In Vitro Effects Of Dutasteride And Finasteride On Prostate Cancer Cell Growth

S Zaslau, A Perlmutter, D Riggs, B Jackson, S Kandzari

Citation

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Abstract

Introduction: Dutasteride and finasteride are used to treat benign prostatic hyperplasia (BPH). The authors hypothesized that dutasteride and finasteride would affect the growth of prostate cells in vitro.

Methods: Three prostate cancer cell lines (LnCaP, PC3, DU145) and one normal line (CRL-2221) were treated in vitro with dutasteride and finasteride. Cell viability was measured at 72 hours by microculture tetrazolium test (MTT).

Results: Dutasteride reduced cell growth (p<0.001), in all cell lines and doses, was tested. Finasteride inhibited cell growth significantly (p<0.05) in the DU145 at 12.5 (57% + 2%), 25 (53% + 4%), and 50 μ g/well (59% + 1%). An increase in cellular proliferation was observed in all cell lines treated with finasteride at doses of 0.4, 0.8, and 1.6 μ g/well (range: 134% to 685%, p<0.001).

Conclusion: Dutasteride could have application in treatment or prevention of prostate cancer. However, the cellular proliferation by finasteride causes great concern in its widespread use.

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INTRODUCTION

The most common type of cancer among American men is prostate cancer. ¹ Although it is estimated that there will be 234,460 new cases of prostate cancer in 2006, ² the estimated number of deaths due to this disease will decline 10% compared to last year. The decline in incidence is directly attributed to increased efficacy in detection, more effective treatment modalities, and increased public awareness. Standard therapies for cancer of the prostate include radical prostatectomy, cryotherapy, radiation therapy, hormonal therapy, and chemotherapy.

Clinical trials have been developed to evaluate different chemopreventive plans regarding prevention of prostate cancer. ¹ A current, ongoing trial is evaluating the effects of selenium and vitamin E on prevention of prostate cancer. Long-term studies also have been assessed in use of nonsteroidal anti-inflammatory drugs (NSAIDS), but with the increase of risk of side effects and toxicity, NSAIDS may be limited as useful chemopreventive agents. ² Another trial, the Prostate Cancer Prevention Trial (PCPT), evaluated the use of finasteride, a 5-alpha-reductase inhibitor, to reduce the risk of prostate cancer. The result of this trial showed a 25% reduction in prevalence of prostate cancer over a 7-year period; however, an increase risk of high-grade disease was observed. 3

Dutasteride, another 5-alpha-reductase inhibitor, is being evaluated in the Reduction by Dusteride of Prostate Cancer Events (REDUCE) to evaluate its effectiveness in preventing prostate cancer. 1 Finasteride and dutasteride are both Federal Drug Administration (FDA) approved for treatment of benign prostatic hyperplasia (BPH), an enlargement of the prostate gland. The prostate gland needs androgens to control growth of the normal prostate and promote BPH and carcinoma of the prostate. 1

The two androgens that have the role in prostate cancer are testosterone and dihydrotestosterone. Testosterone is converted to dihydrotestosterone by the 5-alpha-reductase enzyme. Not only could use of the 5-alpha-reductase inhibitors (finasteride and/or dutasteride) be effective in reducing the risk of prostate cancer, but they also could be a promising treatment for prostate cancer. 1

The purpose of this study is to evaluate the in vitro effects of finasteride and dutasteride on cell growth in both androgendependent and -independent prostate cancer cells and a normal prostate cell line.

MATERIALS AND METHODS DUTASTERIDE

Dutasteride (Avodart) was supplied as soft gelatin capsules that contain 0.5 mg of active dutasteride per capsule, and is distributed by GlaxoSmith Kline (Research Triangle Park, NC). Dutasteride was dissolved to a concentration of 2 mg/mL in 100% ethanol for each cell line, and serial dilutions were performed to concentrations of 0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25.0, and 50.0 g/well.

FINASTERIDE

Finasteride (Proscar) was supplied as 50-mg tablets and distributed by Merck & Co., Inc. (Whitehouse Station, NJ). Finasteride was dissolved to a concentration of 1 mg/mL in 100% ethanol for each cell line, and serial dilutions were performed to concentrations of 0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25.0, and 50.0 g/well.

CELL CULTURE AND REAGENTS

Three prostate cancer cell lines [androgen-independent (PC3 and DU145), androgen-dependent (LnCaP)], and one normal prostate cell line (CRL-2221) were obtained from the American Type Cell Culture (ATCC, Manassas, VA). Cells were maintained as monolayers in their preferred media at 37 C in 5% CO₂. Cells were plated in sterile 96-well microtiter plates at 1 x 10 ⁵ cells/mL and incubated for 24 and 72 hours with the above treatments. An equal volume of drug vehicle was added that represented the controls.

MTT ASSAY

The MTT colorimetric assay was performed to detect tumorcell viability after 72 hours of incubation. MTT, a tetrazolium dye (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide; thiazole blue, SIGMA, St. Louis, MO) was added to each well as described previously. ⁵ Plates were incubated in the presence of MTT dye for 4 hours. Mitochondrial dehydrogenase activity reduced the yellow MTT dye to a purple formazan, then solubilized with acidified isopropanol, and absorbance was read at 570 nm on an enzyme-linked immunosorbent assay (ELISA) plate reader.

STATISTICAL ANALYSIS

Determination of statistical significance was performed by analysis of variance (ANOVA). ₆ Post hoc comparison of individual concentration means with the control was completed using the Tukey-Kramer Multiple Comparison test. ₇ All data are reported as means and standard deviations.

RESULTS CELL PROLIFERATION ANDROGEN-DEPENDENT PROSTATE CANCER CELL LINE (LNCAP)

When the LnCaP cells were treated with dutasteride, significant reductions in cell growth were observed at all doses tested (mean range: $43\% \pm 7\%$) at 72 hours of incubation (p<0.001, Table I).

Figure 1

Table 1: Effects of Dutasteride on Cellular Growth in Androgen-Dependent and Independent Prostate Cancer Cell Lines and Normal Prostate Cells after 72 Hours of Incubation as Measured by the MTT Assay.

Dutasteride	LnCaP	PC3	DU145	CRL-2221		
Concentrations	% Cell	% Cell	% Cell	% Cell		
(µg/well)	Inhibition	Inhibition	Inhibition	Inhibition		
	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)		
0	0 <u>+</u> 0	0 <u>+</u> 0	0 <u>+</u> 0	0 <u>+</u> 0		
0.4	50±5*	78 <u>+</u> 5*	91 <u>+</u> 1*	59 <u>+</u> 1*		
0.8	51 <u>+</u> 7*	84 <u>+</u> 4*	91 <u>+</u> 1*	58 <u>+</u> 1*		
1.6	51±6*	88±2*	91 <u>+</u> 1*	58±1*		
3.2	39 <u>+</u> 6*	86 <u>+</u> 2*	89±0*			
6.3	35 <u>+</u> 5*	84 <u>+</u> 1*	89 <u>+</u> 1*	54 <u>+</u> 2*		
12	36 <u>+</u> 3*	84 <u>+</u> 1*	89 <u>+</u> 1*	54 <u>+</u> 2*		
25	40 <u>+</u> 3*		89 <u>+</u> 1*	55 <u>+</u> 2*		
50	39 <u>+</u> 7*	83±1*	88±1*	54 <u>+</u> 4*		
* = p<0.001						

However, when treated with finasteride, LnCap cells exhibited significant cellular proliferation at the lower concentrations tested. Cellular proliferation was observed at 0.4 µg/well ($173\% \pm 29\%$), 0.8 µg/well ($135\% \pm 23\%$), and 1.6 µg/well ($95\% \pm 20\%$) of finasteride when compared to the control (p<0.001, Table II).

Figure 2

Table 2: Effects of Finasteride on Cellular Growth in Androgen-Dependent and Independent Prostate Cancer Cell Lines and Normal Prostate Cells after 72 Hours of Incubation as Measured by the MTT Assay.

Finasteride	LnCaP	PC3	DU145	CRL-2221
Concentrations	% Cell	% Cell	% Cell	% Cell
(µg/well)	Inhibition	Inhibition	Inhibition	Inhibition
	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)	(Mean <u>+</u> S.D.)
0	0 <u>+</u> 0	0 <u>±</u> 0	0 <u>+</u> 0	0 <u>+</u> 0
0.4	-173 <u>+</u> 29 [#] *	-903 ± 83 ^g *	-662±34 [€] *	-509±54*
0.8	-135 ± 23#*	-726±60 ^g *	-547 <u>+</u> 25 [£] *	-325 ± 25 [£] *
1.6	-95 ± 20 [£] *	-424 ± 74 ^g *	-440±20 [€] *	-157±11#*
3.2	-34 ± 30 ^g	-29 ± 35 ^g	-267 <u>+</u> 73 [£] *	12 <u>+</u> 5
6.3	-12 ± 14 ^g	5 <u>+</u> 15	30 <u>+</u> 13	22 <u>+</u> 1
12	15 <u>+</u> 2	43 <u>+</u> 10	57 ± 2†	23 <u>+</u> 1
25	19 <u>+</u> 3	32 <u>+</u> 7	53±4†	20 <u>+</u> 2
50	19 <u>+</u> 3	48 <u>+</u> 5	$59\pm1\dagger$	20 <u>+</u> 2

A negative value indicates cellular proliferation

* = p<0_001

† = p≤0.05

ANDROGEN-INDEPENDENT PROSTATE CANCER CELL LINES (PC3 AND DU145) PC3 CELL LINE

The androgen-independent cell line, PC3, was treated with dutasteride and significant increases in cell-growth inhibition were observed at all doses tested (mean range: $84\% \pm 3\%$) at 72 hours of incubation (p<0.001, see Table I). The PC3 cells also were treated with finasteride at the same doses. As observed in the LnCap cells, a significant increase in cellular growth was present at the lower doses tested: 0.4μ g/well (903% $\pm 83\%$), 0.8μ g/well (726% $\pm 60\%$), and 1.6μ g/well (424% $\pm 74\%$) of finasteride when compared to the control (p<0.001, see Table II).

DU145 CELL LINE

The androgen-independent cell line DU145 also was tested. Significant reductions in cell growth were observed on all the doses tested (mean range: $90\% \pm 1\%$) at 72 hours of incubation (p<0.001, see Table I). As with the other cells tested, the DU145 cells also were treated with the same doses of finasteride. Cell growth was reduced significantly (p<0.05) with finasteride treatment at 12.5 µg/well ($57\% \pm 2\%$), 25 µg/well ($53\% \pm 4\%$), and 50 µg/well ($59\% \pm 1\%$); however, cell growth also was increased significantly at the

lower doses tested: $0.4 \ \mu$ g/well ($662\% \pm 34\%$), $0.8 \ \mu$ g/well ($547\% \pm 25\%$), $1.6 \ \mu$ g/well ($440\% \pm 20\%$), and $3.2 \ \mu$ g/well ($267\% \pm 73\%$) when compared to the control (p<0.001, see Table II).

NORMAL PROSTATE CELL LINE (CRL-2221)

The normal prostate cell line (CRL-2221) also was treated with various doses of dutasteride. Significant reductions of cell growth were observed at all the doses tested (mean range: $56\% \pm 2\%$) at 72 hours of incubation (p<0.001, see Table I). The CRL-2221 cells also were treated with various doses of finasteride. However, an increase in cell growth was observed in the lower doses of finasteride tested: 0.4 µg/well ($509\% \pm 54\%$), 0.8 µg/well ($325\% \pm 25\%$), and 1.6 µg/well ($157\% \pm 11\%$) when compared to the control (p>0.001, see Table II).

DISCUSSION

With 234,460 new cases of prostate cancer expected to be diagnosed in year 2006, prostate cancer is the most common type of cancer among American men. _{1,2} However, with increased public awareness of the disease, improvements in detection methods, and more effective and, hopefully, less toxic treatments, the number of deaths this year is estimated to decrease to 27,350 cases. This figure represents a 10% decline in estimated deaths attributed to cancer of the prostate.

Standard therapies for cancer of the prostate include radical prostatectomy, cryotherapy, radiation therapy, hormonal therapy, and chemotherapy. The majority of patients with cancer of the prostate respond well to hormonal therapy. Crawford and colleagues reported that the average survival time in patients with metastatic disease who receive hormonal therapy is less than three years, and survival time is only increased by seven months. 8 Despite recent advances in treatment of this malignancy, more effective and potentially less toxic-treatment modalities are urgently needed.

Finasteride and dutasteride are both 5-alpha-reductase inhibitors approved by the Federal Drug Administration (FDA) for treatment of BPH, an enlargement of the prostate gland. Finasteride is a Type II specific inhibitor of 5-alphareductase, whereas dutasteride inhibits both Types I and II isoforms of 5-alpha-reductase. The prostate gland needs androgens to control growth of the normal prostate, and promote BPH and carcinoma of the prostate. 5-alphareductase serves to convert testosterone to dihydrotestosterone (DHT). 5-alpha-reductase is the prime promoter of DHT in the prostate, and converts more than 90% of testosterone to DHT within the prostate gland itself. 4 Not only could use of the 5-alpha-reductase inhibitors (finasteride and/or dutasteride) be effective in reducing the risk of prostate cancer, but they also could be a promising treatment for prostate cancer. 4

Three clinical trials have been developed to evaluate the chemopreventive potential of selenium and vitamin E, finasteride, and dutasteride in prostate cancer. ¹ The ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT) study is a national clinical research trial Sponsored by the National Cancer Institute. The study is designed to review the effects of Selenium and Vitamin E—both separately and together—for prevention of development of prostate cancer. The SELECT study is designed to evaluate these separately, and in combination, and determine their effects in reduction of prostate cancer.

Two of these studies evaluate the use of 5-alpha-reductase inhibitors approved by the FDA for treatment of BPH. The Prostate Cancer Prevention Trial (PCPT) evaluated the use of finasteride, a 5-alpha-reductase inhibitor specific to the Type-2 isoform, to reduce the risk of prostate cancer. The chemopreventive effects of finasteride were evaluated in a multi-center randomized clinical trial conducted by the Southwest Oncology Group in which our institution participated. Thompson and colleages reported that in the 4692 patients randomized to receive the placebo, 1147 (24%) of the men experienced the occurrence of prostate cancer while in the study. Of the 4368 men who had received finasteride during the study, 803 (18%) of these subjects were diagnosed with prostate cancer while participating in the trial. These results indicated that finasteride significantly reduced the prevalence of prostate cancer by 25% more than those randomized to receive placebo. However, 6% of the patients who received finasteride developed prostate cancer during the trial were first seen with high-grade tumors, compared to 5% in the placebo group.

The ongoing use of REDUCE is designed to evaluate the effectiveness of dutasteride in prevention of prostate cancer. ¹¹ Dutasteride, like finasteride, is a 5-alpha-reductase inhibitor, but dutasteride inhibits both Type 1 and Type 2 5alpha-reductase. This study was brought about from the analysis of three randomized clinical trials that evaluated use of dutasteride for treatment of BPH. The men who received dutasteride were reported to have a significantly lower rate of prostate cancer after 27 months on study than those in the control group. ¹¹

Lazier and colleagues compared the effects between dutasteride and finasteride against the LnCaP and PC3 prostate cancer cell lines. The LnCaP and PC3 cells were incubated with testosterone or DHT in steroid-free media with or without increasing concentrations of dutasteride or finasteride at various time points. The results of this study indicated that, when compared, dutasteride was more effective at inhibiting cell growth at higher concentrations $(10 \,\mu\text{M}$ -50 μM) in both cell lines than finasteride. ¹²

Other in vitro studies regarding the effects of 5-alpha reductase inhibitors on prostate cancer have been reported. Schmidt et al. evaluated the effects and mechanisms of action of dutasteride in the androgen-dependent human prostate cancer cells (LNCaP). In their study, LNCaP cells were treated with dutasteride in doses that ranged from $1 \,\mu M$ -100 µM for 24, 48, 72, and 96 hours. The authors reported that after 48 hours of incubation, cell viabilities and cell numbers were reduced with 1 µM of dutasteride, and approximately 50% by addition of 10 µM of dutasteride. Along with the viability and proliferation studies, these authors also reported that gene expression and cellular pathways were altered with treatment of dutasteride, which indicated that this 5-alpha reductase inhibitor may lead to better therapeutic and chemopreventive use in prostate cancer. 4

In this current study, the authors reported significant cellular growth inhibition with various doses of dutasteride in each cell line tested for 72 hours. A mean reduction in cell growth of $43\% \pm 7\%$ was observed in the androgen-dependent prostate cancer cell line, LnCaP; however, the greatest reduction in cell growth was observed in the androgen-independent prostate cancer cell lines, DU145 (mean range: 90% ± 1%), followed by the PC3 cells (mean range: 84% ± 3%). Dutasteride treatment also inhibited cellular growth in the normal prostate cell line, CRL-2221, by 56% ± 2%.

The same authors also evaluated the effects of finasteride on cellular growth in each of the cell lines listed above. Unlike with dutasteride, finasteride, at lower doses ($0.4 \mu M - 1.6 \mu M$) caused significant cellular proliferation in each of the cell lines tested. An increase in cell growth (mean range: $134\% \pm 39\%$) was observed in the LnCaP cell lines. The PC3 cell lines exhibited the greatest increase in proliferation by $685\% \pm 242\%$, followed by a $549\% \pm 111\%$ increase in

the DU145 cells. Significant increases in cell growth also were observed in the normal prostate cell line, CRL-2221, by $330\% \pm 176\%$.

Interestingly, no significant changes in cell growth were observed in the LnCaP, PC3, or CRL-2221 prostate cell lines when treated with higher doses of finasteride. However, at doses of 12.5 µg/well to 50 µg/well, finasteride significantly reduced cell growth by $57\% \pm 3\%$ in the DU145 cells. With these findings, the authors' results support other published data, in that dutasteride may be more effective than finasteride in prevention of prostate cancer growth.

CONCLUSIONS

The significant inhibition of cellular growth exhibited by dutasteride suggests it could have application in treatment or prevention of prostate cancer. The significant difference in decrease in cellular proliferation seen with dutasteride as compared with finasteride suggests that it may be more effective in inhibition of prostate cancer cell growth.

CORRESPONDENCE TO

Stanley Zaslau, MD Associate Professor P.O. Box 9238
Section of Urology, Department Of Surgery Robert C. Byrd
Health Science Center West Virginia University
Morgantown, WV 26506-9238 Phone: (304) 293-2706 FAX:
(304) 293-2807 e-mail: szaslau@hsc.wvu.edu

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Author Information

Stanley Zaslau, MD

Associate Professor, Department of Surgery, Robert C. Byrd Health Science Center, West Virginia University

Adam Perlmutter, DO

Chief Resident, Department of Surgery, Robert C. Byrd Health Science Center, West Virginia University

Dale Riggs, MS

Research Associate, Department of Surgery, Robert C. Byrd Health Science Center, West Virginia University

Barbara Jackson, BA

Research Assistant, Department of Surgery, Robert C. Byrd Health Science Center, West Virginia University

Stanley J. Kandzari, MD

Professor, Department of Surgery, Robert C. Byrd Health Science Center, West Virginia University