Reduction of Ventral Prostate Weight by Finasteride Is Associated with Suppression of Insulin-like Growth Factor I (IGF-I) and IGF-I Receptor Genes and with an Increase in IGF Binding Protein 3

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Abstract

Finasteride, a competitive and specific inhibitor of 5α-reductase, is widely used in the treatment of symptomatic benign prostatic hyperplasia. We demonstrate here that finasteride, when administered in an in vivo experimental system, caused ventral prostate regression. Intraprostatic dihydrotestosterone levels decreased, whereas testosterone levels increased in a dose-dependent manner following finasteride treatment. Finasteride also inhibited the expression of insulin-like growth factor (IGF)-I and IGF-I receptor genes in the ventral prostate. Finasteride significantly increased IGF binding protein-3 and slightly decreased IGF binding protein-2, -4, and -5 gene expression. Because IGFs are potent mitogens for prostate epithelial cells, this newly described activity of finasteride may contribute to its antiproliferative properties, particularly with regard to the inhibition of prostate growth seen clinically and in animal models.

Introduction

The prostate gland requires androgens for proper growth, maintenance, and function. Men with 5α-reductase deficiency (1, 2) and men castrated when young do not develop prostate cancer (3). Androgen-deprivation therapy causes characteristic changes both in the normal prostate and in prostate cancer (4, 5). The 5α-reductase enzyme is a membrane-bound protein dependent on the reduced form of NADP+. It is responsible for the conversion of testosterone to the more potent DHT in androgen-dependent target cells (6, 7). DHT has a greater affinity than testosterone for the androgen receptor and is thought to actively modulate prostate growth. Finasteride acts as a competitive inhibitor of 5α-reductase, resulting in the suppression of serum and intraprostatic DHT concentrations to castrate levels, with subsequent reduction in prostate size (8–10). Inhibition of 5α-reductase activity has been shown in vivo and in vitro (11–14). Finasteride has a safe profile with few side effects (9, 10, 15–17), making it a reasonable candidate for chemoprevention trials in the general and high-risk target populations. Although finasteride is approved for treatment of symptomatic BPH (18), the molecular mechanisms underlying growth inhibition induced by finasteride are incompletely described.

The proliferation of epithelial cells in the prostate is influenced by factors such as epidermal growth factor, TGF-α, TGF-β, nerve growth factor, and members of the IGF and fibroblast growth factor family (19–21). Several studies have indicated that IGFs are mitogenic in prostate tumor cells and normal prostate cells (20). The prostate stroma secretes IGF-I, and the epithelial cells respond to IGFs through the interaction of these growth factors with the type I IGF receptor (20, 22). The principal IGF produced in the rat prostate is IGF-I, whereas in humans, the predominant species is IGF-II (reviewed in Ref. 23).

IGF-I bioactivity in target tissues for IGF-I action is influenced by both serum levels of IGFs and autocrine/paracrine interactions. There is evidence that local expression of IGF-I and various IGFBPs is important in this context and are under complex physiological regulation (24, 25). Both IGF-I and IGF-II have a high affinity for IGFBPs. To date, seven IGFBPs that modulate IGF bioactivity in a complex manner have been characterized (24, 26). These binding proteins are expressed in many tissues and can be found in the conditioned media of a wide variety of cell types. Although the IGFBPs are known to influence the interaction of IGFs with their receptors, their precise physiological roles remain unclear. Both stimulatory and inhibitory effects of IGFBPs on cellular proliferation have been reported under various experimental conditions (27–32).

Normal prostate epithelial cells have been shown to secrete IGFBP-2 and IGFBP-4, whereas stromal fibroblasts produce IGFBP-2, -3, and -4 (20, 33, 34). In the adult prostate, IGFBP-2 is expressed, while IGFBP-5 expression is repressed (19). We have shown previously that the gene expression of IGFBP-2, -3, -4, and -5 increases rapidly in the ventral prostate during castration-induced involution (35). The physiological actions of IGFBPs in prostate cells are not known. It has been hypothesized that IGFBPs attenuate the cellular response to IGF-I through the high-affinity binding of IGF-I to IGFBPs. This interaction sequesters IGF-I away from the receptor, interfering with the normal homeostatic intracellular signaling downstream of the receptor. It is also known that some IGFBPs have intrinsic bioactivity independent of IGFs (31, 36, 37).

We show here that finasteride reduces ventral prostate weight in part by enhancing IGFBP-3 expression, suppressing IGF-I gene expression, and reducing IGF-I receptor expression. Our preliminary data, together with the antiproliferative effect of finasteride in BPH, suggest that finasteride might serve to suppress prostate cell growth by disrupting IGF autocrine/paracrine loops.

Materials and Methods

Animals. Animals were maintained and treated according to the guidelines of the Canadian Council on Animal Care. The experimental protocol was approved by the Local Animal Care Committee. Male Sprague Dawley rats were treated with finasteride by gavage in increasing doses of 0.0, 0.1, 1.0, and 100 mg/kg BW/day. Finasteride 5-mg tablets (Merck Frosst, Montreal, Quebec, Canada) were crushed and suspended in water immediately before gavage. Each group of rats received either 1 ml of the 0.1, 1, and 10 mg/kg BW finasteride suspensions or 3 ml of the 100 mg/kg BW dose, every day for 21 days. Control rats received only water. At the end of the treatment period, rats were killed by decapitation, and the ventral prostate was removed and weighed. Tissues were fixed in formalin, embedded in paraffin, and cut into 6-μm sections for routine histological examination. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1 This work was supported by Grant 778 from the Cancer Research Society and a grant from the Fonds de la Recherche en Santé du Québec (to H.H.). 2 To whom requests for reprints should be addressed, at Lady Davis Research Institute, McGill University, 3755 Cote Ste Catherine Road, Montreal, Quebec, H3T 1E2 Canada. Phone: (514) 340-8260, extension 5263; Fax: (514) 340-7502. 3 The abbreviations used are: DHT, dihydrotestosterone; BPH, benign prostatic hyperplasia; TGF, transforming growth factor; IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; IGFBP, IGF binding protein; BW, body weight.
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REDUCTION OF VENTRAL PROSTATE WEIGHT BY FINASTERIDE

Fig. 1. Effects of finasteride on ventral prostate weight and tissue androgen levels. Male rats (n = 8) received different doses of finasteride, as indicated, daily for 21 days. Ventral prostate weight (A) and tissue androgens (B) are shown. SE (bars) at each point was < 10%. Ventral prostate weight significantly decreased in all finasteride-treated groups compared to vehicle alone (P < 0.05). Testosterone (T) and dihydrotestosterone (DHT) levels were determined, as described in "Materials and Methods." Testosterone levels significantly increased, while DHT levels significantly decreased compared with vehicle alone (P < 0.01).

Fig. 2. Effects of finasteride on IGF-I and IGFBP gene expression in ventral prostate. Rats were treated with various doses of finasteride as indicated. Total RNA from the ventral prostate was extracted, and Northern blotting was performed as described (38). Blots were hybridized with rat IGF-I cDNA (46) and rat IGFBP (from IGFBP-2 to IGFBP-5) cDNAs (47). Representative autoradiograms of IGF-I (A), IGFBP-3 (B), and IGFBP-2, -4, and -5 (D) and a representative densitometric scanning of IGF-I and IGFBP-3 mRNAs (C; bars, SE) are indicated. IGF-I gene expression was significantly inhibited in all finasteride-treated groups compared with vehicle alone (P < 0.01). IGFBP-3 gene expression was significantly enhanced at 0.1, 1, 10, and 100-mg doses compared with vehicle alone (P < 0.05). In Lane U, uterus RNA serves as positive control.

Northern Blot. Total RNA was isolated from ventral prostate using the RAZol B method (Teltest) as described (38). Northern blotting was performed on total RNA to detect any changes in the levels of IGF-I, IGF-IR, and IGFBP (from IGFBP-2 to IGFBP-5) gene expression following treatment. mRNA levels were quantitated by densitometric scanning of autoradiograms.

IGF-IR Binding Assay. Membrane fraction was extracted from the ventral prostate. Affinity labeling experiments were performed on membrane protein using 125I-labeled IGF-I as a ligand to detect changes in IGF-I receptor levels, as described previously (39).

Tissue Androgen Determination. Intraprostatic testosterone and DHT levels were measured in 100–200 mg of homogenized prostate tissue by RIA after extraction in Delsal's solution (4:1 methylal:methanol). The androgens were purified over silica columns and separated by celite chromatography as described previously (40). Mean assay sensitivities (assuming a mean recovery) were 0.62 nmol/kg tissue (0.18 ng/g) for testosterone and 0.72 nmol/kg tissue (0.21 ng/g) for DHT. Interassay coefficients of variation for the entire procedure were 11.1% for testosterone and 9.9% for DHT. Statistical significance of differences was determined by Student's t test.

Results

Male rats given various doses of finasteride daily for 21 days did not show any statistical difference in BW (data not shown). Finasteride treatment, however, caused significant reduction in ventral prostate weight, even at the dose of 0.1 mg/kg BW (P < 0.05; Fig. 1A). Analysis of ventral prostate tissue androgens revealed a dose-dependent increase in testosterone and a decrease in DHT following finasteride administration (Fig. 1B).

Because IGF-I is mitogenic for prostate epithelial cells, changes in the expression of IGF-I, IGF-IR, and IGFBP-2, -3, -4, and -5 in the ventral prostate following finasteride treatment were investigated. Fig 2A shows that finasteride down-regulated IGF-I gene expression in a dose-dependent manner. IGFBP-3 gene expression in this tissue increased in a dose-dependent manner (Fig. 2B). Although mRNA levels of IGFBP-2 were not affected by finasteride treatment, IGFBP-4 and IGFBP-5 mRNA levels slightly decreased (Fig. 2D). There is a positive correlation between IGF-I gene expression and ventral prostate weight and an inverse correlation between ventral
IGFBP-3 gene expression and ventral prostate weight and a reciprocal regulation by finasteride of prostate IGFBP-3 and IGF-I gene expression. The data also provide an additional molecular mechanism by which finasteride affects IGF physiology in the prostate gland. The relative importance of regulation of genes involved in IGF physiology in the mediation of finasteride effects cannot be determined from our data. However, in view of our findings, it is possible that the in vivo antiproliferative effects of finasteride are attributed to the suppression of IGF-1 autocrine/paracrine loops, which in turn reduce ventral prostate weight as described.

We hypothesize that finasteride-induced IGFBP-3 attenuates prostate cell response to IGF-I through the high-affinity binding of IGF-I to IGFBP-3, thereby interfering with the normal homeostatic intracellular signaling downstream of the IGF-I receptor. The combined effects of finasteride to increase IGFBP-3 and decrease IGF-I and IGF-IR expression would be expected to interfere with the activity of IGFs, which are potent mitogens and antiapoptotic agents for many normal and neoplastic cell types (41, 42), including normal and transformed prostate epithelial cells. Furthermore, there is evidence that finasteride-induced IGFBP-3 may inhibit prostate cell proliferation by an IGF-independent pathway (36, 37, 43).

It has been proposed that the combination of finasteride with other antiproliferative agents may decrease prostate cancer risk in target populations such as those who have a family history of prostate cancer. Combination therapy may decrease overall side effects by lowering effective doses and shortening treatment durations. The presence of vitamin D receptors in prostate tissue and prostate cancer cells (44) indicates that the cells are responsive to vitamin D. In light of our present results, it is possible that low doses of finasteride can be used in combination with low doses of vitamin D or vitamin D analogues in the treatment of BPH and prostate cancer. By combining the two, side effects of finasteride related to sexual dysfunction and hypercalcemia associated with high-dose vitamin D administration can be reduced or eliminated while retaining the desired therapeutic goals.

In this report, we observed that finasteride up-regulates IGFBP-3 in the ventral prostate. Because IGFBP-3 has been shown to induce apoptosis in PC-3 cells (36), it will therefore be of interest to determine the effect of finasteride on apoptosis in the ventral prostate. Because previous reports demonstrate that TGF-β induces IGFBP-3 expression in certain tissues (45), it is possible that the up-regulation of IGFBP-3 by finasteride is due to increased TGF-β. However, we observed that finasteride only causes mild changes in TGF-β1 in the ventral prostate (data not shown).

In summary, our data demonstrate that finasteride reduces ventral prostate weight, inhibits prostatic IGF-I gene expression, decreases IGF-IR expression, and up-regulates IGFBP-3 gene expression. These results provide further understanding of the mechanism of action of finasteride in the treatment and prevention of prostate cancer.

Acknowledgments

We thank Merck Frosst (Montreal, Quebec, Canada) for the finasteride, Dr. Shimakasi for the rat IGFBP cDNAs, and Alexis Codrington for manuscript preparation.

References

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