Co-administration of finasteride and the pure anti-oestrogen ICI 182,780 act synergistically in modulating the IGF system in rat prostate

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Abstract

Prostate cancer is the most diagnosed invasive malignancy in males. Androgens and oestrogens have been implicated in the pathogenesis of prostate cancer. We report herein that the pure anti-oestrogen ICI 182,780 (ICI) reduces Ki-67 labelling index and IGF-I receptor levels in rat prostate. Increase of IGF-I mRNA and IGF-binding protein 3 (IGFBP-3) accumulation occur without any effect on prostate weight. Finasteride significantly decreases prostate weight and inhibits IGF-I gene expression. IGFBP-3 mRNA, Akt and phospho-Akt are not affected by finasteride. Co-administration of ICI plus finasteride reduces prostate weight by approximately 50% and causes acinar dilation with decreased luminal epithelial

cell thickness. The acinar epithelial cells became atrophic and inactive with minimal cytoplasm. We also demonstrate a synergistic effect of ICI and finasteride on induction of IGFBP-3 accumulation and inhibition of Akt phosphorylation. Because the IGF and IGFBP-3 system plays an important role in prostate epithelial cell proliferation, apoptosis and tumour progression, the inhibitory effects of finasteride and ICI on IGF system may contribute to their anti-proliferative activity. These observations support a potential use of ICI in conjunction with finasteride in the prevention and/or treatment of prostate cancer.

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Introduction

The prostate gland requires androgens for growth, maintenance and function. Androgen-deprivation therapy causes marked and characteristic changes in both normal prostate and prostate cancer (Murphy *et al.* 1991, Ferguson *et al.* 1994). The 5 α -reductase enzyme is responsible for the conversion of testosterone to dihydrotestosterone (DHT) in androgen-dependent target cells (Gormley 1992, Aquilina *et al.* 1997). DHT has a greater affinity for the androgen receptor (AR) than testosterone, and it plays an important role in the regulation of prostatic growth. Finasteride acts as a competitive and specific inhibitor of 5 α -reductase, resulting in suppression of serum and intra-prostatic DHT concentration to castrated levels, with subsequent reduction in prostatic size (The Finasteride Study Group 1993, Rittmaster 1994, Gormley *et al.* 1992). Finasteride also inhibits AR expression (Wang *et al.* 1997). Inhibition of 5 α -reductase has been shown to inhibit the growth of prostate cancer both *in vivo* and *in vitro* (Bologna *et al.* 1992, Kelloff *et al.* 1992, Lamb *et al.* 1992,

Tsukamoto *et al.* 1995). An attractive feature of finasteride is its excellent safety profile (Gormley *et al.* 1992, Rittmaster 1994, Stoner 1994, Oesterling 1995, Boyle *et al.* 1996), making it a reasonable candidate for chemoprevention in high-risk target populations.

Oestrogen receptors are present in fibroblasts, basal epithelial and acinar epithelial cells (Droller 1997). Both oestrogens and DHT decrease the rate of cell death (Droller 1997). Oestrogens induce stromal fibroblasts to express epidermal growth factor receptor (EGF-R) and fibroblast growth factor receptor (FGF-R), increase AR levels and stimulate prostate basal epithelial cells to produce growth factors that act on the epithelial cells (Droller 1997). In dogs, oestrogens cause marked stimulation of prostate growth (Rhodes *et al.* 2000). Increased serum oestrogens promote prostate growth indirectly by increased growth factor production (Droller 1997).

Several epidemiological studies provided data suggesting that the incidence of prostate cancer is higher in men with higher circulating insulin-like growth factor-I (IGF-I) levels (Chan *et al.* 1998, Wolk *et al.* 1998). IGF-I has been

shown to be an anti-apoptotic agent and acts as a mitogen in the prostate gland. Systemic treatment with IGF-I for 7 days resulted in a 29% increase in the net weight of the ventral prostate (Torrington *et al.* 1997). Persistent expression of IGF-I in the basal epithelium of mouse prostate leads to neoplasia in this tissue (DiGiovanni *et al.* 2000). Suppression of IGF-I expression by finasteride was correlated with decrease in prostate weight (Huynh *et al.* 1998a). Blocking of IGF-II autocrine loop by vitamin D and its analogue EB1089 inhibits PC-3 prostate cancer cell proliferation (Huynh *et al.* 1998b). The role of IGFs in prostate growth is mediated in part by the interrelated components of the IGF system, which includes IGF receptors, IGF-binding protein (IGFBP) receptors and IGFBP proteases. The IGFBPs are a family of seven proteins that bind to IGFs with high affinity (Jones & Clemmons 1995, Oh *et al.* 1996), and control the distribution of IGFs. Normal prostate epithelial cells secrete IGFBP-2 and IGFBP-4, whereas the stromal fibroblasts produce IGFBP-2, 3 and 4 (Cohen *et al.* 1991, 1994a). Following castration, the expression of IGFBP-2, 3, 4 and 5 genes in the ventral prostate is rapidly induced (Nickerson *et al.* 1998). 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 its receptor, and hence interfering with the normal homeostatic intracellular signalling downstream of the receptor. It is also known that some IGFBPs have intrinsic bioactivity, which is independent of IGF (Oh *et al.* 1993, Huynh *et al.* 1996, Rajah *et al.* 1997). IGFBP-3 can have an inhibitory activity, probably through transforming growth factor- β (TGF- β) receptor type V (Leal *et al.* 1997). Changes in IGFBP-3 levels from benign to malignant disease has been reported (Thrasher *et al.* 1996). In the process of neoplastic progression, activation of autocrine loops is a common event. Neoplastic progression is associated with the expression of IGFBP proteases. In the tumour micro-environment, these would tend to increase IGF bioactivity by cleaving IGFBPs and liberating free IGFs. In humans, prostate-specific antigen (PSA) is a proteolytic enzyme that cleaves IGFBP-3 in extracellular fluid (Peehl *et al.* 1996), and an inverse correlation between PSA and IGFBP-3 levels in bone metastases has been reported (Smith *et al.* 1999). Epidemiological observations linking IGF-I to risk of prostate cancer provided potential implications of the IGF system as a target for prevention (Chan *et al.* 1998, Wolk *et al.* 1998).

In this study, we report that blockage of oestrogen action by a pure anti-oestrogen ICI 182,780 (ICI) (Wakeling *et al.* 1991) and reduction of DHT levels by finasteride lead to reduction of IGF-mediated autocrine/paracrine loops and reduction in prostate weight. This approach may offer a clinical utility for prostate cancer and/or benign prostatic hyperplasia.

Materials and Methods

Animals

Animal studies were performed in agreement with the guidelines of the local Animal Care. To block the conversion of testosterone to DHT, male Sprague-Dawley rats ($n=15$) (Charles River, Quebec, Canada) weighing 300–350 g were treated with 5 mg finasteride/kg body weight/day (5 mg/tablet, Merck Frost, Quebec, Canada) by gavage. The finasteride dose was based on our previous studies (Huynh *et al.* 1998a). Control animals ($n=15$) received the same volume of vehicles. To block oestrogen activity, ICI (Wakeling *et al.* 1991) (AstraZeneca Pharmaceuticals, Macclesfield, Cheshire, UK) was supplied at a concentration of 50 mg/ml in castor oil solution. Rats received castor oil ($n=15$) or either 1 mg ($n=15$) or 1.5 mg ($n=15$) ICI per kg body weight per week for 3 consecutive weeks. To investigate the combined effects of ICI and finasteride, rats (15 rats per group) were daily treated with 5 mg finasteride/kg body weight plus either 1 mg ICI or 1.5 mg ICI per kg body weight per week for 3 consecutive weeks. Animals were sacrificed 21 days following the initial treatment and the prostate tissue was collected and weighed. A portion of the prostate tissue was fixed in 10% buffered formalin for paraffin embedding and the remainder was immediately frozen in liquid nitrogen for extraction of RNA and protein.

Immunohistochemistry and histology

Fixed prostate tissue was routinely processed in a tissue processor and embedded in paraffin. Sections of 5 μ m were cut and stained with haematoxylin-eosin. Examination of the slides was performed by light microscopy. The Immunocruz Staining System was used for immunohistochemical study. Briefly, the slides were deparaffinized and rehydrated gradually through graded alcohols: washed in 100% ethanol twice for 10 min each, 95% ethanol twice for 10 min each, and 75% ethanol twice 5 min each. The slides were washed in deionized water for another 3 min and incubated with 3% H₂O₂ for 20 min to block endogenous peroxidase activity. To examine expression of Ki-67, antigens were retrieved by heating the slides in citrate buffer (pH 6.0) at 95 °C for 5 min. After pre-incubation with normal serum for 20 min at room temperature, the primary antibody was applied (2 μ g/ml) and incubated overnight at 4 °C. The section was then incubated with the appropriate biotinylated secondary antibody at 1:500 dilution followed by peroxidase-conjugated streptavidin complex, according to the manufacturer's instruction, and diaminobenzidine. The section was then counterstained with haematoxylin. Between each change of incubation the sections were rinsed three times in PBS for 5 min each. To evaluate the Ki-67 labelling index, 500 epithelial cells were counted for each group in randomly chosen fields at a magnification of 400. The Ki-67

labelling index was expressed as the number of clearly labelled Ki-67 reactive nuclei in 500 cells counted. Significance difference was determined by Student's *t*-test.

Western blotting

To determine the changes in the expression of IGF-IR, IGFBP-3, Akt, phosphatidylinositol-3-kinase (PI-3 kinase) p85 and phospho mitogen-activated protein kinase (MAPK) (Thr²⁰²/Tyr²⁰⁴), prostate tissue was homogenized in lysis buffer (1 mM CaCl₂, 1 mM MgCl₂, 1% NP-40, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 1 µM PMSF, and 100 µM NaVO₄). Proteins were subjected to Western blot analysis as described (Huynh *et al.* 1995). Blots were incubated with either rabbit anti-IGF-IRβ (1 µg/ml) (Santa-Cruz Biotechnology Inc., Santa Cruz, CA, USA), rabbit anti-IGFBP-3 (1:500) (Upstate Biotechnology, Lake Placid, NY, USA), mouse anti-phospho specific MAPK (Thr²⁰²/Tyr²⁰⁴) (1 µg/ml) (New England BioLabs, Beverly, MA, USA), rabbit anti-phospho-Akt (Ser⁴⁷³) (New England BioLabs) and mouse anti-α tubulin (0.5 µg/ml) (Santa-Cruz) antibodies and horseradish peroxidase-conjugated donkey anti-mouse or anti-rabbit secondary antibody (1:7500). Blots were visualized with a chemiluminescent detection system as described by the manufacturer (ECL, Amersham). Quantitative analysis of protein expression was accomplished by scanning autoradiograms and densitometry.

Northern blot

Total RNA was isolated from prostate tissue and Northern blotting was performed as described (Huynh *et al.* 1993). Blots were hybridized with IGF-I (Murphy *et al.* 1987), IGF-IR (ATCC), and IGFBP (1–5) (Shimasaki & Ling 1991) cDNAs. To control for equal RNA loading, blots were rehybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (ATCC). Quantitative analysis of gene expression was accomplished by scanning autoradiograms and densitometry. For each lane, the sum of the density of bands corresponding to transcripts hybridizing with the probe under study was calculated, and normalized the amount of RNA loaded.

Statistical analysis

Differences in prostate weight, Ki-67 labelling index, gene expression and the levels of proteins under study were analysed by the Mann–Whitney *U* test. *P* < 0.01 was considered significant.

Results

Effects of ICI and finasteride on the prostate weight

Treatment of rats with a pure anti-oestrogen, ICI, for 3 weeks had no effect on prostate weight. Daily treatment of

finasteride at a dose of 5 mg/kg body weight caused a 25% reduction in prostate weight (*P* < 0.01) (Fig. 1). Prostate weight was about 50% of controls when ICI was co-administered with finasteride (*P* < 0.01) (Fig. 1). When co-administration with 5 mg of finasteride, the dose of ICI required to yield a maximal reduction in prostate weight was 1 mg per kg body weight.

Effects of ICI and finasteride on the prostate morphology

The effects of ICI, finasteride and the combination on prostate gland morphology are shown in Fig. 2. Control prostate gland presents a cluster of epithelial tubules surrounded by connective tissue. The epithelial ducts had a large lumen, lined by tall cubical cells. The luminal cells were large and apparently active. Most of the nuclei in the finasteride-treated gland were arranged in a basal manner, with some nuclei arranged in an irregular fashion (Fig. 2B). There were no apparent differences in the luminal cell morphology between vehicle and finasteride-treated prostate glands (Fig. 2A vs 2B). Co-administration of ICI and finasteride resulted in marked histological changes of the prostate gland (Fig. 2D). The ICI/finasteride-induced pattern was characterized by an increased size of the lumen (data not shown). The epithelial layer lining the lumen was very thin. The epithelial cells were inactive, with diminished cytoplasm (Fig. 2D).

Effects of ICI and finasteride on the prostate epithelial cell proliferation

We examined the effect of hormonal regulation of Ki-67 expression in prostate tissue. Figure 3 shows the results of an experiment where prostate tissues were collected from rats treated with vehicle, finasteride, ICI, and ICI plus finasteride. Both finasteride and ICI caused an insignificant decrease in the number of epithelial cells expressing Ki-67 as compared with controls (*P* < 0.01). No significant reduction in the Ki-67 labelling index of the epithelium was seen when ICI and finasteride were co-administered as compared with the effect of either ICI or finasteride alone. A significant decrease in Ki-67 index was observed as compared with control gland when the two drugs were co-administered (*P* < 0.01).

Effects of ICI and finasteride on the IGF system

Since over-expression of IGF-I in the basal epithelium of prostate leads to spontaneous hyperplasia (DiGiovanni *et al.* 2000) and systemic injection of IGF-I increased wet weight of the ventral prostate (Torrington *et al.* 1997), the *in vivo* effects of ICI, finasteride and finasteride plus ICI on IGF-I, IGF-I receptor and IGFBP expression were examined. As shown in Fig. 4, daily treatment of male rats with 5 mg finasteride per kg body weight resulted in 30% decrease in IGF-I mRNA (*P* < 0.05). ICI, at a dose of

Figure 1 Effects of ICI, finasteride and ICI plus finasteride on rat prostate weight. Rats were treated with the indicated concentrations of finasteride ($n=15$), ICI ($n=15$) and ICI plus finasteride ($n=15$) for 3 weeks. C represents control rats treated with vehicle ($n=15$). Prostate tissues were collected, weighed individually and plotted. Bars with different letters are significantly different from one another at $P<0.01$ as determined by the Mann-Whitney U test. Data are expressed as the mean of 15 samples $\pm$ S.E.M.

1.5 mg, caused a 1.8-fold increase in IGF-I transcripts as compared with controls ($P<0.01$). The IGF-I gene expression was significantly inhibited when finasteride and ICI were co-administered ($P<0.01$). Neither ICI nor finasteride had significant effects on IGFBP-3 mRNA when administered as a single agent ($P<0.01$). However, when they were given together, the IGFBP-3 mRNA levels were significantly augmented ($P<0.01$) (Fig. 4B).

The IGF-I receptor protein was significantly decreased by ICI ($P<0.01$). One mg of ICI per kg body weight was sufficient to inhibit IGF-IR expression (Fig. 5). Finasteride increased IGF-IR levels non-significantly ($P<0.01$). When ICI was co-administered with finasteride, significant inhibition of IGF-IR expression was observed only at 1.5 mg ICI ($P<0.01$) (Fig. 5). Despite significant reduction in IGF-IR following ICI or combined ICI and finasteride, the levels of MAPK phosphorylation were not significantly affected ($P<0.01$) (Fig. 5).

Since IGF-I is mitogen for prostate cancer cells and IGFBP-3 modulates activity of IGF-I (Clemmons 1997), the effects of ICI, finasteride and finasteride plus ICI on IGFBP-3 levels were examined. Figure 6 shows that IGFBP-3 levels were significantly increased by either ICI or finasteride ($P<0.01$). ICI-induced IGFBP-3 synthesis was dose-dependent. Further significant increase in IGFBP-3 accumulation was observed in ICI plus finasteride treated prostate tissues ($P<0.01$).

Since PI-3 kinase activity is very important for IGF-I-induced mitogenesis and anti-apoptosis (Yenush & White 1997, White & Yenush 1998), the levels of PI-3 kinase p85 and its downstream target Akt were investigated. Figure 7 shows that levels of PI-3 kinase p85 and Akt did not change as a result of ICI+finasteride treatments. However, the basal phosphorylation of Akt was significantly reduced by ICI+finasteride treatment ($P<0.01$).

Discussion

Both oestrogens and androgens are essential for prostate growth. Androgen deprivation therapy is widely used for advanced disease and significant advances have come with luteinizing hormone-releasing hormone (LHRH) analogues and anti-androgens. We report herein that *in vivo* interruption of oestrogen action using a pure anti-oestrogen ICI for 3 weeks had no effect on prostate weight while blockade of the conversion of testosterone to DHT by finasteride reduced prostate weight by approximately 25%. The ability of finasteride to reduce prostate weight was enhanced by combined treatment.

Histological studies revealed that either finasteride or ICI when administered independently exerted insignificant reductions of the thickness of prostate luminal epithelial cells as compared with controls. The luminal

Figure 2 Prostate gland morphology in rats treated with vehicle (A), ICI 1-5 mg/kg body weight/week (B), finasteride (daily 5 mg/kg body weight) (C), or finasteride plus ICI (D). Note the basal nuclei seen in ICI-treated gland (B). Finasteride plus ICI caused acinar dilation. Luminal epithelial cells were atrophic and lost secretory activity. Haematoxylin–eosin stain was used; magnification $\times 1050$.

epithelial cells in ICI-treated gland became columnar and a single layer of regular basal nuclei was seen. This pattern is typical of terminally differentiated epithelial cells. The dilated glandular and luminal epithelial cell atrophy were observed when ICI and finasteride were given together. Although significant reduction in prostate weight following ICI+finasteride treatment was observed, DNA content per mg of prostate tissue was not significantly different among all treatments (data not shown), suggesting that the observed changes in prostate was mainly due to the decrease in cell volume.

In the present study we observed that both finasteride and anti-oestrogen ICI caused similar reduction in Ki-67 index. However, only finasteride significantly reduced prostate weight but not ICI. This observation could be due to a single point measurement (3 weeks post-treatment). It is possible that ICI as a single agent may require a longer period of time to exert significant effects on prostate weight. In addition to the changes in proliferation, the reduction in luminal epithelial cell volume observed in finasteride/ICI treatment is more likely responsible for the decrease in prostate weight.

Oestrogen receptors (ER- α and ER- β) are present in human and rat prostate (Beurden-Lamers *et al.* 1974, Jung-Testas *et al.* 1981). Localization of ER- β mRNA in

prostate epithelial cells, as opposed to stromal localization for ER- α (Prins *et al.* 1998), indicates that ER- β may activate a different set of genes than ER- α in response to oestrogenic stimulation. It is not known whether an ER- α or an ER- β -mediated pathway is involved in accounting for the observed effects of ICI on prostate weight seen in this present study. Although the role of the ER in the prostate is not well understood, it is a member of a superfamily of transcription factors that regulate gene expression in a variety of cells. Oestrogens are, besides androgens, implicated in the growth of the prostate (Griffiths *et al.* 1991), and consequently oestrogens have been implicated in the pathogenesis of benign prostatic hyperplasia (Habenicht *et al.* 1993). There is firm evidence that many biological processes of prostate epithelial tissue are controlled by androgens and oestrogens through the production of stimulatory or inhibitory factors by the stroma and the adjacent epithelial tissue (Cunha *et al.* 1987, Prins & Birch 1995). The observation that anti-oestrogen ICI can decrease Ki-67 labelling index suggests that *in vivo* oestrogens may induce prostate epithelial cell proliferation in an autocrine, intracrine or paracrine fashion.

Anti-oestrogens such as ICI compete with oestrogens for oestrogen receptors and evoke a different receptor

Figure 3 Effects of treatment with ICI, finasteride or ICI plus finasteride on proliferation of prostate epithelial cells. Rats were treated with the indicated dose of ICI, finasteride and finasteride plus ICI as described in Materials and Methods. Ki-67 labelling index of epithelial cells was determined as described in Materials and Methods. Bars with different letters are significantly different from one another at $P<0.01$ as determined by the Mann-Whitney U test. Data are expressed as the mean of eight samples $\pm$ S.E.M.

conformation that results in reduced or no production of oestrogen-dependent genes such as EGF, EGF-R and FGF-R (Droller 1997). Thus, blocking of oestrogen activity can also disrupt paracrine production of growth factors that act on the epithelial cells. The loss of autocrine stimulatory activity on the stromal cells or paracrine activity on the epithelium by co-administration of ICI plus finasteride may inhibit epithelial cell activity as indicated by the decrease in cytoplasmic volume of luminal epithelial cells, with subsequent reduction in the size of the prostate. Since IGF-I has been reported to be required for an increase in the size of prostate epithelial component (Torrington *et al.* 1997), the reduction in IGF-I and upregulation of IGFBP-3 levels by combined treatments would lead to reduction in the size of luminal epithelial cells. These observations suggest that the growth-promoting effects of oestrogens and androgens *in vivo* may be mediated, at least in part, by a local synthesis of growth factors such as IGF-I and IGFBP-3, based on the hypothesis that the structure and function of the epithelium of the adult prostatic gland is dependent on epithelial-stromal interactions (Cunha 1994).

Both prostate cancer and benign prostate stromal cells have IGF-I receptors, and stromal cells produce IGF-I in response to androgen (Cohen *et al.* 1994b). IGF-I could act via an autocrine stimulatory activity on the stroma cells, or paracrine activity on the epithelium. Inhibition of IGF-IR expression may lead to reduction in IGF-I stimulation, which in turn reduces epithelial cell proliferation. IGF-IR has also been shown to play an important role in apoptosis, metastasis of prostate cancer cells (Burfeind *et al.* 1996) and tumorigenesis (DiGiovanni *et al.* 2000); inhibition of IGF-IR by ICI and ICI plus finasteride observed in this

Figure 4 Effects of ICI, finasteride and finasteride plus ICI on the expression of IGF-I and IGFBPs in the prostate gland. Rats were treated with the indicated dose of ICI, finasteride and finasteride plus ICI as described in Materials and Methods. Total RNA derived from prostate gland was subjected to Northern blot analysis. Blots were hybridized with IGF-I, IGFBPs (2–5) and GAPDH cDNAs (A). Densitometric scanning of the IGF-I and IGFBP-3 bands is shown in (B). Data are expressed as the mean of eight samples $\pm$ S.E.M. Bars with different letters are significantly different from one another at $P<0.01$ as determined by the Mann-Whitney U test. Representative samples are shown. ADU, arbitrary densitometric units.

study is important because such treatments may suppress tumour growth and reduce or abolish tumour invasion (Burfeind *et al.* 1996). Inhibition of IGF-IR also disrupts the IGF-II autocrine loop, which is quite common in prostate cancer. It remains to be established whether a decrease in IGF-IR and IGF-I expression does occur in prostate tumours following ICI/finasteride treatment.

Our data suggest that in the prostate, IGF-IR expression is oestrogen-dependent while IGF-I expression is androgen-dependent. Induction of IGF-I gene expression following ICI treatment may provide, in part, an explanation for the failure of ICI to decrease prostate weight. Increase in levels of testosterone and decreased DHT

Figure 5 Effects of ICI, finasteride and ICI plus finasteride on IGF-I receptor and MAPK phosphorylation levels. Rats were treated with the indicated dose of ICI, finasteride and finasteride plus ICI as described in Materials and Methods. Proteins extracted from prostate glands were analysed by Western blot analysis as described in Materials and Methods. Blots were incubated with rabbit anti-IGF-I receptor β (IGF-IR β), mouse anti-phospho MAPK (Thr²⁰²/Tyr²⁰⁴) (Phospho MAPK p44/42) and anti-mouse α -tubulin (α -Tubulin) antibodies and horseradish peroxidase conjugated secondary antibodies as described in Materials and Methods (A). Blots were visualized with a chemiluminescent detection system. Densitometric scanning of the IGF-IR β and phospho MAPK bands is shown in (B). Data are expressed as the mean of eight samples $\pm$ S.E.M. Bars with different letters are significantly different from one another at $P < 0.01$ as determined by the Mann-Whitney U test. Representative samples are shown. ADU, arbitrary densitometric units.

following finasteride treatment has been reported (Huynh *et al.* 1998a). Thus, a slight decrease in prostate weight following finasteride administration may be due to conversion of testosterone to oestrogens, which in turn maintain AR and androgen activity.

The observation that ICI when co-administered with finasteride upregulates IGFBP-3 accumulation is significant given the mitogenic activity of IGF-I and anti-proliferative activity of IGFBP-3. The changes in IGF-I and IGFBP-3 following anti-oestrogen-androgen-based therapies was reported here to be similar to castration (Nickerson *et al.* 1998) and vitamin D analogue-induced apoptosis in rat prostate gland (Nickerson & Huynh 1999). Increase in IGFBP-3 would prevent the interaction of

Figure 6 Effects of ICI, finasteride and ICI plus finasteride on IGFBP-3. Rats were treated with the indicated dose of ICI, finasteride and finasteride plus ICI as described in Materials and Methods. Proteins extracted from prostate glands were analysed by Western blot analysis as described in Materials and Methods. Blots were incubated with rabbit anti-IGFBP-3 (IGFBP-3), and anti-mouse α -tubulin (α -Tubulin) antibodies and horseradish peroxidase conjugated secondary antibodies (A). Blots were visualized with a chemiluminescent detection system. Densitometric scanning of the IGFBP-3 bands is shown in (B). Data are expressed as the mean of eight samples $\pm$ S.E.M. Bars with different letters are significantly different from one another at $P < 0.01$ as determined by the Mann-Whitney U test. Representative samples are shown. ADU, arbitrary densitometric units.

IGF-I with its receptor and decrease mitogenic activity. Furthermore, IGFBP-3 may inhibit prostate epithelial cells through TGF- β receptor type V (Leal *et al.* 1997). At the present time, it is not known whether upregulation of IGFBP-3 by the combination also induced apoptosis in luminal epithelial cells. Experiments are under way to determine this possibility.

It has been reported that IGF-I can rescue cancer cells from doxorubicin-induced apoptosis and the process requires PI-3 kinase (Gooch *et al.* 1999). Recent work into IGF-I anti-apoptosis signalling has demonstrated the importance of PI-3 kinase and its downstream substrate Akt (Kulik *et al.* 1997, Franke *et al.* 1995). Furthermore, a

Figure 7 Effects of ICI, finasteride and finasteride plus ICI on PI-3 kinase p85, Akt and phospho-Akt in rat prostate gland. Rats were treated with the indicated dose of ICI, finasteride and finasteride plus ICI as described in Materials and Methods. Proteins extracted from prostate glands were analysed by Western blot analysis as described in Materials and Methods. Blots were incubated with anti- α -tubulin (A) and anti-PI-3 kinase p85 (B), anti-Akt (C) and phospho-Akt (Ser⁴⁷³) (D) antibodies. Densitometric scanning of the IGFBP-3 bands is shown in (E). Data are expressed as the mean of eight samples $\pm$ S.E.M. Bars with different letters are significantly different from one another at $P < 0.01$ as determined by the Mann-Whitney U test. ADU, arbitrary densitometric units.

direct link between PI-3 kinase and the apoptosis-regulating protein Bcl family of proteins has been established through Akt phosphorylation of BAD (Datta *et al.* 1997, Zha *et al.* 1996). Thus the ability of finasteride/ICI combination to reduce Akt phosphorylation may be important for finasteride/ICI-induced apoptosis. Experiments are under way to determine whether ICI + finasteride combination also induces prostate epithelial cell apoptosis *in vivo*.

Combined treatments have been shown to improve survival (Labrie *et al.* 1983) and disease-free survival (Crawford *et al.* 1989) in prostate cancer patients as compared with monotherapy. Several other studies have confirmed the benefit of adding early adrenal androgen blockage to medical or surgical castration (Crawford 1990, Denis *et al.* 1993, Janknegt *et al.* 1993). Other studies have failed to show survival advantages at early analysis

(Lunglmayr 1990, Beland *et al.* 1991). Our present study shows that ICI acts synergistically with finasteride to reduce prostate weight and IGF bioavailability. These observations may provide insight into fundamental mechanisms by which oestrogens influence normal prostate growth, androgen-independent growth and metastasis of prostate cancer cells. They provide a novel combined therapy for benign prostatic hyperplasia and prostate cancer.

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