BICALUTAMIDE (CASODEX)-INDUCED PROSTATE REGRESSION INVOLVES INCREASED EXPRESSON OF GENES ENCODING INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS

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ABSTRACT

Objectives. To examine the effects of bicalutamide (Casodex), a pure antiandrogen with high specificity for the androgen receptor, on insulin-like growth factor binding protein (IGFBP) expression and apoptotic regression of the rat ventral prostate.

Methods. Rats were treated daily with 10 mg/kg body weight bicalutamide or vehicle alone. Ventral prostates were collected at various days of treatment. Northern blot analysis was performed to quantitate expression of genes encoding IGFBPs, and the TUNEL method was used to determine the extent of apoptosis in ventral prostate.

Results. In rats treated daily with bicalutamide, increases in mRNA levels of IGFBP-2, -3, -4, and -5 were detectable by Northern blotting by 6 hours and reached 6 to 10-fold of control levels after 5 days of treatment. The time-course of induction of apoptosis in the ventral prostate by bicalutamide, as detected in situ by the TUNEL method, corresponded to the time-course of induction of IGFBP expression.

Conclusions. We demonstrate that apoptotic regression of the ventral prostate during bicalutamide treatment is accompanied by increased expression of IGFBP-2, -3, -4, and -5. Rapid induction of IGFBPs, which can limit access of insulin-like growth factors (IGFs) to the IGF-I receptor, may play a role in the induction of apoptosis by antiandrogens, particularly in view of the increasing evidence that IGF-I inhibits apoptosis. These results document a previously unrecognized effect of antiandrogens and extend our previous studies relating IGF physiology to prostate biology. Together with evidence that a strong positive correlation exists between plasma IGF-I levels and prostate cancer risk, our data suggest that IGF physiology may play a key role in prostate cancer biology and is strongly influenced by androgen-targeting therapies.

Prostate cancer is the most common malignancy among men in North America, and approximately one in every nine will be diagnosed with prostate cancer during his lifetime.1 The standard therapy for prostate cancer involves elimination of androgens by orchiectomy or medical castration with luteinizing hormone-releasing hormone (LHRH) agonists. Although these methods dramatically lower serum testosterone concentrations, the intraprostatic concentration of dihydrotestosterone is only reduced by 50% to 60%.2 The use of antiandrogens, which block the interaction of testosterone and dihydrotestosterone with the androgen receptor, is the most effective method of interfering with the action of androgens produced locally in the prostate.3

Bicalutamide (Casodex, AstraZeneca, Macclesfield, England) is an antiandrogen with high specificity for the androgen receptor but without additional hormonal or antihormonal activity.4 Although there is some controversy surrounding the effectiveness of bicalutamide as a monotherapy or in combination therapy with LHRH agonists,5-10 bicalutamide may be beneficial in patients after failure of another antiandrogen. Administration of

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150 mg bicalutamide daily to treat an increasing prostate-specific antigen (PSA) level after a flutamide withdrawal response produced another PSA decrease of more than 50%.1,2

Evidence is increasing that the effects of androgens on growth and maintenance of the prostate are mediated by a number of growth factors.12 Insulin-like growth factor I (IGF-I) is required for the survival of human prostate epithelial cells in vitro and plays an essential role in normal prostate physiology.13 A recent prospective study revealed a strong positive correlation between serum IGF-I levels and prostate cancer risk.14 The well-recognized mitogenic and antiapoptotic effects of IGF-I are mediated through binding to the IGF-I receptor.15 IGF activity is modulated by members of the proposed IGF binding protein (IGFBP) superfamily, which includes six IGFBPs (IGFBPs 1 to 6) that bind IGFs with high affinity and several other IGFBP-related proteins.16 Observations that IGFBP-3 inhibits IGF-induced proliferation of normal prostate epithelial cells17 and that serum levels of certain IGFBPs are changed in patients with prostate cancer18 suggest that IGFBPs19 are important modulators of IGF activity in the human prostate.

IGFBPs have been demonstrated to regulate apoptosis in vitro20,21 and are associated with apoptosis during castration-induced regression of the rat ventral prostate.22 We undertook these experiments to test whether regression of the ventral prostate by the antiandrogen bicalutamide involves changes in local IGF physiology.

MATERIAL AND METHODS

ANIMALS

All animal studies were conducted in accordance with the local committee for humane animal care standards. Bicalutamide was dissolved in 100% ethanol at a concentration of 25 mg/mL. Male Sprague-Dawley rats weighing 400 g (Charles River, Quebec, Canada) received daily oral doses of bicalutamide (10 mg/kg body weight in 0.5% Tween-80 phosphate-buffered saline) by gavage for up to 7 days. Control rats were given 0.5% Tween-80 phosphate-buffered saline containing 16% ethanol. Animals were killed 6 hours after treatment and then every 24 hours for 7 days. The ventral prostate was collected. Part of the tissue was fixed in 10% buffered formalin, embedded in paraffin, and cut into 5-μm sections. The remaining tissue was immediately frozen in liquid nitrogen for RNA extraction.

NORTHERN BLOT ANALYSIS

Total RNA was isolated from tissue using the RNeasy kit (Qiagen). Total RNA (50 μg) was fractionated on 1% agarose gels and transferred onto Zeta-Probe membrane (Bio-Rad) in 50 mM NaOH. The cDNAs for rat TRPM-223 and IGFBPs 2, 3, 4, and 524 were labeled with [α-32P]dCTP using T7 Quick-Prime kit (Pharmacia). Prehybridization, hybridization, and washing were performed as previously described.22 Blots were subjected to autoradiography with intensifying screen at −80°C. Quantitative analysis of gene expression was accomplished by densitometric scanning of autoradiograms, and results were corrected for minor loading differences by normalizing to 28S rRNA.
FIGURE 2. Apoptosis in the rat ventral prostate during bicalutamide treatment. The TUNEL method was used to detect ventral prostate apoptosis in situ. The sections were photographed at a magnification of ×320 under a fluorescence microscope. The nuclei of apoptotic cells contain fluorescein-labeled DNA and appear bright. Results from a representative assay, including ventral prostate tissue from rats treated with (A) vehicle alone or 10 mg/kg bicalutamide daily for (B) 6, (C) 48, and (D) 96 hours are shown.

QUANTITATION OF APOPTOSIS

Apoptosis was detected in formalin-fixed, paraffin-embedded tissue sections using the ApoAlert DNA fragmentation assay (Clontech), which is based on the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method. The 5-μm sections were prepared and treated according to the manufacturer's instructions. Apoptosis was visualized and photographed under a fluorescent microscope equipped with an FITC filter. Labeling indexes were obtained from epithelial cells in the distal ducts by counting the number of labeled cells among at least 100 epithelial cells per region. Three regions per tissue section were counted, and this was repeated on three independent sections. The number of labeled and unlabeled epithelial cells in all regions were taken together and expressed as percentage values.

RESULTS

Rats were treated with a single dose of bicalutamide 10 mg/kg body weight, which is known to cause a reduction in prostate weight within 2 weeks. Animals were killed at various points beginning 6 hours after treatment to detect early changes in gene expression. Northern blot analysis was used to confirm the expression of TRPM-2/clusterin, an androgen-repressed gene known to be associated with involution of the prostate. TRPM-2 mRNA levels were low in control rats receiving vehicle only and increased fivefold by 6 hours of bicalutamide treatment (Fig. 1). TRPM-2 gene expression increased 16-fold by 48 hours and reached 25-fold by 120 hours in the prostate of bicalutamide-treated rats (Fig. 1).

TUNEL assays were performed on ventral prostate sections to determine the extent of bicalutamide-induced apoptosis. We examined the secretory luminal cells in the distal region, as these are the most sensitive to androgen ablation. In the prostates of control rats, the rate of apoptosis was very low (Fig. 2A), in keeping with prior reports that apoptosis occurs in approximately 2% of cells in the rat ventral prostate and is balanced by cell proliferation. After 6 hours of bicalutamide treatment, approximately 45% of prostate epithelial cells were apoptotic and fluoresced with an intensity related to their degree of DNA fragmentation (Fig. 2B). By 48 hours of bicalutamide treatment, approximately 65% of cells were apoptotic (Fig. 2C), and the number of apoptotic cells in the ventral prostate reached approximately 85% by 96 hours of treatment (Fig. 2D).

Northern blot analysis was used to determine whether bicalutamide-induced prostate regression involves changes in IGFBP gene expression. Administration of bicalutamide resulted in time-dependent increases in expression of IGFBP-2, -3, -4, and -5 in the ventral prostate. IGFBP-2 mRNA levels increased threefold by 6 hours and reached approximately 15-fold by 120 hours in bicalutamide-
treated rats (Fig. 3A). IGFBP-3 mRNA was expressed at a very low level in control animals and increased twofold and 10-fold at 6 hours and 120 hours, respectively, of bicalutamide treatment (Fig. 3A). IGFBP-4 and IGFBP-5 mRNAs were both expressed in the normal prostate and increased sixfold and fivefold, respectively, by 6 hours, and 15-fold and 10-fold, respectively, by 120 hours of treatment (Fig. 3B). All differences were statistically significant (P < 0.05) as determined by the Mann-Whitney U test. Only a slight accumulation of bicalutamide was observed in rats dosed daily with 10 mg/kg bicalutamide; therefore, the observed increases in IGFBP gene expression by 120 hours of treatment primarily resulted from the period of treatment, with only small contributions from pharmacokinetic accumulation.

**COMMENT**

It has been recognized for some time that IGFs have a central role in regulating proliferation and apoptosis of prostate epithelial cells. However, more attention has been given recently to the apoptotic effects of IGFBPs. IGFBP-5 expression increases during postlactational involution of the rat mammary gland. We and others have shown that IGFBP-3 induces apoptosis in vitro in MCF7 breast cancer cells and PC-3 prostate cancer cells. In keeping with the hypothesis that IGFBPs trigger apoptosis in the prostate after androgen ablation, we have found that castration-induced apoptosis in the rat ventral prostate involves increased expression of IGFBPs. It has also been demonstrated that reduction of ventral prostate weight by finasteride is associated with increased IGFBP-3 mRNA levels. Our observation that IGFBP gene expression increases during bicalutamide-induced prostate regression is consistent with these previous findings. Taken together, these results extend our previous studies relating IGF physiology to the risk of prostate cancer by demonstrating the effects of androgen-targeting therapies on local IGF physiology in prostate tissue.

Prostatic stromal cells produce IGF-I mRNA but do not express IGF-I receptors. Prostate epithelial cells on the other hand, have IGF-1 receptors that are activated by IGF-1 from the stromal cells in a paracrine manner. Expression of mRNAs for IGFBP-2, 3, and 4 is localized to epithelial cells in human prostate, and IGFBP-5 mRNA is expressed in both stromal and epithelial cells. Immunostaining of IGFBP-2, -3, -4, and -5 in human prostate is localized to epithelium. Within 6 hours of bicalutamide treatment, we observed apoptotic cell death of ventral prostate epithelial cells, as well as increased gene expression of IGFBPs. We hypothesize that rapid induction of IGFBPs may sequester IGF-1, thereby preventing activation of the
IGF-I receptor and promoting apoptosis. Although the individual IGFBP mRNA levels were increased from between two- to fivefold by 6 hours, the magnitude of increase in total IGF binding capacity is potentially much greater. At present, a lack of suitable antibodies against rat IGFBPs prevents us from examining the consequence of increased IGFBP mRNA on prostate IGFBP protein content. Thomas et al. have demonstrated upregulation of IGFBP-5 protein in the rat ventral prostate during regression, although in their study an antihuman IGFBP-5 antibody was used to detect rat IGFBP-5. We have not been able to replicate these results using commercially available antibodies. Our previous findings on IGFBP-3-induced apoptosis in vitro were consistent with the hypothesis that IGFBPs regulate apoptosis by modulating IGF bioavailability. We cannot exclude the possibility that IGFBPs regulate apoptosis directly, as there is growing evidence that IGFBP-3 has IGF-independent effects on cell growth and survival.

To establish that IGFBP induction is necessary for bicalutamide-induced apoptosis, an experimental strategy based on IGFBP knockout mice would be necessary. However, given the redundancy we have observed with induction of multiple IGFBPs, it would be necessary to disrupt multiple IGFBP genes simultaneously.

CONCLUSIONS

In this in vitro study we demonstrated that apoptotic regression of the ventral prostate during bicalutamide treatment is associated with increased expression of IGFBP-2, -3, -4, and -5. Bicalutamide is a pure antiandrogen in widespread use clinically. We presented evidence that apoptosis in the prostate induced by bicalutamide is correlated with changes in IGF physiology. We are currently examining the impact of antiandrogens on IGF physiology in in vivo prostate tumor models that are responsive or nonresponsive to androgen deprivation.

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