The biological basis for the use of an anti-androgen and a 5-α-reductase inhibitor in the treatment of recurrent prostate cancer: Case report and review

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Abstract. Although many prostate cancer cases relapse to a hormone-insensitive state, endocrine therapy involving androgen depletion by orchiectomy or by treatment with LHRH-analogue as well as blockade of the androgen receptor (AR) with anti-androgens remains a primary treatment option. Quality of life (QOL) however, is a prime consideration of men choosing such an approach. In this report we discuss a synergistic combination of 150-mg bicaltumide (Casodex) and 5 mg finasteride (Proscar) in the treatment of a 69-year-old patient with a relapsed (biochemical failure) Gleason score 7 prostate cancer, initially treated with external beam radiation therapy. A successful clinical outcome as evidenced by undetectable serum PSA, bone scan density and overall general well-being was accomplished with minimal side effects. Experiments using an established hormone-dependent prostate cancer cell line (LNCaP) showed that the combination of bicaltumide-finasteride at the same ratio as used clinically, produced synergistic effects on the inhibition of cell proliferation and AR expression/phosphorylation. A more complete inactivation of the AR on this regimen may have had the effect of constraining the ability of the AR to mutate, and/or diminishing the ability of androgen independent clones to evolve. Thus, passage to androgen independence may have been slowed or arrested.

Introduction

Quality of life (QOL) is a prime consideration of men choosing therapy for prostate cancer treatment. Anti-androgen monotherapy, that does not lower testosterone levels in serum, has the potential to be an effective treatment while maintaining a reasonable quality of life. With the availability of the anti-androgen bicalutimide, having both an improved side effect profile, and a possible increased activity over other anti-androgens, monotherapy has been undertaken using this agent as a single high-dose (150 mg/day). Analysis of resulting mature data has shown no significant difference in survival or time to progression between bicalutimide 150 mg and castration in men with locally advanced prostate cancer. There was also greater maintenance of sexual interest, improvement in physical capacity for daily activities, and a reduction in the incidence of hot flashes, when compared with castration (1-8).

Early studies using finasteride and an anti-androgen involved the use of the anti-androgen flutamide. These first trails were based on the fact that flutimide was the predominant available anti-androgen and that finasteride had a well-known ability to decrease intra-prostatic levels of 5-α-dihydrotestosterone (DHT). These early studies strongly suggested that the combination of the two agents could provide an effective form of maximum androgen blockade while maintaining serum concentration of testosterone (9-11).

The PLESS trial (the Proscar Long-term Efficacy and Safety Study) was completed recently (11) but the results of this and other available studies do not yet elucidate the use of finasteride in prostate cancer treatment protocols. In the current study, an attempt has been made to explore the role of a combination of bicalutimide and finasteride and to elucidate a likely mechanism of action in the treatment of prostate cancer. We demonstrated that the combination of bicalutimide and finasteride produced significant synergistic therapeutic effects for a patient with biochemical failure after a primary therapy with external beam radiation. Experiments showed that this combination resulted in synergistic inhibition of AR expression/phosphorylation and cell proliferation in LNCaP cells.

Materials and methods

Patients (case report). A currently 69-year-old patient, with an original Gleason score 6, clinical stage T2bNxM0 prostate cancer, demonstrated biochemical failure after primary therapy with external beam radiation. A graph of the patient's PSA rise subsequent to radiation treatment in 1990 is shown in Fig. 1A. When the PSA reached 6, a repeat biopsy revealed

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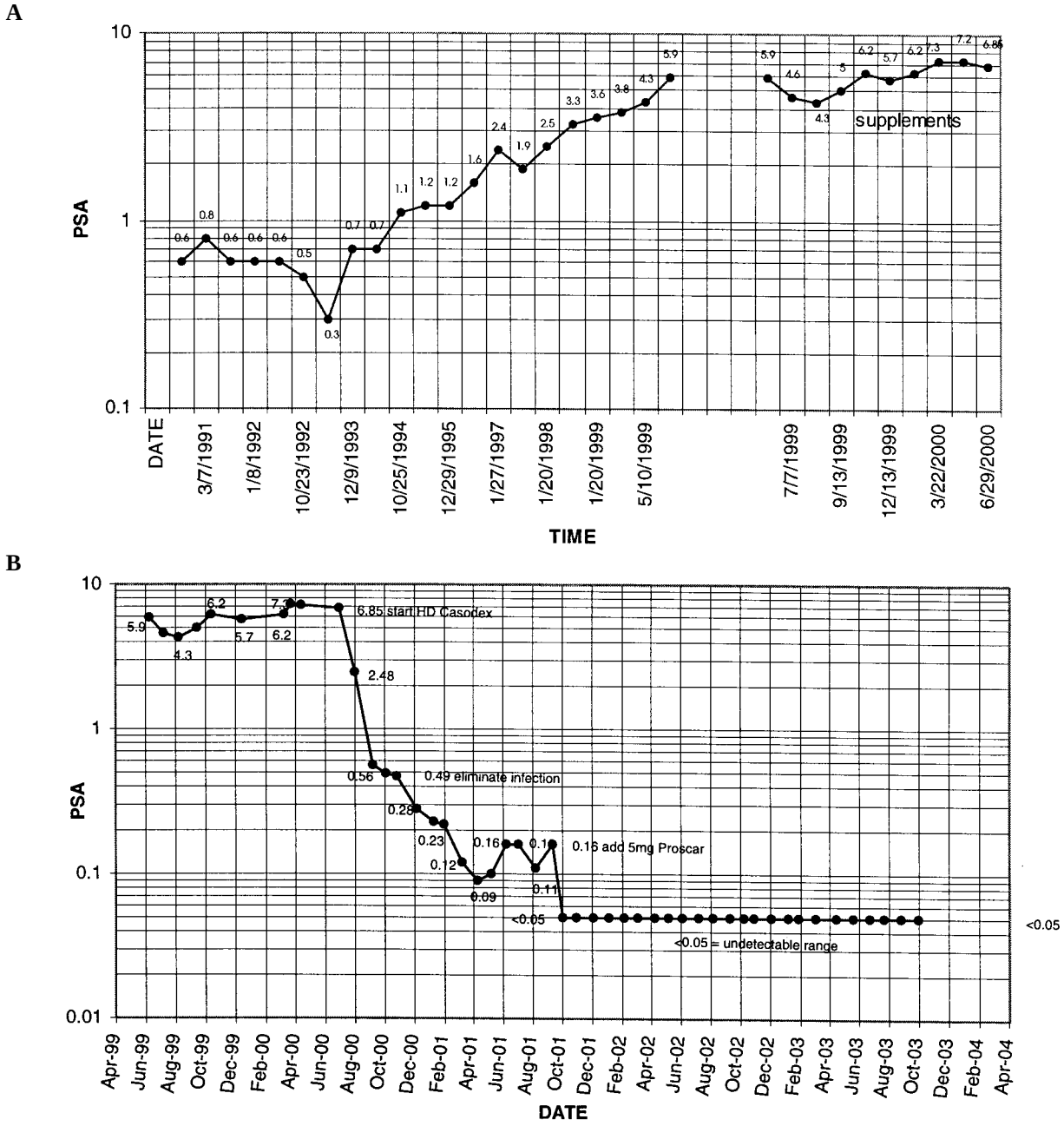

Figure 1. Serum PSA of patient prior to secondary therapy (A) and during the therapy (B). A, PSA vs. time (semi-log scale) (presupplements - then add year of supplements). B, PSA vs. date (semi-log scale) (1 year of supplements only - then 150 mg Casodex only - then add 5 mg Proscar to Casodex).

a Gleason score 7 prostate cancer and a bone scan identified no evidence of gross distant spread. At that time a dietary/anti-oxidant supplement program (Table I) was initiated. Although the slope of the curve of PSA vs. time decreased, the PSA continued to rise (Fig. 1A) and therefore, additional treatment was deemed appropriate.

Based on the initial publications indicating efficacy with minimum side effects, the patient undertook a program of monotherapy with high dose bicalutimide (150 mg/day). Fig. 1B illustrates monthly PSA vs. time as result of this program, from July 2000 until September 2001 when a nadir was reached (basically between 0.1-0.2).

At this point, finasteride was added based on two observations: the first, is the well established role of finasteride as a 5-α-reductase inhibitor, in blocking the intracellular conversion of testosterone to DHT. The second, laboratory experiments showing that finasteride, separate from its ability to reduce DHT, can modulate factors influencing cancer proliferation and evolution to the androgen independent state through its direct action on the androgen receptor (AR). The AR has a role in initiation, progression and resistance to therapy due to its extensive signaling axis, and its tendency toward somatic mutation. These contribute to the evolution of prostate cancer, ultimately leading to androgen independent state (12-26).

Moreover, Wang *et al*, have shown that AR phosphorylation determined whether AR ligands act as agonists or antagonists. For instance, DHT, a natural ligand of AR, significantly stimulated expression and phosphorylation of AR and, as a result, acts as a strong AR agonist (27). The authors also

Table I. Daily dietary supplements.

Supplement	Patient determination based on literature reviews
Vitamin E	400 IU= 270 mg
Vitamin C	500 mg
Vitamin D	400 IU = µg
Selenium	400 µg
Folic Acid	2.4 mg
Soy Protein	114 mg (isoflavones, genistein)
Green Tea	1040 mg polyphenols; + 4 cups/day +
Lycopene	20 mg lycopene; + processed tomatoes
Vegetables/fruits	Yes
Fat	Minimum (and unsaturated)

showed, in another experiment, that finasteride markedly inhibited PSA secretion and expression while the level of AR was dramatically decreased (28).

These findings lead to the following reasoning: i) DHT acts to stimulate AR expression and phosphorylation, ii) finasteride is a competitive and specific inhibitor of Type II 5-α-reductase, prevents the conversion of a large proportion of T to DHT, and iii) a reduction in DHT leads to a reduction in AR expression and phosphorylation. It then follows that finasteride may be an effective addition to bicalutimide.

In our case, the result was that on the combined protocol, the PSA dropped to an undetectable level (Fig. 2) and to date, has remained there for 25 months subsequent to 14 months on bicalutimide monotherapy.

Side effects, to date have been a moderate amount of gynecomastia and a high serum testosterone level (800-1100 NG/DL range). Sequential bone densitometry (Dexa) scan demonstrated a 2.6% increase in the lumbar spine density, a 0.4% decrease in the left femoral neck density, and a 3.7% increase in the right femoral density for >2 years. The results indicate no evidence of osteoporosis, and no progression of mild osteopenia.

Laboratory experiments. Based on the clinical observation of the significant synergy between bicalutimide and finasteride in the treatment of a patient with a biochemical failure, experiments in established hormone-dependent prostate cancer (LNCaP) cells were carried out to explore the mechanisms involved.

Analysis of synergism in the inhibition of cell proliferation of two-drug combination. Exponentially growing LNCaP cells were seeded into 96-well dishes at density of 5,000 cells per well. Twenty-four hours later, the cells were exposed to serial dilution of biocalutimid and finasteride (commercially available tablets) alone or in combination at a ratio of 30:1. After incubation for 3 days, the cell growth was measured by MTT as described previously (28), and the percentages of inhibition (1-T/C)% calculated, and data analyzed by a PC program, CalcuSyn, Biosoft (edited by T.-C. Chou, Memorial Sloan-Kettering Cancer Center, New York, and

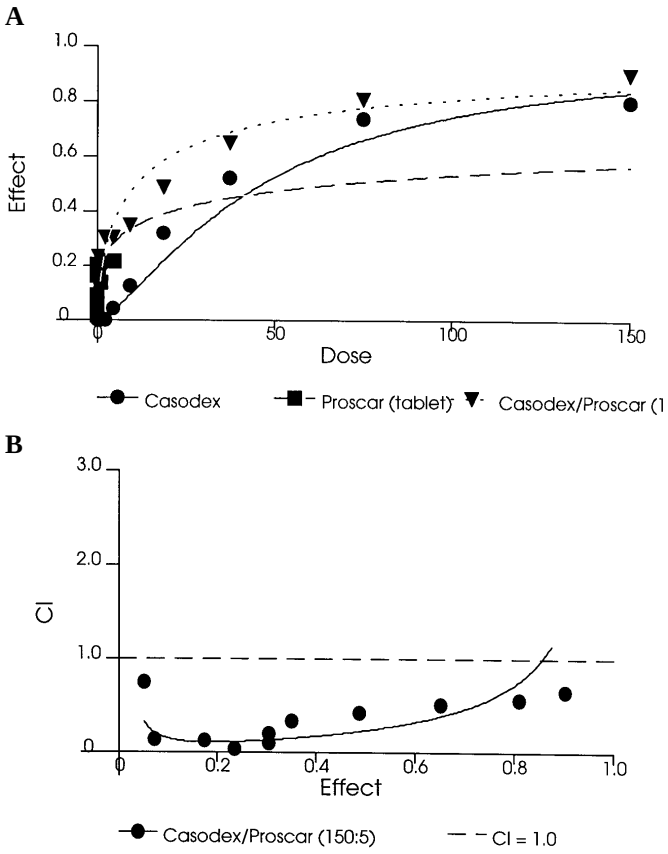

Figure 2. A, dose-effect plot; B, CI-effect plot. LNCaP AD cells grown exponentially were aliquoted into 96-well plates at a density of 5000 cells/200 µl per well in RPMI-1640 medium containing 10% fetal bovine serum (FBS) overnight. The cells in the plates were then exposed to series of dilution of bicalutimide or finasteride alone or both in combinations at different ratio. After 72 h of incubation, 100 µl of the medium was removed from each of the wells and 50 µl of a 1 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and the cells were incubated for additional 4 h. Solution of 0.04 N HCl-isopropanol (200 µl) was added to each well to dissolve the black fromazan participates, and absorbance at 540 nm was measured on a 96-well Microplate Reader. The inhibition of cell growth (1 minus tested over the control or 1-T/C) was calculated as 'effect'. The outcomes of the combinations were evaluated by computer program CalcuSyn (28).

M.P. Hayball, at Cambridge, UK, 1996). Regression and statistical analysis were performed using Sigma plot program. The computer-calculated combination index (CI) was used to judge the outcomes of a combination: CI >1, CI= 1, and CI <1 indicating antagonism, additive, and synergistic, effects respectively.

Western blot assay. LNCaP cells at exponential growth phase in 6-mm dishes were treated with indicated concentrations of bicalutimide and finasteride alone or in combination for 24 h. The cells were then washed, harvested and total proteins extracted for Western blot analysis as described previously using antibodies specific against AR, PSA or ß-actin (as loading control) (29).

Results

Effect on the inhibition of cell proliferation. Using commercially available agents, as shown in Fig 2, we

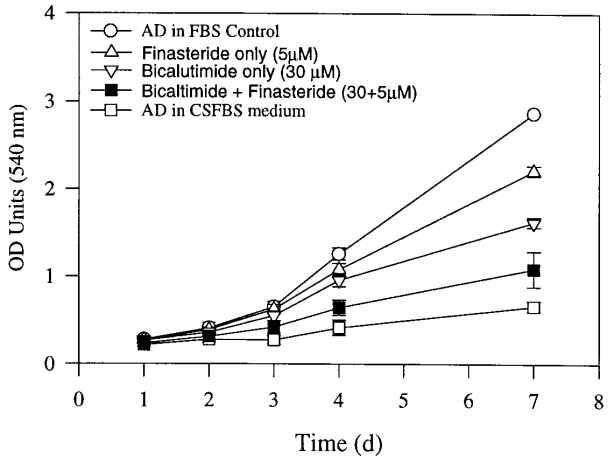

Figure 3. Comparisons of LNCaP (AD) cell growth under conditions of a castration, non-castration, or various drug treatments. Five thousand cells at exponential growth phase were seeded in 96-well dishes in RPMI-1640 medium containing 10% full FBS (normal growth medium, non-castration) or coat-stripped FBS (castration). For drug treatments, the cells were maintained in normal growth medium in the presence of 30 μM of bicalutimide or 5 μM of finasteride alone or both. The cell growth was then measured by MTT method at indicated time points.

demonstrated that at the dose equivalent ratio of 150 mg bicalutimide and 5 mg finasteride, the agents act to strongly inhibit proliferation, in LNCaP cells. Computer analysis using the model of Chou (30), showed that the combination index was <1, indicating that this combination acted synergistically on cell proliferation (Fig. 2A and B).

To evaluate whether the effect of the two-drug combination on the cell proliferation is similar to the effect of castration achieved surgically (bilateral orchiectomy), or medically [using the luteinizing hormone-releasing hormone (LHRH) agonist goserelin], *in vitro* comparisons of the cell growth in a hormone-dependent prostate cancer cell line LNCaP were performed under conditions of castration, non-castration or various drug treatments. As shown in Fig. 3, almost no cell growth was observed when the cells were maintained in medium containing coal-stripped serum (no androgens, similar to castration conditions) while a significant growth was achieved under full serum conditions. Exposure of the cells to either bicalutimide or finasteride alone resulted in a significant inhibition of cell growth that was enhanced synergistically by the combination of those two drugs. The growth curve observed with combination therapy mimicked castration conditions.

Down-regulation of AR and PSA expression. To explore whether the synergistic effect on cell growth achieved by the combination of bicalutimide and finasteride is due to their inhibition of AR expression/phosphorylation, LNCaP cells at exponential growth phase were exposed to bicalutimide, and finasteride alone or in a combination, and the levels of AR proteins as well as AR down-stream target gene, PSA were measured by Western blot. As shown in Fig. 4, the levels of AR proteins were decreased either by bicalutimide or by finasteride alone, and this decrease was enhanced by the two-drug combination. As an AR regulated down-stream gene, the expression of PSA paralleled the decrease of AR expression/phosphorylation. Thus, our data suggest that the synergistic effect on cell proliferation by the two-drug combination is a result of the down-regulation of AR expression/phosphorylation.

Discussion

We herein report a therapeutic outcome for a patient with biochemical failure of prostate cancer after primary therapy with external beam radiation using a regimen of 150-mg bicalutimide and 5-mg finasteride in combination. The serum level of PSA has been undetectable since the first month of the therapy and has remained undetectable during therapy for 24 months to the time of writing this report. Overall health conditions of this patient, such as feeling of well-being, increase in bone density, and lack of other side effects

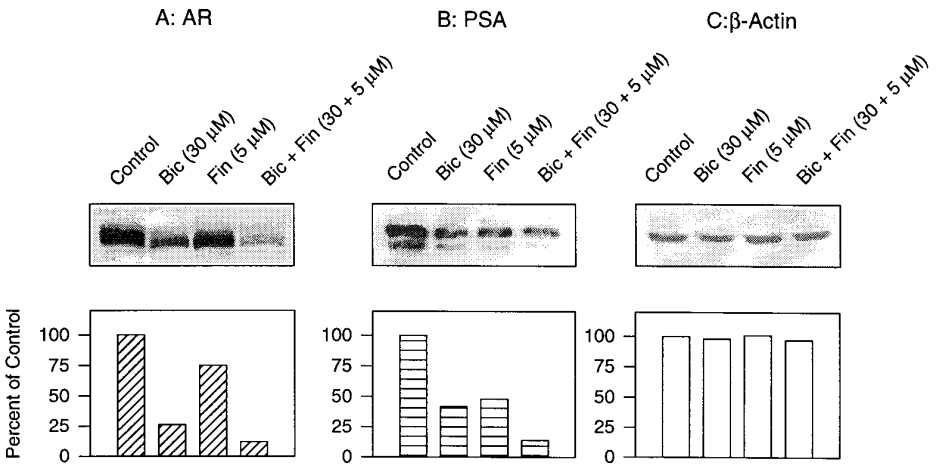

Figure 4. Effect of bicalutimide or finasteride alone or both in a combination on the levels of androgen receptor in LNCaP cells by Western blot analysis. LNCaP cells, grown exponentially, were treated with indicated concentrations of bicalutimide or finasteride alone or both for 24 h. The cells were harvested, washed, and proteins extracted. Proteins (50 μg) were subjected to Western blot assay using specific antibodies against either AR or PSA or ß-actin as an equal loading control (upper panel). The percent of control of AR or PSA protein levels were calculated from density of ECL film measured by Model GS-700 Imaging Densitometer (lower panel). *P <0.01.

associated with total androgen ablation are notable. Our findings suggest that the bicalutimide-finasteride combination regimen may be a good choice for the treatment of patients with biochemical failure of prostate cancer after primary radiation therapy. Although a large-scale clinical evaluation is needed to confirm our observations, this suggestion is supported by laboratory findings that the combination of bicalutimide and finasteride produced significant synergistic effect on the inhibition of cell proliferation and AR expression/phosphorylation (Figs. 2-4).

As seen in the laboratory, bicalutimide-finasteride curtailment of cell proliferation, compared with castration, shows the combination to be somewhat weaker than that of castration (Fig. 3). However, laboratory data can only parallel, not duplicate behavior of drugs in humans.

However, PSA can be considered as a reasonable surrogate of prostate cancer activity, considering finasteride is also an independent regulator of PSA expression (28). Bicalutimide alone, lowered the patient's PSA 97-99%. Therefore, since the patient's PSA dropped markedly on the combination of drugs, to a consistently maintained undetectable level, it may be reasonable to surmise that the synergistic combination of the drugs seen in the laboratory, also reduced patient's AR expression. Further, a substantial down-regulation of the AR may also have had the effect of constraining AR mutation and/or diminishing the ability of androgen-independent clones to evolve. Thus, passage to androgen independence might have been slowed or arrested.

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