The Prostate 42:203–210 (2000)

Prostatic Involution in Men Taking Finasteride Is Associated With Elevated Levels of Insulin-like Growth Factor-Binding Proteins (IGFBPs)-2, -4, and -5

Lynn N. Thomas,[1,2]* A. Stuart Wright,[1,2] Catherine B. Lazier,[3] Pinchas Cohen,[4] and Roger S. Rittmaster[1,2]

[1]*Department of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada*
[2]*Department of Physiology/Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada*
[3]*Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada*
[4]*Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania*

BACKGROUND. Insulin-like growth factor-binding proteins (IGFBPs)-2, -4, and -5 are associated with upregulation of apoptosis in the ovary. The purpose of this study was to assess the roles of IGF-I and IGFBPs during involution of the prostate. Frozen and fixed tissue was collected by transurethral prostatectomy from Caucasian men, aged 52–82 years, scheduled for prostatectomy for benign prostatic hyperplasia, who took either placebo (n = 7) or the 5α-reductase inhibitor finasteride for 6 days to 6 years (n = 15) prior to surgery.
METHODS. Intraprostatic androgen levels were measured by radioimmunoassay. Tissues were immunostained for IGF-I and IGFBP-2, -3, -4, and -5, and staining was quantitated by computerized image analysis. Serial sections were stained for markers of apoptosis (TUNEL and tissue transglutaminase) and IGFBP-2, -4, or -5.
RESULTS. IGF-I staining was significantly decreased in the medium-term (18–43 days) treatment group and remained so for the duration of the study ($P = 0.026$). IGFBP-3 staining was unchanged in the early and medium-term treatment groups; however, a transient earlier rise in the level of this proapoptotic protein cannot be ruled out. The percentage of epithelial cell area staining positively for IGFBP-2 increased significantly, from 1.6 ± 0.5 in the placebo group to 12.0 ± 2.0 ($P < 0.0001$), and 7.6 ± 1.9 ($P = 0.003$) in the short (6–13 days) and medium-term treatment groups, respectively. IGFBP-4 staining increased from 2.2 ± 0.6 to 9.8 ± 1.9 ($P < 0.0001$) and 7.4 ± 1.2 ($P = 0.004$) in the short and medium-term groups, respectively, and IGFBP-5 staining increased from 0.2 ± 0.1 to 3.8 ± 2.0 ($P = 0.004$) in the medium-term group. The results from serial sections showed that IGFBP-2 and -4 costained with markers of apoptosis, while IGFBP-5 did not.
CONCLUSIONS. These results indicate that IGFBP-2, -4, and -5 are associated with prostatic involution. Because of the timing and distribution of expression, we hypothesize that IGFBP-2 and -4 have a role as signals for apoptosis, but that IGFBP-5 likely does not. *Prostate 42:203–210, 2000.*

KEY WORDS: **apoptosis; TUNEL; immunocytochemistry; IGF-I**

INTRODUCTION

Testosterone, the major circulating androgen, must be converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase for normal prostatic growth and

*Correspondence to: Lynn N. Thomas, Department of Physiology and Biophysics, 11K Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.
E-mail: lnthomas@is.dal.ca
Received 12 April 1999; Accepted 7 September 1999

secretory activity [1]. The 5α-reductase inhibitor finasteride prevents the conversion of testosterone to DHT, resulting in involution of the prostate through a combination of atrophy and apoptosis [2].

The stimulatory effect of DHT on prostatic growth and secretory activity is likely due in part to modulation of intraprostatic growth factors such as insulin-like growth factor-I and -II (IGF-I and -II). IGFs promote cell growth and differentiation in many tissues, including the prostate, through interactions with IGF receptors [3]. Regulation of IGF activity occurs to some extent via hormonal control of insulin-like growth factor-binding protein (IGFBP) concentrations. IGFBPs are a family of at least six proteins that modify IGF action; they can inhibit IGF activity by reducing its bioavailibility through receptor competition [4–7], or enhance IGF activity by an unknown mechanism which may be related to an ability to facilitate ligand presentation to IGF receptors [8–10]. IGFBP-4 and -5 have been proposed by Erickson et al. [11,12] to be signals for apoptosis during follicular atresia in the ovary. Several studies support this hypothesis and indicate that IGFBP-2 also may be associated with follicular atresia [13–15]. In rats, we found that increased IGFBP-5 staining occurred during prostatic involution, but that the timing and distribution of staining indicated that its expression was a result of, rather than a trigger for, apoptosis [16].

The purpose of this study was to assess the role of IGF-I and IGFBPs during involution of the prostate in men taking finasteride, and to investigate the relationship between apoptosis and IGFBP-2, -4, and -5 in the human prostate. Immunocytochemical techniques were used to examine the prostates of men taking placebo or 5 mg finasteride daily for 6 days to 6 years. Tissues were collected at the time of prostatectomy and immunostained for IGF-I or IGFBP-2, -3, -4, or -5. Image analysis was used to quantitate the changes in IGF-I and IGFBP protein expression during involution. The relationship between programmed cell death and IGFBPs was further clarified by staining serial sections for IGFBP-2, -4, or -5 and for markers of apoptosis (DNA fragmentation [17] and tissue transglutaminase [18]) and comparing the distribution of staining.

MATERIALS AND METHODS

Patient Selection

The prostates used for this research were collected from patients who underwent transurethral prostatectomy for symptoms of benign prostatic hyperplasia (BPH). Six patients received finasteride (for 23–92 days) as part of a double-blind, placebo-controlled study previously done to investigate the effects of finasteride on intraprostatic hormone concentrations [19]. Those patients randomized to placebo in that study, plus two additional patients, provided the control prostates for the current work. One patient received finasteride for 4 years as part of a phase III clinical study, and other patients received finasteride for up to 6 years outside of any research protocol before undergoing prostatectomy. Patients who were treated with finasteride received 5 mg daily. Prostatectomies were performed for relief of BPH symptoms; patients with previously diagnosed or suspected cancer were excluded from the study. The decision to undergo prostatectomy was made before and independent of participation in this study. The Camp Hill Medical Centre Research Ethics Committee (Halifax, Nova Scotia, Canada) approved all protocols, and those patients involved in clinical studies provided written informed consent. All patients were Caucasian, and their ages ranged from 52–82 years, with a mean age of 68. No patient was taking other medication known to influence androgen action or prostate function.

Tissue Collection

Prostate tissue was collected at the time of transurethral prostatectomy. The men who received finasteride were divided into three groups, based on the duration of treatment: 6–13 days (n = 5), 18–43 days (n = 4), and 70 days to 6 years (n = 6). The placebo group contained seven prostates. The first chips of periurethral prostatic tissue resected were recovered. A piece of each tissue was placed immediately into liquid nitrogen and frozen at −80°C in order that intraprostatic testosterone and DHT could be assayed to confirm patient compliance. Intraprostatic hormone levels for patients taking placebo ranged from 0.01–0.7 ng/g of tissue for testosterone and 4.23–8.07 ng/g for dihydrotestosterone (DHT). Intraprostatic hormone levels for patients taking finasteride ranged from 1.76–4.01 ng/g for testosterone and 0.09–0.71 ng/g for DHT. The remaining tissue was prepared for histological examination by formalin fixation followed by paraffin embedding. Five-micron sections were cut from the blocks and dried on slides for 1 hr at 57°C, or overnight at room temperature. Sections were deparaffinized in xylene and rehydrated through decreasing concentrations of alcohol to phosphate-buffered saline (PBS).

Immunostaining for IGFBPs

Deparaffinized sections were incubated with 5% horse serum for 60 min at room temperature to block nonspecific binding activity, and then incubated with

primary antibody overnight at 4°C in 100% humidity. The antibody for IGF-I was a rabbit polyclonal directed against human IGF-I, which was obtained from the National Hormone and Pituitary Program (Torrance, CA). It was used at a dilution of 1:200. The antibody for IGFBP-2 was a rabbit anti-bovine polyclonal from Upstate Biotechnology, Inc. (Waltham, MA), which cross-reacts with the human species [20]; it was used at a dilution of 1:500. The antibody for IGFBP-3, which was a gift from Dr. Ron Rosenfeld (Oregon Health Sciences University, Portland, OR), was a rabbit anti-human polyclonal [21], and it was used at a dilution of 1:200. The antibodies for IGFBP-4 and -5 were rabbit anti-human polyclonals supplied by Dr. Michael Kiefer (Chiron Corp., Emeryville, CA) [22]. Both were used at a dilution of 1:500. None of these antibodies has greater than 1% cross-reactivity with IGFs or IGFBPs other than the one for which each was developed. After incubation with the appropriate antibodies, tissues were washed in PBS, incubated with biotinylated goat anti-rabbit IgG (Dimension Laboratories, Mississauga, Ontario, Canada) for 60 min at room temperature, washed in PBS, and incubated for 60 min at room temperature with streptavidin-alkaline phosphatase (Dimension Laboratories). Alkaline phosphatase activity was detected with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The tissue was counterstained with methyl green. Ducts were randomly selected, and image analysis was performed on those selected. Negative controls included tissues from which the primary antibody was excluded and those for which normal rabbit serum was substituted for the primary antibody. No specific staining was seen in either case.

Immunostaining for Tissue Transglutaminase (tTG)

Deparaffinized sections were immersed in 2% hydrogen peroxide to inactivate endogenous peroxidase activity, and then treated as described above for IGFBPs, with the following alterations: the primary antibody was rabbit anti-human tTG, used at a dilution of 1:1,000, and it was produced by NeoMarkers (Fremont, CA); streptavidin-HRP was used instead of streptavidin-alkaline phosphatase, and peroxidase activity was visualized with 3-amino-9-ethyl carbazole; counterstaining was done with Mayer's hematoxylin. The primary antibody has been shown by the manufacturer to be highly specific to tissue transglutaminase; it does not cross-react with other transglutaminases.

Staining for DNA Breaks

A modification of the TUNEL technique for in situ end-labeling of fragmented DNA, an early apoptotic event, was used as previously described [17,23].

Image Analysis

A JVC model TK-1070U video camera (JVC Canada, Scarborough, Ontario, Canada) and a Nuvista+ frame grabber board (Truevision, Indianapolis, IN) were used to capture microscopic images of immunostained tissues to a computer. Five areas containing epithelium were randomly selected for each tissue, and Adobe Photoshop (Adobe Systems, Inc., Mountain View, CA) was used to divide the epithelial and stromal fractions of the images so that they could be analyzed separately. In the epithelial fraction of the images, Adobe Photoshop was also used to exclude the lumen of the ducts. The software package NIH Image (Web site: rsb.info.nih.gov/NIH-IMAGE/) was used on a Power Macintosh 6100/60 computer to analyze the images. A threshold was chosen which best represented all of the significant staining, and NIH Image calculated the area of all pixels that met or exceeded the threshold; the results were displayed as a percent of total area. Higher and lower thresholds were also utilized to determine whether variations in staining intensity would affect the results. The results were unchanged except for degree of significance; therefore, only the results from the original threshold are reported here. Because epithelial cell area decreases with finasteride treatment, the staining percentages obtained from NIH Image were adjusted appropriately, depending on which group they were in. Data from a previous study [2] allowed us to determine that epithelial cell area was reduced by 10.2%, 15.7%, and 57.9% in the short-, medium-, and long-term groups, respectively.

Comparison of Apoptosis and IGFBP-2, -4, and -5 Staining

Pairs of 5-μm serial sections from the prostates of 20- and 23-day finasteride-treated patients were in situ end-labeled for fragmented DNA (TUNEL staining) and stained for IGFBP-2, -4, or -5, or stained for tTG and IGFBP-2, -4, or -5. Identical fields of view were photographed using a JVC model TKF7300U high-resolution frame-capture camera on a Leitz (Wetzlar, Germany) research-grade microscope connected to a Macintosh Power PC 7100/80 computer. TUNEL and tTG staining were compared to IGFBP-2, -4, and -5 staining to determine whether the cells undergoing apoptosis were the same cells staining for the IGFBPs.

Statistics

Results are expressed as mean ± SEM. Statistics were performed using StatView (Abacus Concepts, Berkeley, CA) on a Macintosh computer. Group means were compared using analysis of variance followed by Fisher's protected least significant difference test. $P < 0.05$ was considered statistically significant.

RESULTS

Immunostaining for IGF-I and IGFBPs

Staining for IGF-I was significantly reduced, compared to placebo, in the medium ($P = 0.026$) and long ($P = 0.022$)-term treatment groups (Fig. 1a). IGFBP-3 was significantly reduced only in the long-term treatment group ($P = 0.005$) (Fig. 1b). The percentage of epithelial cell area staining positively for IGFBP-2, -4, and -5 was increased by finasteride treatment as follows: staining for IGFBP-2 significantly increased from 1.6 ± 0.5 for the placebo group to 12.0 ± 2.0 ($P < 0.0001$) and 7.6 ± 1.9 ($P = 0.003$) for the short- and medium-term groups, respectively (Fig. 1c). Staining for IGFBP-4 significantly increased, from 2.2 ± 0.6 in the placebo group to 9.8 ± 1.9 ($P < 0.001$) and 7.4 ± 1.2 ($P = 0.004$) in the short- and medium-term groups, respectively (Fig. 1d). The percentage of epithelial cell area staining positively for IGFBP-5 significantly increased, from 0.2 ± 0.1 in the placebo group to 3.8 ± 2.0 ($P = 0.004$) in the medium-term treatment group (Fig. 1e). In the stroma, no significant changes were seen in IGF-I or any of the IGFBP proteins.

Comparison of Staining in Serial Sections

Staining for IGFBP-2, -4, and -5 was elevated in at least one of the finasteride-treatment groups, and because a previous study demonstrated that the number of apoptotic cells is also increased with finasteride treatment [2], the relationship between the distribution of IGFBPs and the distribution of apoptotic cells was examined. This was done by comparing staining for apoptosis, as assessed by both the TUNEL method and by tTG staining, and staining for IGFBP-2, -4, or -5 in serial sections of the 20- and 23-day finasteride-treated specimens. These time points were chosen, because staining for both markers of apoptosis, as well as for all of the IGFBPs of interest, was at least partially elevated. IGFBP-2, which peaked in the short-term treatment group, costained with TUNEL and tTG, although not exclusively (Figs. 2b, 3b). IGFBP-4 staining was less discrete; it appeared, at least faintly, in most ducts during the early stages of involution and also costained with TUNEL and tTG (Figs. 2c, 3c). IGFBP-5 was more selectively expressed than both IGFBP-2 and -4, as can be concluded from the relatively low percentages of epithelial cell area staining for IGFBP-5 (Fig. 1e). IGFBP-5, which peaked in the medium-term treatment group, did not costain with either TUNEL or tTG (Figs. 2d, 3d). The areas of tissue which showed extensive apoptosis showed virtually no staining for IGFBP-5, and the areas of tissue which were heavily stained for IGFBP-5 showed little or no staining for TUNEL or tTG.

DISCUSSION

IGFBP-2, -4, and -5 have been implicated in several studies as possible signals for apoptosis in the ovary. Erickson et al. [11,12] discovered that IGFBP-4 and -5 mRNAs are expressed in atretic follicles but not in dominant follicles of the rat ovary. Monget et al. [13] determined that low molecular weight IGFBP (IGFBP-2, -4, and -5) concentrations are increased in the follicular fluid of atretic follicles compared to dominant follicles in the ewe ovary, and Cataldo and Giudice [15] reported similar results from human follicular fluid. The accumulated evidence of these and other studies has led to the theory that some IGFBPs are signals for apoptosis in the ovary.

In a previous study, we induced prostatic involution in rats, either by castration or by treatment with the 5α-reductase inhibitor finasteride, and examined the possibility that IGFBP-5 may be a signal for apoptosis during prostatic regression; we concluded that while IGFBP-5 was intimately associated with the process of involution, it probably acts by inhibiting cell proliferation rather than by initiating programmed cell death [16]. Finasteride also induces prostatic involution in men. The current study used immunocytochemistry techniques to examine the relationship between DHT withdrawal and IGFBP and IGF-I protein expression in the human prostate. Results indicate that IGFBP-2, -4, and -5 protein levels increase within a specific time period during involution. This is consistent with results, discussed above, of studies done on the ovary which have associated increased IGFBP-2, -4, and -5 concentrations with decreased IGF activity. It does, however, differ from the results obtained by Huynh et al. [24] and Nickerson et al. [25] in their studies on the rat prostate. They determined that IGFBP-2, -4, and -5 mRNA levels decrease slightly after finasteride treatment. Two factors probably account for the differences between these studies: Huynh et al. [24] examined rat prostates while we examined human prostates, and Huynh et al. [24] studied mRNA, which does not always reflect changes at the physi-

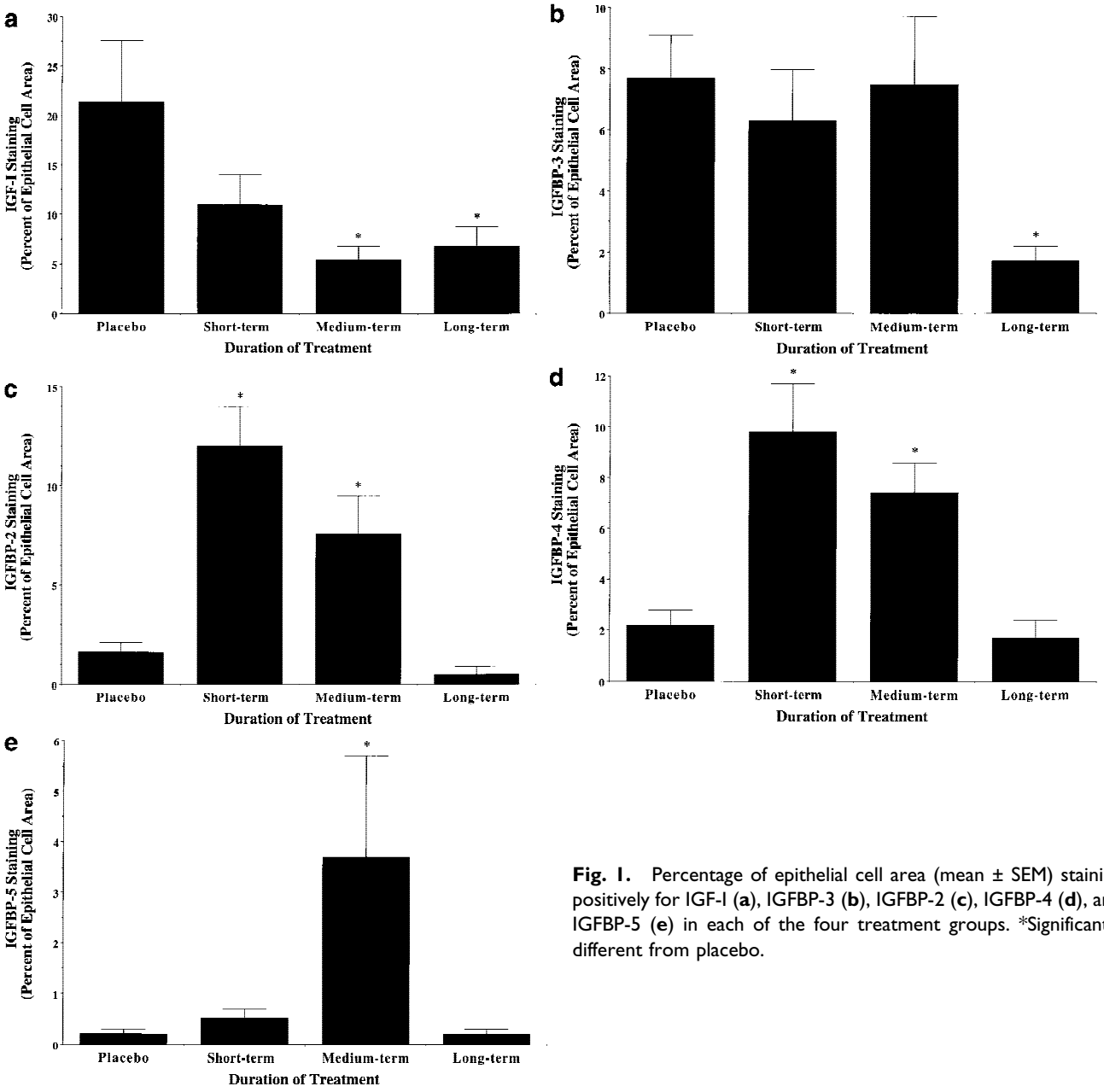

Fig. 1. Percentage of epithelial cell area (mean ± SEM) staining positively for IGF-I (**a**), IGFBP-3 (**b**), IGFBP-2 (**c**), IGFBP-4 (**d**), and IGFBP-5 (**e**) in each of the four treatment groups. *Significantly different from placebo.

ological level, whereas we examined protein expression. IGFBPs often undergo post-translational modifications, particularly by proteases [21,26].

Tennant et al. [27,28] demonstrated that IGFBP-2, -4, and -5 significantly increase in prostate carcinoma vs. benign prostate tissue. Although this appears to conflict with the results of the current study, which indicate that IGFBP-2, -4, and -5 increase during prostate involution, their suggestion that IGFBP-4 actually functions as a growth suppressor expressed in response to the uncontrolled cell growth which occurs in a malignancy is consistent with our results. This also may be true for IGFBP-2 and -5, although these binding proteins may have differential abilities to either potentiate or inhibit IGF action, depending on whether they are membrane-bound [4,10,29].

Comparison studies on serial sections were done in the present study to examine further the relationship between apoptosis and IGFBP-2, -4, and -5 during prostatic involution. Results indicate that IGFBP-5 is not directly related to apoptosis, whereas IGFBP-2 and -4 may be. IGFBP-5 did not costain with either marker of apoptosis, and staining for IGFBP-5 peaked in the middle treatment group; from a previous study, we know that TUNEL staining peaks in the early treatment group [2]. IGFBP-2 and -4 did costain with

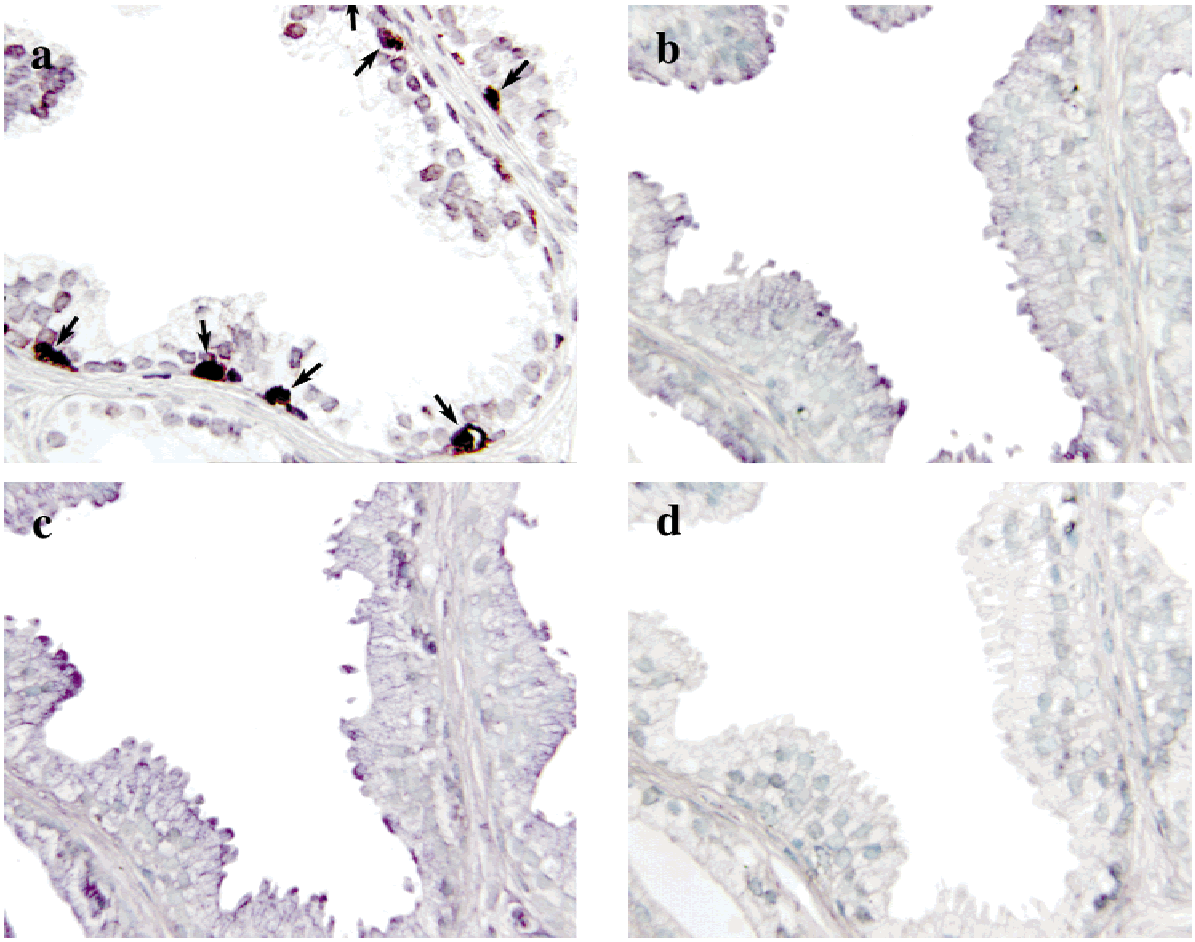

Fig. 2. Tissue from the prostate of a patient treated for 20 days with finasteride, stained for DNA fragmentation (**a**, arrows), IGFBP-2 (**b**), IGFBP-4 (**c**), and IGFBP-5 (**d**). Staining for IGFBPs is purple with green nuclear counterstain. IGFBP-2 and -4 are present in the ducts that show extensive DNA fragmentation, whereas IGFBP-5 does not appear in these ducts. Other areas that showed extensive IGFBP-5 staining, showed no TUNEL (or tTG) staining (data not shown). Although a separate TUNEL-stained section was used for each comparison, only one example of TUNEL staining is shown, to avoid repetition.

TUNEL and tTG staining, although not exclusively, and both peaked in the early treatment group.

In contrast with IGFBP-2, -4, and -5, IGFBP-3 protein expression did not increase with finasteride-treatment. It is known that IGFBP-3 induces apoptosis in the prostate cancer cell line PC-3 and in the breast cancer cell line MCF-7 [30,31]. It has also been shown that IGFBP-3 mRNA levels are increased within 6–24 hr in the rat ventral prostate after castration [25]. The fact that no increase in IGFBP-3 staining was seen in the current study with humans may be because an increase occurred at an earlier time point, possibly associated with a transient wave of apoptosis. Alternatively, it is possible that an increase in IGFBP-3 occurred that went undetected because the antibody used recognizes at least some proteolytic fragments [21]. Prostate-specific antigen (PSA) is known to be an IGFBP-3 protease [21], and finasteride treatment causes PSA levels to drop [32]; therefore, IGFBP-3 activity may be low in the untreated prostate due to proteolytic cleavage by PSA, and increased during involution because it is no longer being degraded. Tennant et al. [27] published results that support the hypothesis that controlled proteolysis may be an important factor in determining the amount of IGFBP-3 activity in the prostate.

CONCLUSIONS

IGFBP-2, -4, and -5 increase following finasteride-induced involution of the human prostate. Results are consistent with the hypothesis that IGFBP-2 and -4 may have a role in triggering apoptosis. However, IGFBP-5 probably does not have such a role because it does not stain in ducts with apoptotic activity and is expressed later, relative to apoptosis.

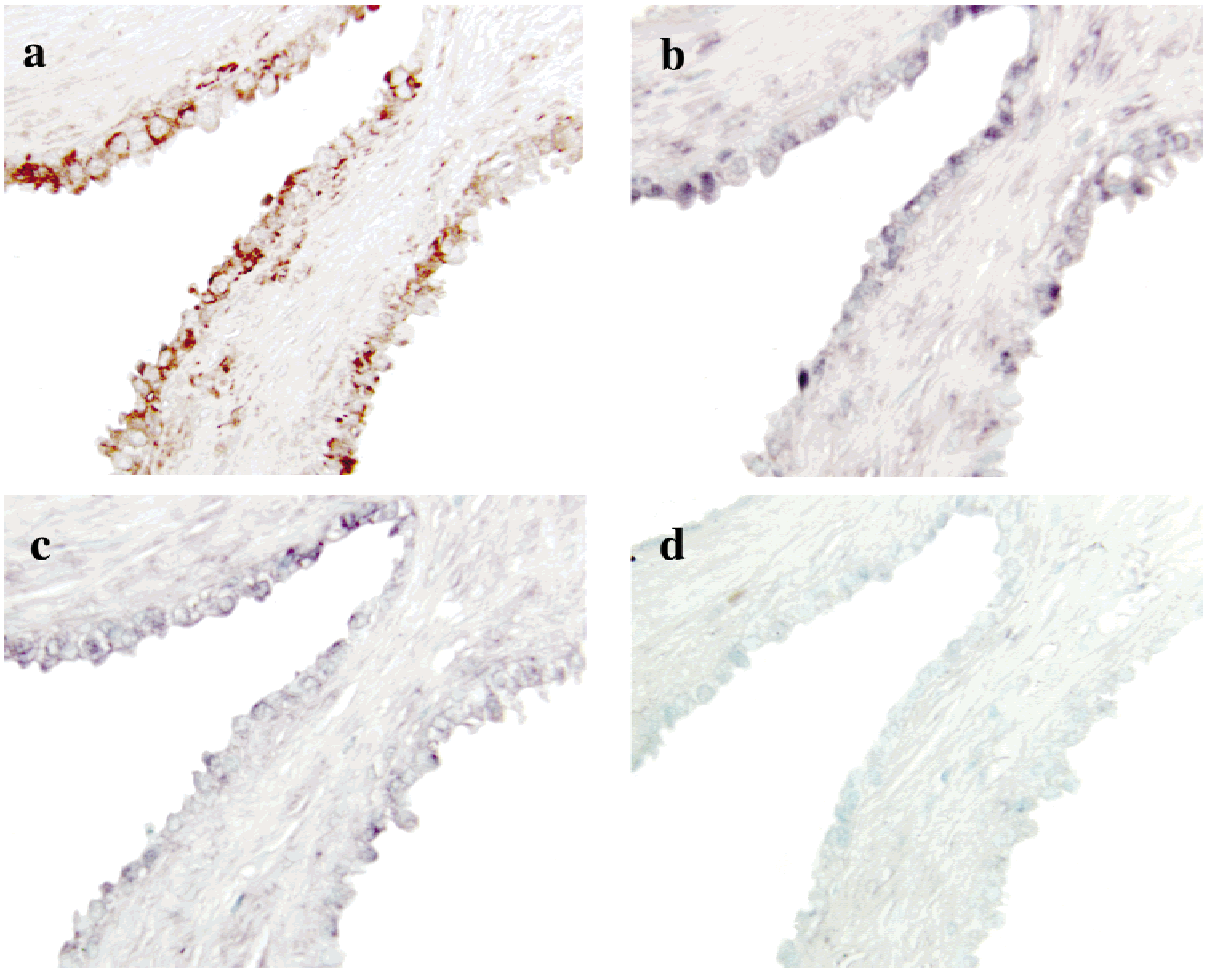

Fig. 3. Tissue from the prostate of a patient treated for 20 days with finasteride, stained for tTG (**a**), IGFBP-2 (**b**), IGFBP-4 (**c**), and IGFBP-5 (**d**). Staining for tTG is red with blue nuclear counterstain, and staining for IGFBPs is purple with green nuclear counterstain. IGFBP-2 and -4 are present in the same ducts as tTG, whereas IGFBP-5 is not present in these ducts. As with Figure 2, only one example of tTG staining is shown.

REFERENCES

1. Cunha GR, Donjacour AA, Cooke PS, Mee S, Bigsby RM, Higgins SJ, Yoshiki S. The endocrinology and developmental biology of the prostate. Endocr Rev 1987;8:338–362.
2. Rittmaster RS, Norman RW, Thomas LN, Rowden G. Evidence for atrophy and apoptosis in the prostates of men given finasteride. J Clin Endocrinol Metab 1996;81:814–819.
3. Cohick WS, Clemmens DR. The insulin-like growth factors. Annu Rev Physiol 1993;55:131–153.
4. Conover CA, Kiefer MC. Regulation and biological effect of endogenous insulin-like growth factor binding protein-5 in human osteoblastic cells. J Clin Endocrinol Metab 1993;76:1153–1159.
5. Perkel VS, Mohan S, Baylink DJ, Linkhart TA. An inhibitory insulin-like growth factor binding protein (In-IGFBP) from human prostatic cell conditioned medium reveals N-terminal sequence identity with bone derived In-IGFBP. J Clin Endocrinol Metab 1990;71:533–535.
6. Burch WM, Correa J, Shively JE, Powell DR. The 25-kilodalton insulin-like growth factor (IGF)-binding protein inhibits both basal and IGF-I-mediated growth of chick embryo pelvic cartilage in vitro. J Clin Endocrinol Metab 1990;70:173–180.
7. Conover CA, Ronk M, Lombana F, Powell DR. Structural and biological characterization of bovine insulin-like growth factor binding protein-3. Endocrinology 1990;127:2795–2803.
8. Conover CA. Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3: studies of underlying mechanism. Endocrinology 1992;130:3191–3199.
9. Clemmons DR, Gardner LI. A factor contained in plasma is required for IGF binding protein-1 to potentiate the effect of IGF-I on smooth muscle cell DNA synthesis. J Cell Physiol 1990;145:129–135.
10. Jones JI, Gockerman A, Busby WH Jr, Camacho Hubner C, Clemmons DR. Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-I. J Cell Biol 1993;121:679–687.
11. Erickson GF, Li D, Sadrkhanloo R, Liu, Shimasaki S, Ling N. Extrapituitary actions of gonadotropin-releasing hormone: stimulation of insulin-like growth factor-binding protein-4 and atresia. Endocrinology 1994;134:1365–1372.
12. Erickson GF, Nakatani A, Ling N, Shimasaki S. Localization of insulin-like growth factor-binding protein-5 messenger ribonucleic acid in rat ovaries during the estrous cycle. Endocrinology 1992;130:1867–1878.

13. Monget P, Monniaux D, Pisselet C, Durand P. Changes in insulin-like growth factor-I (IGF-I), IGF-II, and their binding proteins during growth and atresia of ovine ovarian follicles. Endocrinology 1993;132:1438–1446.
14. Mondschein JS, Etherton TD, Hammond JM. Characterization of insulin-like growth factor-binding proteins of porcine ovarian follicular fluid. Biol Reprod 1991;44:315–320.
15. Cataldo NA, Giudice LC. Follicular fluid insulin-like growth factor binding protein profiles in polycystic ovary syndrome. J Clin Endocrinol Metab 1992;74:695–697.
16. Thomas LN, Cohen P, Douglas RC, Lazier C, Rittmaster RS. Insulin-like growth factor binding protein 5 is associated with involution of the ventral prostate in castrated and finasteride-treated rats. Prostate 1998;35:273–278.
17. Gavrieli Y, Sherman Y, Ben Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
18. Piacentini M, Autuori F, Dini L, Farrace MG, Ghibelli L, Piredda L, Fesus L. "Tissue" transglutaminase is specifically expressed in neonatal rat liver cells undergoing apoptosis upon epidermal growth factor-stimulation. Cell Tissue Res 1991;263:227–235.
19. Norman RW, Coakes KE, Wright AS, Rittmaster RS. Androgen metabolism in men receiving finasteride before prostatectomy. J Urol 1993;150:1736–1739.
20. Cohick WS, Clemmons DR. Regulation of insulin-like growth factor binding protein synthesis and secretion in a bovine epithelial cell line. Endocrinology 1991;129:1347–1354.
21. Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 1992;75:1046–1053.
22. Kiefer MC, Schmid C, Waldvogel M, Schlapfer I, Futo E, Masiarz FR, Green K, Barr PJ, Zapf J. Recombinant human insulin-like growth factor binding proteins 4, 5, and 6: biological and physiochemical characterization. Growth Regul 1993;3:56–59.
23. Rittmaster RS, Manning AP, Wright AS, Thomas LN, Whitefield S, Norman RW, Lazier CB, Rowden G. Evidence for atrophy and apoptosis in the ventral prostate of rats given the 5 alpha-reductase inhibitor finasteride. Endocrinology 1995;136:741–748.
24. Huynh H, Seyam RM, Brock GB. 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. Cancer Res 1998;58:215–218.
25. Nickerson T, Pollak M, Huynh H. Castration-induced apoptosis in the rat ventral prostate is associated with increased expression of genes encoding insulin-like growth factor binding proteins 2, 3, 4 and 5. Endocrinology 1998;139:807–810.
26. Iwashita M, Kudo Y, Takeda Y. Effect of follicle stimulating hormone and insulin-like growth factors on proteolysis of insulin-like growth factor binding protein-4 in human granulosa cells. Mol Hum Reprod 1998;4:401–405.
27. Tennant MK, Thrasher JB, Twomey PA, Birnbaum RS, Plymate SR. Insulin-like growth factor-binding protein-2 and -3 expression in benign human prostate epithelium, prostate intraepithelial neoplasia, and adenocarcinoma of the prostate. J Clin Endocrinol Metab 1996;81:411–420.
28. Tennant MK, Thrasher JB, Twomey PA, Birnbaum RS, Plymate SR. Insulin-like growth factor-binding proteins (IGFBP)-4, -5, and -6 in the benign and malignant human prostate: IGFBP-5 messenger ribonucleic acid localization differs from IGFBP-5 protein localization. J Clin Endocrinol Metab 1996;81:3783–3792.
29. Bourner MJ, Busby WH Jr, Siegel NR, Krivi GG, McCusker RH, Clemmons DR. Cloning and sequence determination of bovine insulin-like growth factor binding protein-2 (IGFBP-2): comparison of its structural and functional properties with IGFBP-1. J Cell Biochem 1992;48:215–226.
30. Rajah R, Valentinis B, Cohen P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism. J Biol Chem 1997;272:12181–12188.
31. Nickerson T, Huynh H, Pollak M. Insulin-like growth factor binding protein-3 induces apoptosis in MCF7 breast cancer cells. Biochem Biophys Res Commun 1997;237:690–693.
32. Guess HA, Heyse JF, Gormley GJ. Effect of finasteride on prostate-specific antigen in men with benign prostatic hyperplasia. Prostate 1993;22:31–37.