, all procedures were performed in accordance with institutional animal care committee guidelines. Four- to six-week-old nude athymic BALB/c female mice were obtained from the National Cancer Institute and maintained in pathogen-free conditions.

Established androgen-independent CWR22R and MSKPC9 tumor xenografts (16) were propagated in nude mice using the following ratio: one excised 2500 mm³ tumor to five animals. Each animal was injected subcutaneously into the subcapsular area with 500 μL of the prepared tumor suspension consisting of one part minced tumor tissue, one part RPMI-1640 medium (Invitrogen, Carlsbad, CA), and one part reconstituted basement membrane (Matrigel; Collaborative Research, Bedford, MA).

After 5 days, mice with established tumors of approximately 5 × 5 mm³ received either PC-SPES (n = 9; 250 mg/kg, administered orally five times per week in PBS, 1.5% carboxymethylcellulose with 0.2% Tween-20 [Sigma Chemical Co.]), paclitaxel (n = 7; 6.25 mg/kg, administered subcutaneously five times per week), a combination of PC-SPES and paclitaxel (n = 6; 250 mg/kg of PC-SPES and 6.25 mg/kg of paclitaxel, administered five times per week), or vehicle control (n = 9; 100 μL of PC-SPES vehicle, administered orally five times per week, and 100 μL of 0.9% NaCl, administered subcutaneously five times per week). Tumors were measured twice weekly with Vernier calipers, and tumor volumes were calculated by the formula (π/6) × (larger diameter) × (smaller diameter)². Mice were euthanized 24 days after tumor injection.

Cell Growth Assay

LNCaP cells were plated in 96-well culture plates at a density of 5 × 10³ cells per well, allowed to attach overnight, and treated with vehicle control or PC-SPES (concentrations ranging from 0.5 to 10 μL/mL) for 48 and 72 hours. DU-145 cells were plated in a similar fashion and treated for 48 and 72 hours with vehicle control, 0.5–10 μL/mL PC-SPES, 10 nM or 100 nM paclitaxel, or combinations of 0.5 μL/mL PC-SPES and 10 nM or 100 nM paclitaxel. During the last 4 hours of incubation, the medium was replaced with fresh complete medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 1 mg/mL. The MTT reaction was stopped after 4 hours by replacing the medium with 100 μL of isopropanol, and the cells were incubated overnight at 37°C. The conversion of soluble yellow MTT to an insoluble blue formazan product was measured with a μQuant spectrophotometer at 570 nm. The amount of formazan dye is a direct indication of the number of metabolically active cells in the culture (17). Each experiment included eight replicates for each treatment, and each experiment was repeated four times. The results are reported as the average percent change in cell growth for each treatment relative to the vehicle control.

Statistical Analysis

Analysis of variance (ANOVA) was used to assess the overall group mean differences in tumor volume across the four comparison groups on day 24. Equality of variance across the four groups was assessed using Levene’s homogeneity of variance test (18). Contrasts (each with one degree of freedom) were used to compare all treatment groups with the control group. Dunnett’s multiple comparisons procedure (19) was also used to compare all treatment groups with the control group. Dunnett’s procedure was used to create simultaneous (adjusted for multiple comparisons) 95% confidence intervals (CIs). P values were reported for all significance tests. All statistical tests were two-sided.

RESULTS

Microarray Analysis

Because PC-SPES is composed of numerous known and unknown chemical constituents that could induce multiple cellular effects, we hypothesized that assessing cellular gene expression profiles could identify unanticipated molecular alterations resulting from PC-SPES treatment. To test this hypothesis, we performed cDNA microarray analysis to simultaneously measure changes in the expression levels of several thousand genes in response to PC-SPES treatment. LNCaP prostate adenocarcinoma cells were treated with 5 μL/mL PC-SPES for various time periods, a dose that reproducibly killed between 50% and 75% of the cells by 72 hours (data not shown). After LNCaP cells were treated with PC-SPES for 24 and 48 hours, the levels of 156 and 319 distinct transcripts, respectively, were reproducibly altered (6). (Complete microarray data may be viewed at www.pedb.org/PC-SPES.) We detected changes in the expression of genes involved in cell structure (Fig. 1, A), cell cycle control, immune response, cell stress, and androgen regulation (data not shown), providing support for the concept that the complex chemical makeup of PC-SPES may lead to cytotoxicity through multiple mechanisms. PC-SPES was cytotoxic to prostate carcinoma cells (data not shown) and induced changes in cell structure gene expression toward several other cancer cell lines derived from tissues other than prostate, including breast (MCF-7 and T47D), liver (HepG2), and brain (U-87) (Fig. 1, A).

PC-SPES and α-Tubulin Gene Expression

The microarray studies determined that the expression of one gene, α-tubulin, decreased after PC-SPES treatment of all seven
cancer cell lines tested (Fig. 1, A). PC-SPES treatment of LNCaP cells resulted in changes in the expression of 17 additional genes that encode cell structural proteins (Fig. 1, A). Because of the critical roles for tubulin proteins in the process of cell division, and as targets for chemotherapeutic drugs, we further studied tubulin expression by northern blot analysis. Compared with the mRNA levels in control cells, α-tubulin mRNA levels were reduced approximately 2.8-fold and sevenfold in LNCaP cells treated with PC-SPES for 8 and 48 hours, respectively (Fig. 1, B). Similar decreases in β-tubulin mRNA levels were also detected (data not shown). Northern analysis confirmed that α-tubulin mRNA levels were reduced in the nonprostate cell lines after treatment with PC-SPES for 24 hours (Fig. 1, C). These results suggest a possible mechanism to explain a previous report demonstrating cytotoxic effects of PC-SPES toward tumor types other than prostate (7).

Several chemotherapeutic drugs active against prostate cancer—including taxanes, vinca alkaloids, and estramustine—function in part by impairing microtubule organization and dynamics (20). Taxanes such as paclitaxel act as microtubule stabilizers, inducing G2/M cell cycle arrest by promoting microtubule assembly and interfering with microtubule depolymerization (21). By contrast, vinca alkaloids such as vinblastine are known as mitotic spindle poisons because of their ability to inhibit microtubule polymerization (22). The net effect of these antimitotic drugs is cell cycle arrest and often apoptosis in dividing cells.

We next compared the effects of various drugs (PC-SPES, paclitaxel, vinblastine, doxorubicin, and DES) used to treat patients with prostate cancer on α-tubulin mRNA levels in LNCaP cells (Fig. 1, D). Compared with mRNA levels in vehicle-treated control, after 24 hours of PC-SPES treatment, α-tubulin mRNA levels were nearly undetectable. Similarly, vinblastine treatment led to a fourfold reduction in α-tubulin mRNA levels compared with treatment with vehicle control. α-Tubulin mRNA levels were not reduced by more than twofold in cells treated with paclitaxel, doxorubicin, or DES (Fig. 1, D), even though the cells were treated with drug concentrations that resulted in greater than 50% cell growth inhibition (data not shown). Cells treated with PC-SPES and paclitaxel had an intermediate level of α-tubulin mRNA expression. DES is an estrogenic compound used clinically for the treatment of advanced prostate cancer (23). Although one proposed mechanism for PC-SPES activity involves an estrogenic effect (2), PC-SPES and DES clearly had different effects on tubulin expression. Doxorubicin is an anthracycline with cytotoxic activity mediated in part by DNA intercalation (24). The lack of changes in α-tubulin mRNA levels with cytotoxic doses of doxorubicin indicates that the decreased α-tubulin expression is not simply a response to cell death.

**PC-SPES Effects on Cellular Microtubule Structure**

We next visualized cellular microtubule networks by indirect immunofluorescence with an anti-α-tubulin antibody to determine whether changes in tubulin mRNA expression were associated with changes in levels of tubulin proteins and their assembly into microtubules. Nuclear chromatin was simultaneously stained with TOTO-3 to visualize intact nuclei. In untreated LNCaP cells, microtubules formed a fine extensive network throughout the cytoplasm that was generally aligned with the cell axis (Fig. 2, A). By contrast, within 8 hours, microtub-
PC-SPES Effects on Tubulin Polymerization

The immunohistochemical findings prompted us to examine whether PC-SPES could directly interfere with tubulin polymerization, and we tested this hypothesis by using an in vitro assay that measures GTP-induced assembly of purified tubulin monomers. We monitored the polymerization of microtubules by measuring the change in solution turbidity (350 nm absorbance) over time. We tested PC-SPES concentrations ranging from 0.1 to 10 µL/mL and plotted absorbance curves over 20 minutes (Fig. 2, I). Increasing concentrations of PC-SPES led to a progressive decrease in both the rate and overall amount of tubulin polymerization. The effect of PC-SPES on tubulin polymerization in this assay indicates that a compound or compounds within the extract may directly interact with tubulin. We also compared the ability of paclitaxel and the combination of PC-SPES and paclitaxel to alter tubulin polymerization (Fig. 2, J). In agreement with a previous study (25), 10 µM paclitaxel increased both the rate and the overall amount of tubulin polymerization. However, the combination of PC-SPES and paclitaxel reduced tubulin polymerization to 63% (95% CI = 38% to 87%) of the level induced by the paclitaxel alone treatment, suggesting that PC-SPES and paclitaxel have opposite influences on tubulin dynamics.

PC-SPES and Paclitaxel Effects on Prostate Tumor Growth In Vivo

The ability of PC-SPES to disrupt microtubule dynamics suggested that it might interfere with the effects of chemotherapeutic agents that exert their cytotoxic effects by interacting with microtubules. We used the CWR22R prostate cancer xenograft model (26) to evaluate the efficacy of PC-SPES, paclitaxel, and the combination of both agents against prostate tumor growth in vivo. CWR22R tumors were implanted subcutaneously into nude mice, and tumor volumes were measured twice weekly during a 24-day treatment regimen (Fig. 3, A). Compared with tumor growth in control mice (n = 9; mean volume = 2983 mm³, 95% CI = 2380 to 3586 mm³), tumor growth was statistically significantly inhibited in mice that received PC-SPES...
Effects of PC-SPES and paclitaxel on androgen-independent prostate cancer cell growth. A) Androgen-independent CWR22R xenograft prostate tumors were established in female BALB/c athymic nude mice, and the mice were treated five times per week with vehicle control (oral administration, closed squares; n = 9 mice), PC-SPES (250 mg/kg, oral administration; open squares; n = 9 mice), paclitaxel (6.25 mg/kg, subcutaneous administration; closed triangles; n = 7 mice), or a combination of PC-SPES and paclitaxel at the same dosing schedule as single agents (open triangles; n = 6 mice). Results are expressed as mean tumor volume (mm$^3$) versus time after tumor inoculation. Data points represent the mean tumor volume of all treated mice, with upper 95% confidence intervals indicated on the day 24 time points. B) Androgen-independent DU-145 prostate cancer cells were treated with vehicle, PC-SPES (0.5 µL/mL), paclitaxel (10 nM or 100 nM), or combinations of PC-SPES and paclitaxel for 48 hours. Cell growth was measured by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data are expressed as the percentage of cell numbers in the treatment group relative to cell numbers in the control group. Bars represent the mean of four replicates with 95% confidence intervals.

PC-SPES Interference With Paclitaxel-Mediated Cell Growth Inhibition In Vitro

We further studied the ability of PC-SPES to interfere with paclitaxel-mediated cytotoxicity in vitro by using the DU-145 androgen-independent prostate cell line (Fig. 3, B). In dose–response experiments, PC-SPES concentrations of 5 µL/mL and 1 µL/mL for LNCaP and DU-145 cells, respectively, were sufficient to inhibit growth by 50%, indicating that DU-145 cells are more susceptible to PC-SPES cytotoxic effects than are LNCaP cells (data not shown). Thus, in experiments with DU-145 cells, lower doses of PC-SPES were used in combination experiments with paclitaxel to avoid simply observing PC-SPES-mediated cytotoxicity. DU-145 cells were treated with 0, 10, or 100 nM paclitaxel for 48 hours in the presence or absence of 0.5 µL/mL PC-SPES. The growth of DU-145 cells treated with 0.5 µL/mL PC-SPES was 54% (95% CI = 50% to 58%) that of the cells treated with the vehicle. After 48 hours, the growth of cells treated with 10 nM or 100 nM paclitaxel was reduced to 33% (95% CI = 22% to 45%) and 25% (95% CI = 23% to 27%), respectively, that of cells treated with the vehicle (Fig. 3, B). The growth of cells treated with the combination of 0.5 µL/mL PC-SPES and 100 nM paclitaxel was reduced to 40% (95% CI = 38% to 43%) that of cells treated with the vehicle. This result represents a 61% increase in tumor cell growth relative to cells treated with 100 nM paclitaxel alone (Fig. 3, B)—a finding that further supports the possibility that components of PC-SPES may attenuate paclitaxel-mediated cytotoxicity.

Discussion

In vitro and in vivo studies suggest that PC-SPES influences multiple biochemical pathways (1,2,6,27). In this investigation, we sought to determine molecular mechanisms of action that could contribute to the antitumor activity associated with PC-SPES. We determined that PC-SPES treatment markedly decreased the expression of genes encoding α- and β-tubulins. Tubulins are the monomers that assemble into microtubules, which are essential for maintaining cell shape, cell transport, cell motility, and cell division (28). Several chemotherapeutic drugs active against prostate cancer, including the taxanes and estramustine, function in part through the impairment of microtubule organization and polymerization (21,29). It is possible that the inhibition of microtubule formation or a reduction of cellular tubulin monomers by PC-SPES could modify the efficacy of these and other tubulin-modulating drugs. The extensive use of taxanes and estramustine for the treatment of advanced prostate cancer, coupled with the prevalent unregulated and often unrecognized use of PC-SPES, implies that many patients will be exposed concurrently to these agents.

The consequences of combining herbal medications and cytotoxic chemotherapeutics have been largely unexplored. Our results suggest that PC-SPES and paclitaxel may have conflicting effects if administered together in the clinical setting. The combination of PC-SPES and paclitaxel reduced the effectiveness of paclitaxel treatment in both androgen-sensitive and androgen-independent models of prostate carcinoma. The microtubule network in LNCaP cells treated with both agents appeared largely intact, suggesting that the microtubule-inhibitory activity of PC-SPES interferes with the microtubule-stabilizing activity of paclitaxel. Previous studies have examined combinations of taxanes with specific vinca alkaloids and demonstrated that such combinations can be antagonistic or synergistic, de-
pending on the cell type or timing of treatments. When paclitaxel and vinblastine were administered sequentially to breast carcinoma cells in vitro, each drug retained its microtubule-altering activity, and the two drugs exhibited synergistic cytotoxicity (30). However, when added simultaneously, the maximal effect of each drug was reduced, antagonizing the antitumor activity. Other reports support the concept that the timing of paclitaxel and vinca alkaloid drug combination therapy is critical for therapeutic efficacy (31,32). Additional studies will be required to determine whether a sequential schedule of PC-SPES and paclitaxel administration could produce synergistic or additive effects.

In summary, the molecular and biologic activity of PC-SPES show similarity to those of vinblastine. Further purification of PC-SPES to its active component(s) may yield a vinca alkaloid-like compound or possibly a novel class of tubulin-modulating agents. The demonstrated effectiveness of PC-SPES as a treatment for prostate cancer warrants additional trials with therapeutic compounds that operate through complementary rather than antagonistic mechanisms. In addition, the studies reported here provide a cautionary note emphasizing the potential hazards of combining complex poorly-defined botanical compounds with conventional medical therapies.

References


Notes

Note added in proof: Following the submission of this manuscript, a study by Sovak et al. (33) evaluated the active principles in various lots of PC-SPES, including one of those used in the study. In addition to very low levels of DES in this particular lot, other agents were identified that could possibly be responsible for some of the effects seen.

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