

Testosterone is a potential augmentor of antioxidant-induced apoptosis in human prostate cancer cells

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Abstract

We have investigated the effect of antioxidant-induced apoptosis in human prostate cancer cell lines that is augmented by testosterone (T). In this study, DU-145 (androgen unresponsive), ALVA-101 (partially androgen responsive), and LNCaP (androgen responsive) were grown in tissue culture with RPMI 1640 medium, 5–10% fetal bovine serum (FBS), antibiotics and 5% CO₂. Treatment with 2.5–20 µg/ml of PDTC significantly ($P < 0.05, n = 6$) lowered cell growth in all three cells 2–60% following treatment for 1–7 days. T (10⁻¹² M) alone enhances cell growth in androgen responsive cells. In contrast, the combination of PDTC and T significantly ($P < 0.05, n = 6$) augmented the PDTC induction of apoptosis in the androgen responsive cells, (ALVA-101 and LNCaP), but not in the androgen unresponsive cells (DU-145). PDTC reduced the nuclear NF-κB, as determined with an electrophoretic mobility shift assay (EMSA), to 50% of the control in LNCaP cells, 65% in ALVA-101 cells and 45% in DU-145 cells, but the combination of PDTC and T was not more potent than PDTC alone in any of the cell lines. PDTC suppressed both the AR mRNA and protein expression and reversed the stimulatory effect of T on androgen receptor (AR) protein synthesis in LNCaP and ALVA-101 cells. In conclusion, PDTC is a potent growth inhibitor and an inducer of apoptosis in human prostate cancer cells by reducing nuclear NF-κB and AR protein expression. PDTCs suppression of AR synthesis and nuclear NF-κB in response to T may contribute to its enhancement of apoptosis observed with T and PDTC compared to PDTC alone. © 2002 International Society for Preventive Oncology. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Prostate cancer; DU-145; ALVA-101; LNCaP; Antioxidants; PDTC; Testosterone; Apoptosis; NF-κB

1. Introduction

The development, growth and maintenance of the prostate gland are dependent on sex hormones and growth factors [1–17]. In aging men, a shift between the ratio of testosterone and estrogen occurs. By age 40, testosterone (T) levels start to decline and estrogen levels remain relatively constant. Coinciding with these changes is an increased probability of benign prostatic hyperplasia and prostate cancer. By poorly understood control mechanisms, some prostate cells escape natural growth regulatory process (apoptosis) and become cancerous [9]. Deaths due to prostate cancer are about 14% of all cancer deaths in males, and prostate cancer is the second leading cause of death in men [18]. Risk factors for developing prostate cancer include aging, familial traits, Afro-American heritage, a Western life style and high fat diet [9,18–21].

Growth factors and T have a growth-regulating role in the normal prostate gland by stimulating growth and dif-

ferentiation. However, human prostate cancer cells can be growth-suppressed by protein factors such as TGFβ-1 and TNFα (tumor necrosis factor alpha) through stimulating apoptosis [22–24].

Androgen withdrawal has also been used to retard prostate cancer growth. This reduction of androgens results in a rise in TGFβ-1 production that then normally inhibits prostate cell growth possibly by inducing apoptosis [25,26]. However, TGFβ-1 also stimulates angiogenesis and may facilitate prostate tumor growth [2,13].

TNFα release in vivo occurs as a therapeutic response to inflammation and even cancer [24]. Some of the multi-faceted responses stimulated by TNFα are mediated through the activation of nuclear factor κB (NF-κB) in many tissues including prostate cancer cells [27,28]. Activation of NF-κB appears to occur with phosphorylation and degradation of the inhibiting factor, IκB. This allows rapid nuclear translocation of NF-κB where it is a pleiotrophic transcription factor [29].

Some prostate cancer cells may escape cell death by activating mechanisms that override apoptosis signals. Cancer cells that are non-responsive to cell death mechanisms

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activated by TNF α treatment have been identified. These cells were found to have high levels of activated NF- κ B [27,30,31]. In these cases active NF- κ B remains elevated, which in turn inhibits the apoptosis processes. This suggests that treatment of prostate cancer should include TNF α plus an inhibitor of NF- κ B activation. With this insight investigations using combinations of apoptosis stimulators plus NF- κ B inhibitors have become of interest recently [27,28,30,31]. Other proposed mechanisms that induce TNF α resistance in tumor cells include: differences in TNF α receptor expression, productivity of TNF α mRNA, and the possible existence of a gene related to TNF α resistance [32,33].

Androgens act clinically as potentiators to many other hormones and growth factors [9]. Some of these androgen effects on prostate cancer cells include expression regulation of S100P gene [34], and prostate-specific antigen gene [35]. Keller et.al. have reported on another mechanism where androgens repress interleukin-6 activity [36]. They suggested that DHT repressed interleukin-6 activity by inhibiting NF- κ B by maintaining I κ B α in LNCaP cells, thereby blocking NF- κ B activation to the nucleus.

We have investigated the interaction of androgen function and PDTC on NF- κ B and subsequently postulated that T would reverse the effect of PDTC on NF- κ B and apoptosis. However, we have verified a novel increase in apoptosis in response to both PDTC and T. This study presents yet another function of androgens that promises to be a new and novel approach to preventing and treating prostate cancer.

2. Methods

Cell culture: The ALVA-101 human prostate epithelial cancer cell line (androgen moderately responsive, isolated by Steve Loop at American Lake VA Hospital, Tacoma, WA), was used in this study. DU-145 cells (androgen unresponsive) and LNCaP (androgen responsive) cells, obtained from the American Type Culture Collection (ATCC, Rockville, MD) were also studied. The cells were grown in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) 10,000 U/ml penicillin G sodium, 10,000 μ g/ml streptomycin sulfate, 25 μ g/ml amphotericin B, and the medium was changed three times a week. LNCaP cells were grown in RPMI-1640 supplemented with 10% FBS. The cultures were maintained at 37 °C with 5% CO₂ atmosphere. Cells were split into either 75 cm² flasks (concentration of 25,000 cells/ml), 96 multiwell plates (concentration of 5000 cells/well) or in culture plates (100 mm × 20 mm; concentration of 2.5 × 10⁴ to 5.0 × 10⁴ cells/ml). In experiments using T, cells were washed and switched to charcoal-stripped medium.

Cell growth was determined using the Cell Titer 96 AQ assay (Promega, Madison, WI) an ELISA method successfully used previously [22,37]. As described previously, this

assay correlates highly with cell growth determined by cell counting and thymidine incorporation [37]. Cells were plated in 96-well plates using 5000 cells/well in 200 μ l of medium, treated, and then assayed. This assay measures dehydrogenase activity that converts MTS tetrazolium into formazan, and this product is determined by measuring absorbance at 490 nm compared to controls. Absorbance was measured using an ELISA plate reader (Vmax Kinetic Microplate Reader, Molecular Devices, Menlo Park, CA). Cell death detection ELISA plus kit (quantitating histone-associated DNA fragments in the cytoplasm of cells; Boehringer Mannheim, Indianapolis, IN) determined apoptosis.

Nuclear NF- κ B activation was determined in DU-145, LNCaP and ALVA-101 cells by the EMSA. Nuclear extracts were prepared from cells following treatments with and without testosterone (10⁻¹² M) and PDTC (10 μ g/ml) for 1 day. Nuclear isolation procedures were carried as outlined by Dignam et al [38].

The EMSA was used to measure NF- κ B activity from the isolated nuclear extracts. A 42 bp synthetic oligonucleotide (DNA peptide facility, Huntsman Cancer Institute) (5'-GATC-CAA-GGG-GAC-TTT -CCA-TGG -ATC-CAA-GGG-GAC-TTT-CCA-TG-3') containing NF- κ B consensus sequence (GGGGACTTTCC) [38] was labeled with (α -³²P) dCTP using prime-it II Random Primer Labeling Kit (Stratagene, La Jolla, CA). Labeled DNA was purified using a quick spin column (Boehringer-Mannheim Corporation, Indianapolis, IN). Ten micrograms of nuclear extract, determined by a BCA protein assay, was incubated for 15 min at 4 °C with 5 μ l of gel shift binding buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 1 mM DTT, and 1 mM EDTA) and 2 μ l of poly-{d (I-C)}. After incubation, 10 μ l of ³²P labeled NF- κ B (diluted 1:10 in water) was added and the mixture was incubated 20 min at room temperature. Finally, 5 μ l of loading buffer (267.6 mM Tris base, 267.6 mM boric acid, 6 mM EDTA, 32% w/v glycerol, 0.03% bromophenol blue) was added, and the mixture was loaded onto a 5% non-denaturing polyacrylamide gel (Bio-Rad, Hercules, CA). Ten micrograms of HeLa cell nuclear extract was loaded on the gel as a positive control. Electrophoresis was done at 150 v for 30 min. Gel was then exposed on a Phosphor-Imager cassette screen for 4 h. Gel signals were quantitated using a Bio-Rad Molecular Imager FX (Bio-Rad, Hercules, CA).

Reverse transcription/polymerase chain reaction (RT-PCR) was used to isolate the probe for androgen receptor and prostate specific antigen (PSA). Total cellular RNA was extracted using the Tri-Reagent RNA isolation protocol (Molecular Research Center, Cincinnati, OH). Primers used in the androgen receptor assay are as same as reported by Taplin [39]. Primers used in the PSA assay are 5'-AAGTTCATGCTGTGCTGG-3' and 5'-AGGGAGTTGATGGGGTGC-3'. The primers were synthesized by the DNA peptide facility, Huntsman Cancer Institute (University of Utah, Salt Lake City, UT). Reverse

transcription and PCR were performed using the RNA PCR kit (Perkin-Elmer, Branchber, NJ).

Northern blots were used to quantify the expression of androgen receptor and PSA genes. Isolated RNA concentrations were determined spectrophotometrically. Total RNA (20 µg per lane) was size-fractionated on a 1% agarose-formaldehyde gel. The RNA was transferred to nitrocellulose using the blot transfer system (Life Technologies Inc.; Gaithersburg, MD) cross-linked (UV Stratalinker 1800; Stratagene, La Jolla, CA) and then incubated at 42 °C for 2 h in pre-hybridization mixture (50% (v/v) formamide, 25% (v/v) of 20× SSPE, 4% (v/v) 50× Denhardt solution, 0.5% (v/v) sodium dodecyl sulfate (SDS), 1% (v/v) (concentration; 10 mg/ml) denatured salmon testes DNA, 19.5% (v/v) diethyl pyrocarbonate (DEPC) treated water). The nitrocellulose membrane was hybridized overnight at 42 °C with a ³²P-labeled cDNA probe isolated from a PCR product (see above). Following hybridization, the membrane was washed in 1× SSC/0.1% SDS for 10 min at room temperature and then washed in 0.25× SSC/0.1% SDS for 10 min at room temperature. The membrane was air dried and then exposed on an intensifying screen in a Phosphor-Imager cassette (Molecular Dynamics, Sunnyvale, CA) for about 4 h. To standardize the RNA load, the membrane was sequentially hybridized with β-actin cDNA probe 1.1 kb insert (American Type Culture Collection, Rockville, MD). Hybridization signals were quantitated using Bio-Rad Molecular Imager FX.

Western blot was used to quantify the expression of androgen receptor protein in ALVA-101 cells. ALVA-101 cells were plated in culture plates at 2.5×10^4 cells/ml in RPMI 1640, 1% antibiotics and 5% stripped FBS. After 24 h, the cells were treated alone and in combination with testosterone (10^{-12} M) and PDTC (10 µg/ml). After 24 h of incubation, cells were washed with PBS and resuspended in 300 µL of electrophoresis loading buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS and 5% β-mercaptoethanol), boiled for 5 min and passed through 20 gauge needle and then through a 26 gauge needle. Samples were centrifuged at 10,000 g for 10 min, the supernatant recovered and the pellets discarded. The protein concentrations were determined by using Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Electrophoresis was carried out in 7.5% Tris-HCl gel (Bio-Rad, Hercules, CA) according to the method of Laemmli [40] using 20 µg of protein in each lane. Electrophoretic transfer to nitrocellulose filter was performed using a Bio-Rad mini protein 11 apparatus at 25 V for 2 h. The filter was then blocked with 5% nonfat dry milk in PBS-Tween 20 at room temperature for 2 h. Then the filter was incubated for 1 h at 37 °C with 2 µg/ml of mouse anti-human androgen receptor monoclonal antibody (BD PharMingen, San Diego, CA). After washing four times with PBS-Tween 20, the filter was incubated with HRP-conjugated goat anti-mouse immunoglobulin specific polyclonal antibody (BD PharMingen, San Diego, CA) for 45 min at 37 °C. Then the filter was washed four times with

PBS-Tween 20, one time with PBS and immersed in Super Signal West Dura Extended Duration Substrate working solution for 5 min (Pierce, Meridian Road, Rockford, IL). Protein signals were quantitated using a Bio-Rad Molecular Imager FX.

Statistical analysis: The data were analyzed using ANOVA or Student's *t*-test to determine statistical differences with significance of $P < 0.05$ or less. Data are expressed as mean ± standard deviation or mean ± standard error.

3. Results

3.1. Effect of PDTC and testosterone on cell growth

We have previously shown that PDTC is a more potent inhibitor of cell growth than Vitamin E. Apoptosis activity was verified in these experiments with an ELSA kit specific for apoptosis and by microscopic techniques (bisbenzimidazole DNA staining and TUNEL) [37]. DNA damage was documented by 24 h. Androgen dose response studies have shown that LNCaP cells have peak androgen responsiveness at concentrations of 10^{-12} to 10^{-14} M [41]. In ALVA-101 cells as shown in Fig. 1, T concentrations between 10^{-6} and 10^{-14} M modestly but significantly ($P < 0.05$) increased cell growth. For our experiments, we therefore performed studies in all human prostate cancer cells using T concentrations of 10^{-12} M.

To determine if T might counteract the effect of PDTC on apoptosis and cell growth, we treated androgen-unresponsive and androgen-responsive human prostate cancer cells with both PDTC and T. As shown in Fig. 2, PDTC treatment of DU-145 cells at 10 µg/ml for 4–6 days significantly ($P < 0.05$, $n = 6$) reduced cell growth. The addition of T had no influence alone or in combination with PDTC on cell growth inhibition. These findings might suggest that the cells did not

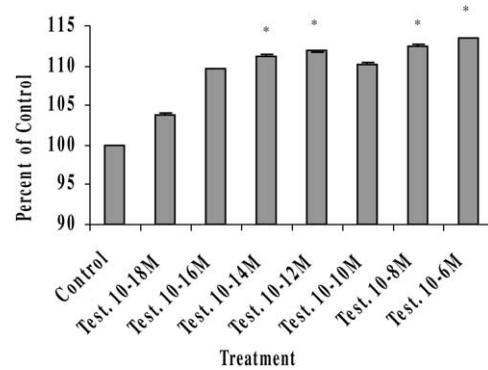


Fig. 1. Effect of testosterone (T) on ALVA-101 cell growth. ALVA-101 human prostate epithelial cancer cells were monitored for cell growth using a metabolic assay (ELISA Cell Titer 96 AQ assay noted in methods). Cells treated with various concentrations of testosterone (10^{-6} to 10^{-18} M) were investigated following 5 days of treatment. Data are compared against control values set at as 100%. The stimulatory effect was enhanced significantly (* $P < 0.05$, mean ± S.D., $n = 6$) with the higher concentrations tested.

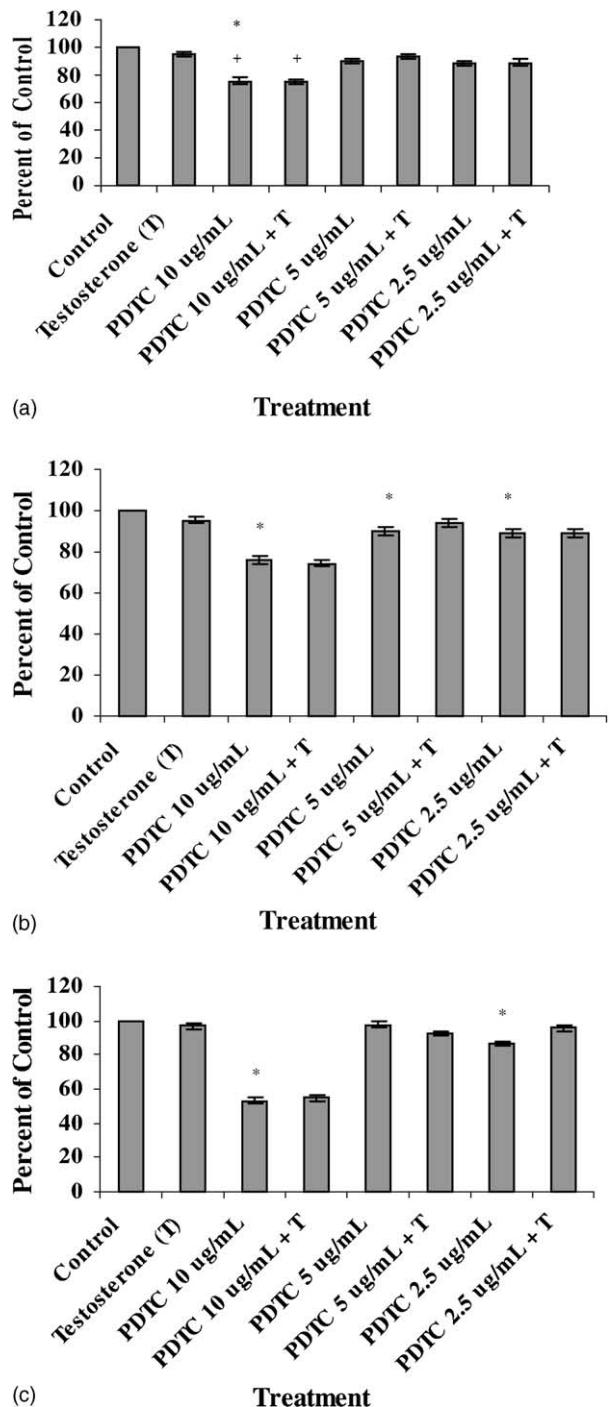


Fig. 2. Effect of PDTC and T on DU-145 cell growth. DU-145 human prostate epithelial cancer cells were monitored for cell growth using a metabolic assay. (a) Cells treated for 4 days with various concentrations of PDTC alone or combined with 10^{-12} M T. Data are compared against control values set at 100%. PDTC treatment of DU-145 cells at 10 μ g/ml significantly ($*P < 0.05$, mean \pm S.D., $n = 6$) reduced cell growth. (b) Cell growth in DU-145 cells was also determined following 5 days of treatment with PDTC and T. Cell growth was significantly reduced by all three PDTC treatments ($*P < 0.05$, mean \pm S.D., $n = 6$). (c) Cell growth in DU-145 cells was also determined following 6 days of treatment with PDTC and T. PDTC treatment of DU-145 cells at 10 and 2.5 μ g/ml greatly reduced cell growth ($*P < 0.05$, $n = 6$). The addition of T had no significant further influence.

respond to T because this cell line is known to be androgen unresponsive.

We then studied the androgen-responsive cells. Fig. 3 demonstrates the effects of PDTC alone or in combination with T treatment of ALVA-101 cells for 4–6 days. T significantly ($P < 0.05$, $n = 6$) increased the inhibition of cell growth in combination of PDTC at 5 and 10 μ g/ml at 4–6 days whereas the 2.5 μ g/ml dose of PDTC in combination with T was ineffective in inhibiting cell growth either alone or with T. This T augmentation of growth inhibition of PDTC seemed paradoxical.

The LNCaP cell line is also known to be androgen-responsive. Fig. 4 shows that PDTC at 5–20 μ g/ml was effective in inhibiting cell growth in LNCaP cells alone at 4–6 days. T only augmented the inhibition ($P < 0.05$, $n = 6$) at doses of PDTC at 10–20 μ g/ml on days 4–6 of treatment. These results also show a T augmentation of the growth suppression in response to treatment with PDTC.

To investigate whether the combined effect of PDTC and T on reduction of ALVA-101 cell growth was caused by apoptosis, we assessed apoptosis as shown in Fig. 5. This demonstrates that apoptosis was increased in response to PDTC alone and combined with T, but not with T alone. These results indicate that the reductions in cell count observed with PDTC were the result of apoptosis.

3.2. Effect of PDTC and testosterone on nuclear NF- κ B

We have observed that PDTC suppresses nuclear NF- κ B in prostate cancer cells and postulated that the paradoxical response to T might be related to an augmentation of the PDTC induced suppression of nuclear NF- κ B. Fig. 6 indicates that T alone while not affecting DU-145 or ALVA-101 cells has a modest but significant influence on lowering nuclear NF- κ B only in LNCaP cells treated for 1 day. However, when T was combined with PDTC (10 μ g/ml) further suppression of nuclear NF- κ B did not occur in the LNCaP cells or in the other two cell lines. Thus, while T would not be expected to produce apoptosis, it seems possible that growth of cells is regulated to protect against excessive growth. LNCaP cells appear to have retained the ability to down regulate nuclear NF- κ B, which would be expected to slow their rate of growth in response to androgen.

3.3. Effect of PDTC and testosterone on androgen receptor mRNA, protein and PSA mRNA

Androgen therapy is known to down regulate AR in androgen-responsive cells [42]. As shown in Fig. 7, T incubated for 0–8 h also resulted in a suppression of AR mRNA except at 4 h when it was increased. PDTC suppressed AR mRNA at all time points as compared to the control. Fig. 8 shows the effect of T and PDTC at 24 h on AR in DU-145 (Fig. 8a), AVLA-101 (Fig. 8b) and LNCaP (Fig. 8c). T alone decreased AR mRNA in both ALVA-101 (Fig. 8b)

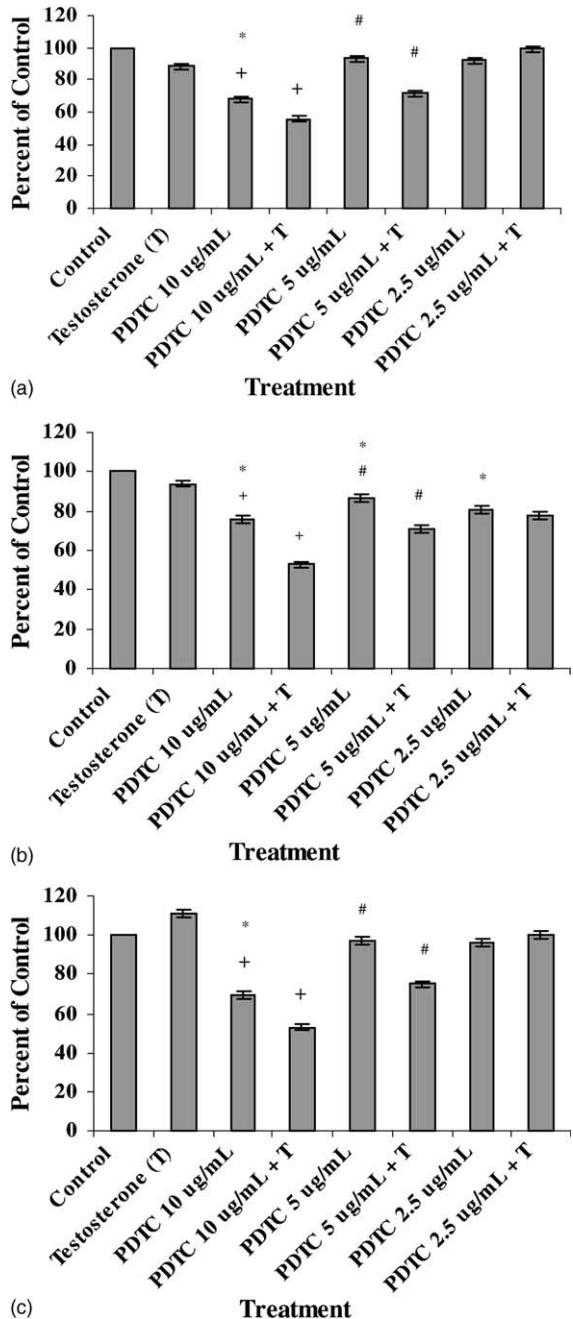


Fig. 3. Effect of PDTC and testosterone on ALVA-101 cell growth. Cell growth was measured using a metabolic assay as described in the Section 2. (a) Cells treated with various concentrations of PDTC alone or combined with 10^{-12} M T and were investigated following 4 days of treatment. PDTC (10 μ g/ml) alone significantly reduced cell growth in these cells (* $P < 0.05$; mean \pm S.D., $n = 6$). A combination of PDTC plus T demonstrated a greater inhibition on cell growth with 10 and 5 μ g/ml of PDTC plus 10^{-12} M T (+, #, $P < 0.05$, mean \pm S.D., $n = 6$). (b) Cell growth in ALVA-101 cells was also determined following 5 days of treatment with PDTC and T. Cell growth was significantly reduced by all three PDTC treatments (* $P < 0.05$, mean \pm S.D., $n = 6$). A combination of PDTC plus T demonstrated a greater inhibition on cell growth with 10 and 5 μ g/ml of PDTC plus 10^{-12} M T (+, #, $P < 0.05$, mean \pm S.D., $n = 6$). (c) After 6 days of treatment with the combination of PDTC (10 and 5 μ g/ml) plus T was more effective inhibitors than PDTC alone (+, #, $P < 0.05$, mean \pm S.D., $n = 6$). PDTC (10 μ g/ml) alone significantly reduced cell growth in these cells (* $P < 0.05$, mean \pm S.D., $n = 6$).

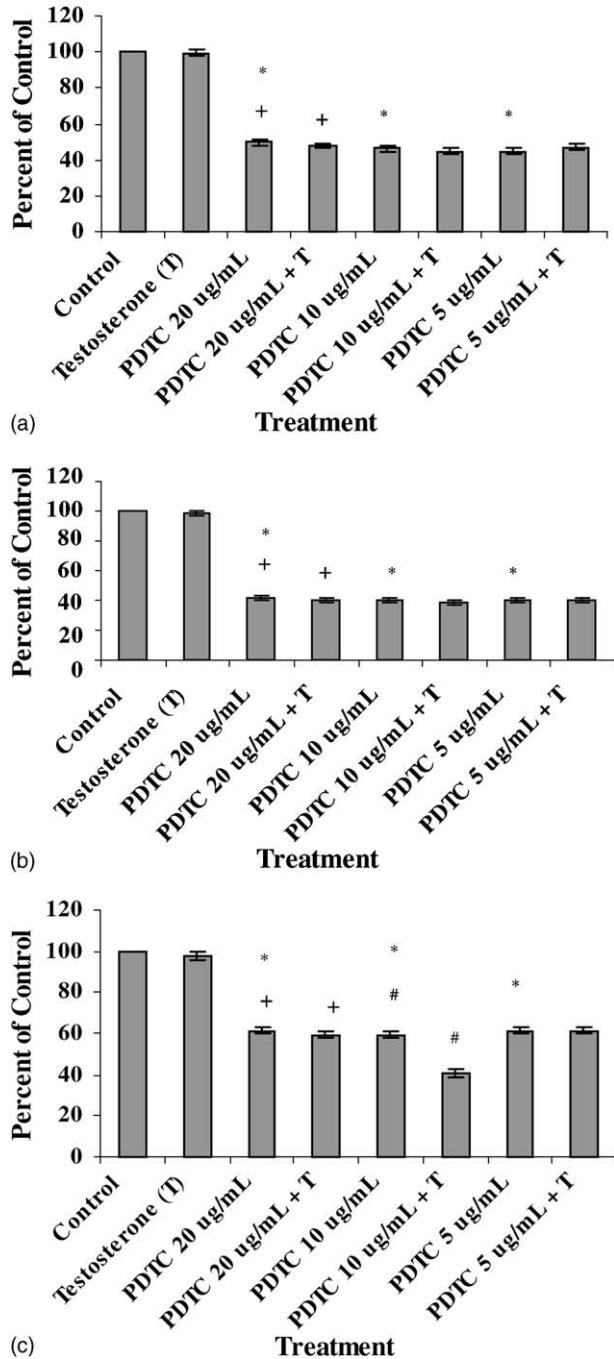


Fig. 4. Effect of PDTC and testosterone on LNCaP cell growth. (a) Cells treated with various concentrations of PDTC alone or combined with 10^{-12} M T were investigated following 4 days of treatment. Data are compared against control values set at a 100% growth rate. Cell growth was significantly reduced by all three PDTC treatments (* $P < 0.05$, mean \pm S.D., $n = 6$). A combination of PDTC plus T demonstrated significant additive inhibition on cell growth with 20 μ g/ml of PDTC plus 10^{-12} M T (+, $P < 0.05$, mean \pm S.D., $n = 6$). (b) Cell growth in LNCaP cells was also determined following 5 days of treatment with PDTC and T was similar to results in a. (c) Cell growth in LNCaP cells was also determined following 6 days of treatment with PDTC and T. A combination of 20 and 10 μ g/ml PDTC plus 10^{-12} M T demonstrated a significant additive inhibition on cell growth (+, #, $P < 0.05$, mean \pm S.D., $n = 6$). Cell growth was significantly reduced by all three PDTC treatments (* $P < 0.05$, mean \pm S.E., $n = 6$).

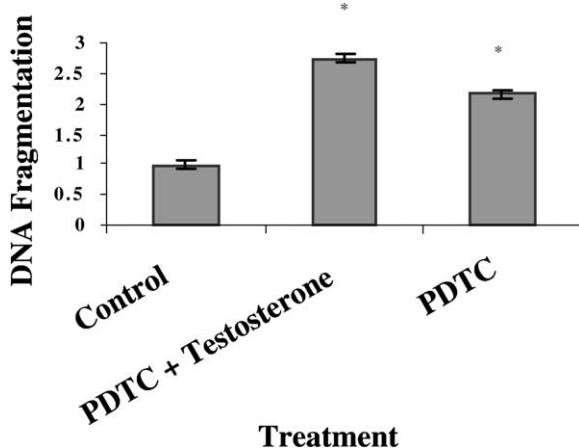


Fig. 5. Effect of PDTC and testosterone on ALVA-101 cell apoptosis. Apoptosis was measured in ALVA-101 human prostate epithelial cancer cells by using cell death detection ELISA plus kit following treatment with T (10^{-12} M) and PDTC (10 μ g/ml) for 5 days. T and PDTC alone and in combination produced a significant increase of apoptosis (* $P < 0.05$; mean \pm S.E., $n = 6$) compared to the control.

and LNCaP (Fig. 8c) but not in the androgen-unresponsive cells (Fig. 8a). In contrast, PDTC alone also significantly ($P < 0.05$, $n = 3$) lowered AR mRNA in both ALVA-101 and LNCaP cells and not in DU-145 cells. In both androgen responsive cells, T appeared to partially reverse the suppression of AR mRNA produced by PDTC as observed by treatment with both PDTC and T, whereas no such interaction was seen in the androgen-unresponsive DU-145 cells.

AR protein expression is known to be increased in LNCaP cells by androgen treatment [42]. We observed that T in-

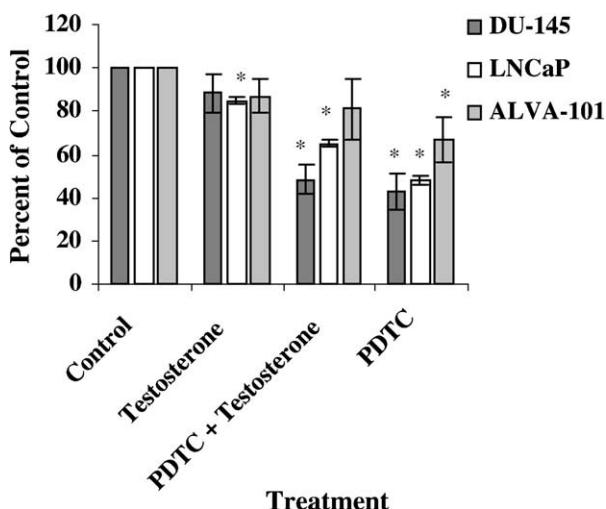


Fig. 6. Effect of PDTC and testosterone on nuclear NF- κ B activation in DU-145, LNCaP and ALVA-101 cells. Nuclear NF- κ B activity was determined by EMSA (as outlined in the Section 2) in DU-145, LNCaP and ALVA-101 human prostate cancer cells following treatment with PDTC 10 μ g/ml and T 10^{-12} M for 1 day. PDTC alone demonstrated significant inhibition of nuclear NF- κ B in all three cell lines and T significantly (* $P < 0.05$, mean \pm S.E., $n = 3$) inhibited nuclear NF- κ B activity in LNCaP cells but not ALVA-101 and DU-145 cells.

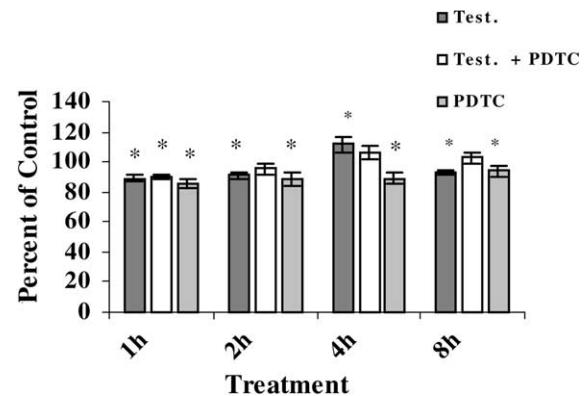


Fig. 7. Effect of PDTC and testosterone on androgen receptor mRNA in ALVA-101 cells. Expression of androgen receptor mRNA was determined by Northern blot analysis in ALVA-101 cells following treatment with PDTC (10 μ g/ml) and T (10^{-12} M) at various time intervals. T treatment resulted in a significant (* $P < 0.05$, mean \pm S.E., $n = 3$) decrease or increase in androgen receptor mRNA expression at the times shown. PDTC treatment only resulted in a decrease in AR mRNA expression.

Table 1
The effect of T and PDTC on prostate specific antigen mRNA expression

Treatment	None	T (10^{-12} M)	PDTC (10 μ g/ml)	T + PDTC
LNCaP	100%	119 \pm 6.6 ^a	58 \pm 5.9 ^a	96 \pm 7.1 ^b
ALVA-101	100%	124 \pm 7.9 ^a	100 \pm 11.6	96 \pm 8.6 ^b

Values are mean \pm S.E.

^a $P < 0.05$, $n = 3$, compared to control.

^b $P < 0.05$, $n = 3$, compared to T.

creased AR protein expression in ALVA-101 cells determined by Western blot as shown in Fig. 9.

We also observed that T significantly ($P < 0.05$, $n = 3$) increased PSA mRNA in the ALVA 101 and LNCaP cell lines (Table 1). PDTC therapy significantly ($P < 0.05$, $n = 3$) decreased the PSA mRNA stimulated by T. These results indicate that T modulates PSA mRNA in the androgen responsive cell lines and that PDTC as a nuclear transcription regulator suppress PSA mRNA expression.

4. Discussion

Although the prevalence of prostate cancer varies among societies, it is universal in aging men [9]. Proposed etiologies for prostate cancer have included aging, race, and environmental and dietary factors [9,43]. Differences in rates of prostate cancer in different societies and the influence of migration on those rates have sparked an interest in diet and particularly antioxidant intake because antioxidant therapy has been associated with reduced prostate cancer incidence rates [9,44–46]. We have previously reported that antioxidants including PDTC, DETC and α -tocopherol induced apoptosis and cell death in prostate cancer cell lines [37]. In

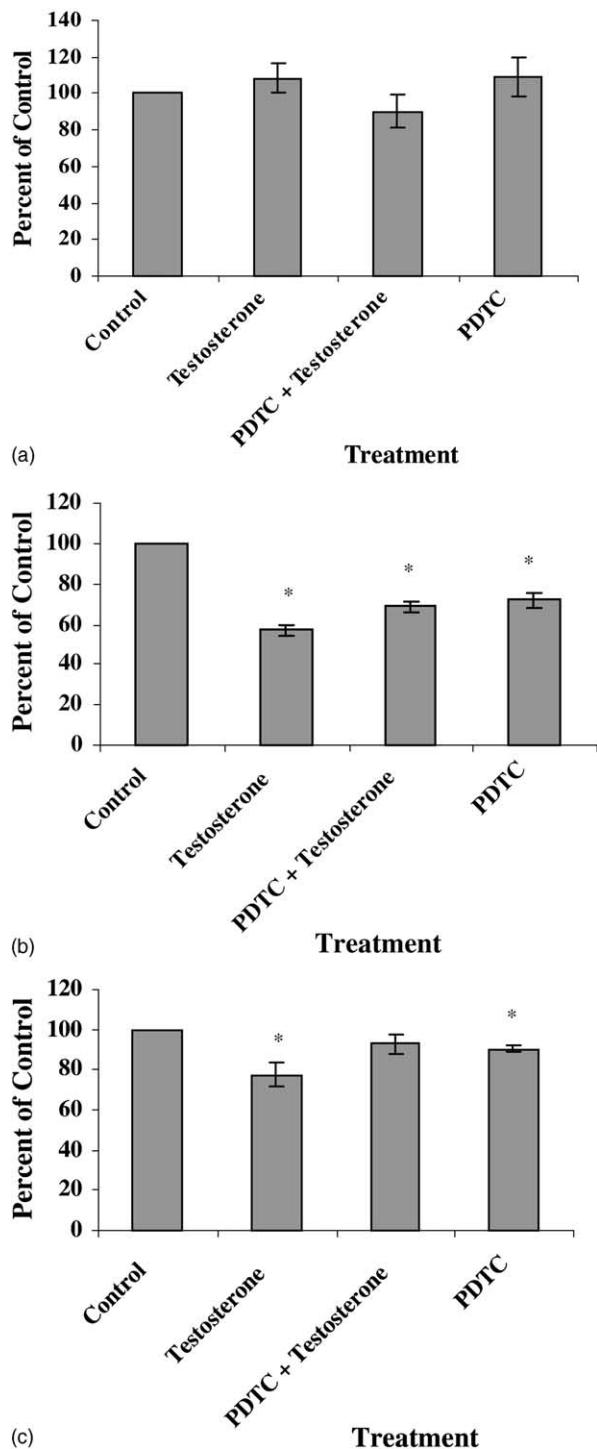


Fig. 8. Effect of PDTC and testosterone on androgen receptor mRNA in DU-145, ALVA-101 and LNCaP cells. (a) Androgen receptor mRNA expression was determined by Northern blot analysis in DU-145 cells following treatment with PDTC (10 μ g/ml) and T (10^{-12} M). T and PDTC alone and in combinations did not significantly affect androgen receptor mRNA in DU-145 cells. (b) Androgen receptor mRNA was also measured with ALVA-101 cells for 1 day following treatment with T and PDTC. T and PDTC alone and in combination produced a significant decrease of androgen receptor mRNA in ALVA-101 cells (* $P < 0.05$, mean \pm S.E., $n = 3$). (c) LNCaP cells treated for 1 day with T and PDTC. T and PDTC alone demonstrated significant inhibition of androgen receptor mRNA but T reversed the PDTC suppression (* $P < 0.05$, mean \pm S.E., $n = 3$).

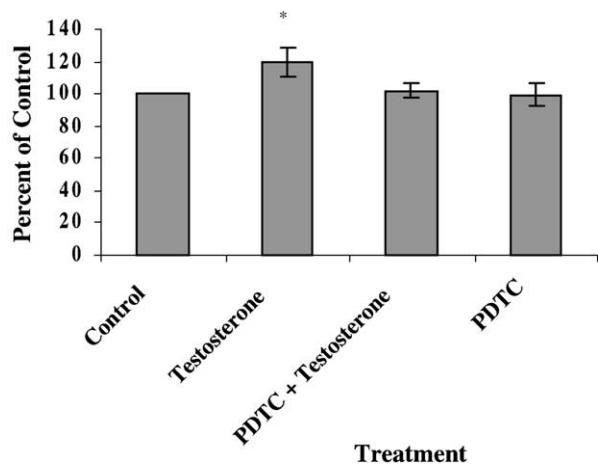


Fig. 9. Effect of PDTC and testosterone on androgen receptor protein expression in ALVA-101 cells. Androgen receptor protein levels were determined by Western blot (as outlined in the Section 2) in ALVA-101 human prostate cancer cells following treatment with PDTC (10 μ g/ml) and T (10^{-12} M) for 1 day. T alone produced a significant increase in androgen receptor protein levels (* $P < 0.05$, mean \pm S.E., $n = 3$).

in this study, we have reported that PDTC and T reduce human prostate cancer cell growth through apoptosis.

A concern with prostate cancer androgen withdrawal therapy is that prostate cancer develops resistance to the growth suppressive effects of the withdrawal [47]. Thus, the withdrawal therapy often provides a short interval of remission before the development of androgen-independent prostate cancer emerges.

One mechanism for emergence of androgen-independent prostate cancers might be that cells are unresponsive to cell death produced by factors such as TNF- α , because cells have high levels of NF- κ B. The elevated levels of NF- κ B may counteract the effects of TNF- α as suggested in some tumor cells by several investigators [27,30,31]. This suggests that measures to reduce the activation of NF- κ B might then lead to apoptosis and retard cell growth.

We have investigated such a combination by using PDTC therapy in three prostate cancer cell lines (as reported in this study and as reported elsewhere) and found that the combination of PDTC and TNF α was more effective than PDTC alone. Sumitomo [28] reported that the combination of TNF- α and PDTC treatment for 24 h effectively inhibited the growth of DU-145 cells, but that PDTC therapy alone for 48 h did not have an effect on cell growth. Our results do indicate that longer intervals of therapy with PDTC are growth-suppressive in DU-145 cells. ALVA-101 cells were even more growth suppressed by treatment with PDTC for 3–5 days than the DU-145 cells. In both cell lines, apoptosis caused the growth inhibition. In the current study using EMSA, PDTC appears to stimulate apoptosis by blocking NF- κ B activation.

The most unique, surprising, and interesting result of this investigation is the effects with testosterone. It is the current dogma prostate cancer treatment involved androgen

suppression. Our observation that T therapy decreases AR mRNA and increases AR protein expression in androgen responsive cells is in agreement with the report of Krongrad et al. [42] who studied the effects of androgen in the LNCaP cell line. However, we have in vitro results in ALVA-101 cells that testosterone potentiates cell death induced by PDTC through an apoptosis mechanism(s). The ability of testosterone to amplify the inhibition of cell growth when combined with PDTC suggests a previously undescribed action of testosterone. The mechanism for the paradoxical T augmentation of growth suppression of PDTC is unresolved.

We did not observe growth suppression in response to PDTC until after 2 days of treatment. T therapy lowers AR mRNA expression and appears to lower nuclear NF- κ B in LNCaP cells only slightly but significantly. Our results in LNCaP cells are in agreement with those reported by Keller et al. [36] who observed that DHT decreased nuclear NF- κ B in LNCaP cells without affecting apoptosis. It is possible that T treatment for only 1 day is not sufficiently long enough to detect a down regulation of nuclear NF- κ B in both ALVA-101 and LNCaP cell lines. The one day T treatment effects of LNCaP cells hint that the T augmentation of PDTC suppressed cell growth may not be paradoxical but may be affecting nuclear NF- κ B. While androgens do increase prostate cell growth, continued stimulation does not always result in a progressive increase in cell numbers. This is probably because androgen transiently down regulates AR mRNA and elevates AR protein expression in androgen responsive prostate cells as observed in our study. We cannot exclude from our studies that the apparent disparity between the effect of T on AR mRNA and protein expression is from posttranslational effects of T. If T therapy were enhancing the PDTC induced cell death by reducing nuclear NF- κ B, a combination of both PDTC and T should have been more effective in suppression of nuclear NF- κ B than PDTC alone, which was not observed. Another possible T enhancement of apoptosis may be that androgen primes the prostate cell making them more responsive to growth inhibitors without directly suppressing growth.

PDTC may be a modulator of nuclear transcription factors affecting AR, which to our knowledge has not been reported previously. This is supported by our observation that PDTC reduces both AR mRNA and protein expression and nuclear NF- κ B. This effect was observed in both androgen-responsive and androgen-unresponsive prostate cancer cells. There is agreement that a mutated but functional AR is present in LNCaP cells [48–50]. This is the first study to our knowledge showing the ALVA-101 cells have the AR mRNA and protein expression as well as PSA mRNA. However, it would be expected that they do since they are androgen-responsive, as demonstrated by an increase in PSA mRNA and AR protein expression in response to T. While there is agreement that DU-145 cells are androgen unresponsive, controversy exists about whether DU-145 cells have a low-level expression of AR mRNA. Some reports suggest they do [51] have unresponsiveness to

the cAMP of the AR response element, and others suggest they do not [52] have AR. Our findings indicate that these cells are androgen unresponsive, but do express low levels of AR mRNA.

In summary, our studies document that PDTC enhances apoptosis by reducing NF- κ B and that T enhances PDTC-induced apoptosis in androgen responsive prostate cancer cell lines. PDTC was also observed to decrease AR mRNA and protein expression and PSA mRNA, possibly by its effects on suppressing NF- κ B, a transcription regulator. Further study is needed to assess the mechanism for this interaction between androgen therapy and antioxidant induced reduction in cell growth. They may have considerable potential in cancer prevention and therapy.

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