Estrogen Effects on Tubulin Expression and Taxane Mediated Cytotoxicity in Prostate Cancer Cells

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BACKGROUND. The present study was designed to determine if estrogens change microtubule polymerization and modulate cell cycle progression in vitro, related to modulation of tubulin expression and to determine if estrogens had antagonistic or synergistic effects with microtubule active agents.

METHODS. cDNA array analysis of LNCaP cells treated with the estrogens, estradiol, estrone, diethylstilbestrol (DES), and 2-methoxyestradiol (2-ME) was carried out and the results confirmed by PCR and Western blotting. Microtubule arrays in cells treated with estrogens were assessed using indirect immunofluorescence. The effects of combining estrogens with taxane was assessed by MTT assay and flow cytometry for cell cycle kinetics. Human prostate cancer xenografts were treated with DES and docetaxel to assess the effects of combining estrogens and taxane in vivo.

RESULTS. Treatment of LNCaP cells with DES and 2-ME suppressed transcripts and protein for β-tubulin isotype IVa. This effect on tubulin synthesis was not blocked by estrogen or androgen receptor modulators. Other estrogens had no effect on β-tubulin expression. 2-ME and DES decreased the density of microtubules. The administration of DES or 2-ME with paclitaxel enhanced cytotoxicity and G2-M arrest in vitro. DES enhanced tumor suppression in a human prostate cancer xenograft model when combined with the taxane docetaxel.

CONCLUSION. The use of DES and 2-ME enhances the effects of taxanes and may be a novel and important means of increasing therapeutic efficacy of cytotoxic chemotherapy against prostate carcinoma.

KEY WORDS: prostate cancer; tubulin; estrogen; paclitaxel; diethylstilbestrol

INTRODUCTION

Estrogens are important therapeutic agents for the treatment of prostate cancer. The original report by Huggins and Hodges [1] which established the testosterone dependence of prostate cancer also documented the efficacy of estrogen administration for the treatment of patients with metastatic disease. The synthetic ethinyl estrogen, diethylstilbestrol (DES), continues to be a standard for defining the efficacy of hormonal therapy in the treatment of androgen dependent prostate cancer, and DES also exhibits significant activity in androgen independent disease [2,3]. DES and the estrogen metabolite 2-methoxyestradiol (2-ME) modulate microtubule dynamics in prostate cancer cells. DES has concentration dependent effects on tubulin polymerization in vitro which are

Abbreviations: DES, diethylstilbestrol; 2-ME, 2-methoxyestradiol.
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independent of effects on the androgen axis. Sharp et al. demonstrated that DES at concentrations of 10^{-6}–10^{-3} M accentuated polymerization of purified tubulin, however, at 10^{-3} M, DES blocked formation of microtubule arrays [4]. Robertson et al. [5] showed that DES induced apoptosis in androgen independent prostate cancer cell lines in vitro in an estrogen receptor independent manner, and that this apoptosis correlated with cell cycle arrest at G2-M. 2-ME, another cytotoxic estrogen suppresses microtubule polymerization and has substantial cytotoxicity against prostate cancer cell lines, while also blocking cells at G2-M [6,7]. Advanced prostate cancer appears to be singularly sensitive to agents which modulate microtubule arrays, such as the taxanes docetaxel and paclitaxel [8]. Taxanes kill cells by inducing cell cycle blockade at G2-M and the use of agents which accentuate that G2-M arrest or which modulate the taxane mediated stabilization of microtubules would be expected to improve sensitivity to taxane. Little is known about how estrogens might modulate the efficacy of other microtubule active agents. This study was undertaken to define the effects of cytotoxic and non-cytotoxic estrogenic agents on microtubule related genes and proteins in prostate carcinoma and to evaluate in vitro and in vivo effect when combined with taxane chemotherapy.

MATERIALS AND METHODS

Cell Culture

The LNCaP prostate carcinoma cell line was cultured in dye-free RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Rockville, MD). For evaluation of estrogen exposure on gene expression, medium was changed to RPMI 1640 medium with 10% charcoal stripped FBS (CS-FBS) (Gemini Biosystems, Woodland, CA) 24 hr before treatments. Cells were then supplemented with DES 5 and 10 μM, estradiol 10 μM, and estrone 10 μM for 48 hr. Cells were harvested and RNA isolated using TRIzol (Life Technologies) according to the manufacturer’s directions. Cells were also treated with DES, estrone, estradiol at the same concentrations and 2-ME (1 μM, Sigma, St. Louis, MO) or ethanol control, with or without ICY 182,780 (1 μM, Tocris, Ellisville, MO) or hydroxyflutamide, 1 μM. Cells were harvested for RNA or protein isolation at indicated time points. Paclitaxel was from Sigma and docetaxel was kindly provided by Aventis (Bridgewater, NJ).

Microarray Fabrication, Probe Construction, and Hybridization

cDNA microarrays were constructed as previously described [9]. Briefly, a non-redundant set of 3,000 distinct prostate-derived cDNA clones was identified from the Prostate Expression DataBase (PEDB), a public sequence repository of expressed sequence tag (EST) data derived from human prostate cDNA libraries. Individual clone inserts were amplified by the PCR, purified, and spotted in duplicate onto Type IV glass microscope slides (Amersham, UK) using a GenII robotic spotting tool (Molecular Dynamics, Sunnyvale, CA). Fluorescence-labeled probes were made from 30 μg total RNA in a reaction volume of 20 μl containing 1 μl 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 Super script II reverse transcriptase (Life Technologies). Reactants were incubated at 42 °C for 120 min followed by the hydrolysis of RNA and cDNA probe purification by chromatography (Qiagen, Valencia, CA) as previously described [9]. Labeled probes were placed onto a microarray slide with a coverslip, hybridized in a humid chamber at 52 °C for 16 hr, and washed with SSC gradients. Cy3-labeled cDNA from treated cells was directly compared against Cy5-labeled cDNA from the vehicle-treated control at each time point. Fluorescent dye labeling was reversed and a replicate experiment was performed for each sample to control for dye-effects. Fluorescence intensities of the immobilized array targets were measured using a Gen II slide scanner (Molecular Dynamics). Quantitative data were obtained with the SpotFinder V 2.4 program. Local background hybridization signals were subtracted prior to comparing spot intensities and determining expression ratios. Intensity ratios for each cDNA clone hybridized with treated and control probes were calculated. Gene-expression levels were considered significantly different between the two conditions if replicate spot ratios for a given cDNA demonstrated a ratio >1.5 or <−1.5 and the average signal was greater than 800 intensity units.

β-Tubulin Isotype Analysis by RT-PCR

RT-PCR analysis for tubulin isotypes was carried out essentially as previously described [10]. The gene specific oligonucleotide primers for isotype classes I–III were as published by Kavallaris et al. [11], and isotypes IVa and IVb were as published by Ranganathan et al. [12] (Table I). Total RNA isolated from LNCaP cells was treated with RNase-free DNase to remove contaminating genomic DNA [13]. Reverse transcription of 2 μg of total RNA was performed to synthesize cDNA using specific primer pairs. PCR reactions were carried out with 50 ng of cDNA, and products were amplified for 35 cycles in a programmable thermocycler. Effective removal of genomic DNA was verified by amplification of intron spanning...
Transcripts were >1.5-fold changed in duplicate arrays. Genes selected showed an average ratio of at least 1 SD greater than 1.5-fold over the control. The fold increase or decrease in transcript abundance relative to the corresponding control experiment is shown. Estradiol and estrone showed no change in transcripts for tubulin or microtubule related genes.

GAPDH primers. Isotype classes were quantified by comparing intensity of PCR products generated in the linear range of amplification to 18S RNA (from parallel samples). PCR products were electrophoresed on 6% TBE gels and visualized by ethidium bromide staining. Densitometric measurements were performed on negatives of the gels. The value of the band of interest was divided by the value of the control band (18S RNA). Data for the figures were expressed as the signal intensity ratios compared to 18S. Because isotype III levels were consistently undetectable, the results were not plotted. The assays were performed in triplicate and standard error of the mean determined.

### Western Analysis

Thirty micrograms of protein were loaded into a precast 8%–16% gel (Invitrogen) run and transferred to nitrocellulose. The membranes were blocked overnight at 4°C in 5% milk/PBS. Anti-tubulin antibodies were added at a 1:1,000 dilution for 1 hr in 3% BSA/PBS. Anti-mouse HRP antibody (Pierce) was added at 1:1,000 dilution for 1 hr in 3% BSA/PBS. TBE gels and visualized by ethidium bromide staining. Densitometric measurements were performed on negatives of the gels. The value of the band of interest was divided by the value of the control band (18S RNA). Data for the figures were expressed as the signal intensity ratios compared to 18S. Because isotype III levels were consistently undetectable, the results were not plotted. The assays were performed in triplicate and standard error of the mean determined.

### Immunocytochemistry

LNCaP cells grown on Superfrost+ slides were washed in cold PBS three times and treated with microtubule fixative (0.1M PIPES, 4% PEG-8000, 1 mM EGTA, 1 mM MgCl₂, 1% Triton X-100, 2% paraformaldehyde (pH 6.8) for 20 min at 37°C. Slides were washed with PBS for 10 min at room temperature and 10 min in cold PBS, extracted with methanol at –20°C for 5 min and washed for 10 min in cold PBS. Slides were blocked with 5% normal goat serum in PBS for 30 min at room temperature. Slides were incubated with the primary, monoclonal anti-α-tubulin antibody (Sigma, DM 1A) (1:5,000) in 0.1% bovine serum albumin in PBS at 4°C. Slides were washed three times in cold PBS and incubated at room temperature for 1 hr with 10 μg/ml goat anti-mouse Alexa 488 (Molecular Probes) as a secondary antibody. The secondary antibody solution also contained 1 μg/ml TOTO-3 (Molecular Probes). Slides were washed in PBS and mounted with aqueous mounting medium (Biomed, M01) using glass coverslips. Cells were studied by confocal microscopy: Alexa 488 was excited at 488 nm using an argon laser, and TOTO-3 at 633 using a helium–neon laser.

### Cell Proliferation Assay

Cells were incubated for 48 hr in 96-well plates with the test compounds. Cell proliferation was measured by replacing the culture media with RPMI media containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) at 1 mg/ml. Isopropanol was added after a 4 hr incubation and cells were left overnight at 37°C. The conversion of yellow MTT to a blue formazon dye product was measured with a spectrophotometer at 570 nM. The amount of formazon dye is a direct indication of the number of metabolically active cells in the culture. Each data point represents the average of four separate experiments containing 8 wells for each experimental condition.

### LuCap 35 Xenografts in Nude Mice

Four to 6-week-old male BALB/c athymic (nu/nu) mice were purchased from Charles River Laboratories. All procedures were performed in compliance with the University of Washington Institutional Animal Care and Use Committee and NIH guidelines. The LuCap35 human prostate cancer xenograft model has been described by Corey and Vessella [14]. LuCap 35 tumor bits (~25 mm³) were implanted s.c. Tumor growth was monitored by measuring tumor volume three times per week. Tumor volume was calculated as length × height × width × 0.5236. Once tumors reached 250 mm³, treatment was initiated. Animals were then treated with control vehicle (n = 8), DES (n = 7, 1 mg/kg SC three times a week for 4 weeks), docetaxel (n = 7, 0.5 mg IP q week × 2 then 0.25 mg IP q week × 2) or DES plus docetaxel (n = 6). Once the tumors reach 1,000 mm³ the experiment was concluded. The significance of differences in tumor growth rate was determined using Student’s unpaired t-test.

### Flow Cytometry

To determine cell cycle changes, cells were cultured according to the treatment schedule noted above. Cells were seeded at 2.5 × 10⁶ cells/75 cm² plate in 15 ml of complete medium and then placed in serum containing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change</th>
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<tbody>
<tr>
<td>β-Tubulin-isotype IVa</td>
<td>–1.78</td>
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<tr>
<td>β-Tubulin-isotype I</td>
<td>–2.38</td>
</tr>
<tr>
<td>γ-Tubulin</td>
<td>–2.52</td>
</tr>
<tr>
<td>γ-Tubulin</td>
<td>–2.72</td>
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### Table I. Alterations in the Expression of Tubulin Related Genes Resulting From DES Exposure

<table>
<thead>
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<th>Gene</th>
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<td>–2.52</td>
</tr>
<tr>
<td>γ-Tubulin</td>
<td>–2.72</td>
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10% charcoal stripped FBS for 24 hr. Cells were then treated with agents at their IC<sub>50</sub> concentration—paclitaxel (2.5 nM), DES (5 μM), 2-ME (1 μM) or the combinations for 48 hr. Cells were harvested after treatment (day 2) and fixed in 95% ethanol. For PI staining, samples were washed with PBS, resuspended in PI solution (1 mg/ml RNase A, 33 μg PI/ml in PBS, 0.5 mM EDTA, and 0.2% NP40), and then incubated at room temperature for 30 min. Flow cytometry was performed using a Becton Dickinson FACS Calibur Cytometer. Flow cytometry data were analyzed for cell cycle profiles using Multicycle LT version 3.0 software (Phoenix Flow Systems, San Diego, CA).

### Statistical Analysis

The dose–response interactions between paclitaxel and DES and 2-ME were evaluated utilizing CalcuSyn (Biosoft, Ferguson, MO) based on the method of Chou and Talalay and expressed in combination index format [15]. The IC<sub>50</sub> was defined as the concentration of drug that produced 50% cell growth inhibition, i.e., 50% reduction in absorbance. Cells were exposed to drugs at 0.25, 0.5, 0.75, and 1 x the IC<sub>50</sub> concurrently for 48 hr, and cell viability was determined by the MTT assay. Because estradiol and estrone did not inhibit LNCaP growth, they were not evaluated in detail.

### RESULTS

#### DES Induced Changes in Prostate Gene Expression

We performed cDNA microarray analysis on LNCaP cells to determine alterations in hormone dependent prostate cancer cell gene expression resulting from exposure to estrogens. After 48 hr of exposure to DES, 77 genes exhibited a ≥1.5-fold increase and 115 genes exhibited ≥1.5-fold suppression in expression level compared to controls. Twenty-nine genes exhibited a ≥2-fold increase and 34 genes exhibited ≥2-fold suppression. Among the genes suppressed over 2-fold by DES, tubulin genes were prominent, making up 4 of the 25 identified signals. The tubulin genes whose expression was suppressed were β-tubulin isotypes I, Iva, and α-tubulin isotypes I and IV (Table I). In addition, glyceraldehyde-3-phosphate dehydrogenase, which is a microtubule associated protein and plays a role in bundling of microtubules [16] was suppressed. Estradiol and estrone treatment had no effect on any microtubule or tubulin related genes (data not shown). We subsequently analyzed mRNA and protein levels of the tubulin isotypes in order to confirm the cDNA array analysis.

#### DES and 2-ME Suppress β-Tubulin Isoype Transcripts

To confirm microarray measurements of tubulin transcript alterations by DES we performed semi-quantitative PCR to estimate suppression of the distinct β-tubulin isotypes by estrogens. Differential expression of β-tubulin isotypes has been proposed as a mechanism for either de novo or acquired taxane resistance [17]. At least seven β-tubulin isotypes have been identified, constituting five evolutionarily conserved isotype classes. These differ primarily in the carboxy-terminal 15 amino acids. Microtubules selectively enriched for isotype class III and IV β-tubulin are significantly more resistant to paclitaxel suppression of microtubule dynamics than are microtubules composed of unfractionated tubulin [18]. RNA levels for class I-IVb β-tubulin were determined using semi-quantitative RT-PCR as previously described [10]. The primer sets used for these assays are shown in Table II. Tubulin isotype RNA levels were normalized to PCR products for 18S RNA in each gel. Intensity of bands for β-tubulin isotypes in cells treated with DES and 2-ME were quantitated (Fig. 1B). The LNCaP cells treated with vehicle control, estradiol and estrone in charcoal stripped medium showed similar amounts of β-tubulin I-IVb with very low levels of class III (Fig. 1A, type III not shown). Cells treated with DES (5 μM) and 2-ME (1 μM) showed significant suppression of class IVa tubulin transcripts.

#### DES and 2-ME Suppress β-Tubulin Isoype I/II and IV Protein Expression

To confirm the magnitude and specificity of DES and 2-ME suppression of tubulin levels, we performed

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### TABLE II. Primers for RT-PCR Amplification of β-Tubulin Isootypes

<table>
<thead>
<tr>
<th>Tubulin Isotype class</th>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>β&lt;sub&gt;I&lt;/sub&gt;</td>
<td>HM40</td>
<td>5'-ACCTCCGTCTGCTCCAGCCTCT-3'</td>
<td>5'-CGGCCCTTGATGTCAGCAT-3'</td>
</tr>
<tr>
<td>β&lt;sub&gt;II&lt;/sub&gt;</td>
<td>Hβ9</td>
<td>5'-CGCATCTCGAGCAGCTTAC-3'</td>
<td>5'-TCGCCCTTCCCTCTCTA-3'</td>
</tr>
<tr>
<td>β&lt;sub&gt;III&lt;/sub&gt;</td>
<td>Hβ4</td>
<td>5'-CTGCTCGAGCAGCTGAG-3'</td>
<td>5'-CATATACTGAGCAGGCCC-3'</td>
</tr>
<tr>
<td>β&lt;sub&gt;IVA&lt;/sub&gt;</td>
<td>Hβ5</td>
<td>5'-AAGTAGCAGGAGGTAAGAGG-3'</td>
<td>5'-CAACAGACAGCAGCATCAGAC-3'</td>
</tr>
<tr>
<td>β&lt;sub&gt;IVB&lt;/sub&gt;</td>
<td>Hβ2</td>
<td>5'-CTTCTCCTGCAGCTGTAAG-3'</td>
<td>5'-CAACAGACAGCAGCATCAGAC-3'</td>
</tr>
<tr>
<td>18S RNA</td>
<td></td>
<td>5'-ATGCTCTTAGCTGAGTGTC-3'</td>
<td>5'-AAGTACAGAGGTATATGATC-3'</td>
</tr>
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</table>
Western blotting with antibodies to β-tubulin isotype classes. These antibodies cannot distinguish between β-tubulin isotype classes IVa and IVb because of the very high homology of the amino acid sequence and Western analysis therefore reflects total isotype class IV. In addition, the antibody used in this experiment does not distinguish between β-tubulin isotype classes I and II. To determine if the DES and 2-ME effects were mediated through hormone receptor dependent or independent effects, LNCaP cells were treated with the same concentrations of estrogens with or without antagonists to the estrogen receptor and the androgen receptor. The LNCaP cell line contains a mutation in codon 877 of the androgen receptor which confers the ability to bind estrogens as well as androgens. LNCaP cells also express low levels of estrogen receptor [19]. For this reason inhibitors to both the estrogen receptor (ICI 182,780) and a control for activation of the androgen receptor (hydroxyflutamide) were included as controls with DES and 2-ME. Control treatment, estradiol and estrone did not substantially suppress protein levels for α- or β-tubulin, and there is a suggestion that estradiol may increase levels of β-tubulin isotype class I (Fig. 2). DES suppressed isotype class IV protein substantially by 12 hr. 2-ME inhibited both isotype class I/II and IV protein expression to a similar degree. Expression of isotype III protein was tested under the same conditions and consistent with the PCR results, no protein could be detected. Of interest is the observation that total β-tubulin levels were not significantly suppressed. This suggests that the total amount of tubulin is not substantially diminished by treatment with DES or 2-ME, but rather that there is selective suppression of specific isotypes. The addition of estrogen antagonists and androgen receptor agonist had no effect on suppression of tubulin isoforms, suggesting that the effect of these estrogens occurs independently of hormone receptors.

**Treatment With DES and 2-ME Disrupts Microtubule Arrays**

The suppression of tubulin isotype levels could potentially mediate a variety of changes in cellular...
microtubule networks. To assess how DES and 2-ME inhibition of tubulin levels affects microtubule arrays in cells, we performed indirect immunofluorescence using an α-tubulin antibody. Untreated cells had extensive fine microtubules throughout the cytoplasm which were generally aligned with the cell axis (Fig. 3A). LNCaP cells exposed to 2-ME 1 μM (Fig. 3C) and DES 5 μM (Fig. 3D) demonstrated similar changes in the microtubule network consisting of an overall decrease in the density of microtubules, with smaller numbers of thicker microtubule bundles which were less polarized.

Paclitaxel treated cells have increased levels of polymerized tubulin arrayed along the cell axis (Fig. 3B). Negative controls including no primary tubulin antibody showed no epifluorescence. These data suggest that DES and 2-ME selectively suppressed, but did not eliminate, microtubule formation and did not result in depolymerization of microtubules as occurs with vinca alkaloids and PC-SPES [20,21].
total soluble tubulin heterodimers. The agents assessed in this study did not induce any signal to suggest increases in soluble tubulin, rather the arrangement and polarization of microtubules differed from control. Taxane cytotoxicity may require polymerized microtubules for effect and DES and 2-ME suppress expression of specific tubulin isotypes, but do not appear to suppress the total tubulin level.

DES and 2-ME Increase Arrest at G2-M

We postulated that the augmented taxane cytotoxicity additionally due to effects on tubulin expression. However, it is possible that in the cell cycle effects of DES and 2-ME which substantially increase the numbers of cells which arrest at G2-M [7,25]. The ability of taxanes to arrest cells at G2-M appears critical to achieving cell death, and any additional arrest at this phase of cell cycle might improve cytotoxicity [26]. To evaluate this possibility, LNCaP cells were treated with DES, 2-ME, and paclitaxel, alone or concurrently at their IC50 concentrations and analyzed for effect on cell cycle. The percentages of cells in the pre-G1, G1, S, and G2-M phases were determined by flow cytometric analysis of propidium iodide-stained cells as described in “Materials and Methods.” All three agents increased the number of cells in G2-M compared to control (Fig. 5). The combination of DES or 2-ME with paclitaxel substantially increased the number of cells that accumulated at G2-M. This increase in G2-M arrest was not consistently accompanied by an increase in the apoptotic, pre-G1 cells, suggesting that cells may be dying by a non-apoptotic mechanism.

DISCUSSION

Estrogen administration has been a mainstay of prostate cancer treatment through its central inhibition of gonadotropin releasing hormone secretion and subsequent decreases in testosterone synthesis. In randomized clinical studies carried out through the Veteran’s Administration Cooperative Urological Research Group, DES had a better control rate for advanced prostate cancer than orchiectomy, but with an increased risk of cardiovascular mortality [27]. In addition to their anti-androgen effects, estrogens have long been believed to exert direct growth-inhibitory effects on prostate cancer cells via induction of apoptosis or cell cycle arrest [4,28]. The evidence that synthetic cytotoxic estrogens such as DES and 2-ME modulate microtubule stabilization and induce G2-M cell cycle arrest in vitro suggested the potential for combination with taxanes in the treatment of prostate cancer [4,25,29,30]. The taxanes paclitaxel and docetaxel have the highest response rates reported for chemotherapeutic agents in advanced prostate carcinoma, and docetaxel is the first agent which has achieved a survival benefit for patients with androgen independent prostate cancer [31]. Building on these results by improving the efficacy of taxanes could...
achieve greater palliative and survival benefits for patients with this disease.

The results of the study demonstrate that DES and 2-ME suppress specific β-tubulin isotypes without altering total tubulin levels. The suppression of tubulin gene transcripts could potentially be due to several different levels of regulation. Typically when the proportion of soluble tubulin heterodimers increases in the cell, gene transcription is modulated in order to maintain homeostatic level of free and polymerized tubulin [32]. This may be due to active dissolution of microtubule polymers or through blocking addition of heterodimers to intact microtubule arrays. Although we did not quantitate the amount of polymerized and unpolymerized tubulin in these cells, the immunofluorescence studies did not show dramatic increases in cytoplasmic tubulin signal, but rather indicated that the total number of microtubule arrays was diminished. Given that DES and 2-ME primarily alter isotype IV, which is resistant to taxane effect, we anticipated that

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**Fig. 5.** Cell cycle effect of paclitaxel combination with estrogens. Cell cycle analysis for LNCaP cells treated with vehicle control (CTL) paclitaxel (PTX), DES, DES plus PTX, 2-ME (2ME), or 2-ME plus PTX (2ME/PTX) was carried out as described in “Materials and Methods.” Cell cycle fraction was determined using propidium iodine staining. The combination of paclitaxel with either agent induced additional accumulation of cells at G2-M beyond that achieved with any agent alone. The percentages of cells arrested in G2-M representative of two independent experiments are indicated on each panel. SEM was less than 10% for all conditions.
these agents might sensitize cells to taxanes. The suggestion of synergism between 2-ME and taxane is somewhat surprising given that these drugs are proposed to work in opposing fashion. Paclitaxel stabilizes intact microtubule arrays, whereas 2-ME has been shown to inhibit tubulin polymerization in a manner similar to the vinca alkaloids [6]. Combining agents which appear to have opposing mechanisms of action might either antagonize or synergize with one another. In a previous study, we demonstrated that PC-SPES impaired polymerization of microtubules and that this resulted in antagonism to taxane effects in vitro and in vivo [20,21]. In the current study, we did not see depolymerization of tubulin with 2-ME, consistent with other reports describing significant inhibitory effects without depolymerization of tubulin [33]. True antagonism between microtubule active agents may require that the balance of polymerized and depolymerized tubulin be dramatically altered, and this effect may not be achieved except in vitro. Jordan et al. have proposed that both tubulin stabilizing and depolymerizing agents mediate their cytotoxic effects by suppressing microtubule spindle dynamics without significantly altering the distribution of tubulin between soluble and insoluble forms [34]. The apparent synergism between paclitaxel and 2-ME is consistent with this model. The effects of DES and 2-ME on tubulin may mediate similar changes in cell cycle, as both DES and 2-ME induce cell cycle arrest at the G2-M boundary [5,29]. This effect might then synergize with taxanes which require G2-M arrest for effective cytotoxicity.

In this study, we have shown that both DES and 2-ME can augment the effect of taxanes on prostate cancer cells, potentially through modulation of tubulin isotype expression and induction of aberrant microtubule arrays or through enhanced G2-M arrest. Exploitation of this synergy carries significant promise for the treatment of prostate cancer as this malignancy appears remarkably resistant to the majority of chemotherapy agents except the taxanes. These studies form the basis for an ongoing phase II clinical trial of DES with docetaxel in the treatment of patients with metastatic, androgen independent prostate cancer. Further studies to explore the potential for combining estrogens with agents targeting microtubules may provide patients with more effective options for chemotherapy in the treatment of advanced prostate carcinoma.

REFERENCES


