Histochemistry of Steroid Receptors in Prostatic Diseases*

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ABSTRACT

Tissue obtained from 55 men with prostatic disease was assayed for estrogen and androgen receptors by a newly developed histochemical technique. The material studied consisted of 45 specimens of benign nodular prostatic hyperplasia and 10 specimens of prostatic adenocarcinoma. The results obtained were compared to those of parallel biochemical assays in 17 cases and successfully correlated in 85 percent. The new procedure is rapid, inexpensive and accurate, allowing for the detection of receptor in cytoplasm and/or nucleus and evaluation of receptor heterogeneity. The histochemical method may offer an alternate to biochemical assay of prostatic tissue as contamination with steroid binding globulins does not appear to be a problem at this time.

It is well known that the majority of men with advanced prostatic carcinoma respond favorably to palliative ablative or additive endocrine therapy.7-11,15,16,17 Although it has been clearly shown that high affinity, low capacity tissue-specific estrogen receptors (ER) and androgen receptors (AR) are present in the human prostate,4,5,8,18 orchiectomy or other endocrine therapy is seldom based on knowledge of the steroid receptors present, and such treatment is administered on an empirical basis.

Many different biochemical assay techniques have been used to measure human prostatic ER and AR.4,6,10,14 However, in addition to the expense and sophisticated equipment necessary, biochemical methods are often complicated by difficulty in preparing cytosol from specimens with an abundance of fibro-
muscular stroma as well as by contamination of the cytosol with testosterone-estrogen binding globulin (TeBG). A new method is reported here for the histochemical detection of ER and AR in human prostatic tissue which is unaffected by the amount of stroma present and apparently by contamination with TeBG.

Materials and Methods

Surgically resected prostatic tissue in excess of that needed for adequate histologic diagnosis was frozen in liquid nitrogen and stored at either −70°C or in liquid nitrogen.

Conjugates of bovine serum albumin (BSA), fluorescein isothiocyanate (FITC) and β-estradiol-17-hemisuccinate, and of BSA, FITC and β-testosterone-17-hemisuccinate were prepared. The estradiol (E₂) conjugate contained four moles of E₂ and five moles of FITC per mole of BSA. The testosterone (T) conjugate contained nine moles of T and five moles of FITC per mole of BSA. Each was diluted with phosphate buffered saline (PBS) containing 10 percent ethanol and applied to four micron thick tissue sections in a concentration range of 47 to 94 pmoles per section. Incubations were for two hours in a humidifying chamber. Sections were then rinsed with PBS, fixed in acetone-ethanol for 10 min and triple washed in PBS for 30 min. In vivo endogenous bound E₂ and T were identified by indirect immunofluorescence techniques employing monospecific antiserum.*

Control sections were exposed to FITC-BSA unlinked to steroid. Concurrent competitive binding studies were performed with FITC-BSA-E₂ plus 50 to 500 fold excess unlabeled E₂, diethylstilbestrol (DES) or nitromifene citrate† as described.‡ Competitive binding studies for AR consisted of tissue sections exposed to FITC-BSA-T plus 50 to 500 fold excess unlabeled T, the antiandrogen cyproterone acetate, and the antiandrogen 6a,7a-difluoromethylene - 1a,2a-methylene-20-spirox-4-ene-one.§

In conjunction with serial tissue sections stained with hematoxylin and eosin, each specimen was assessed for the ratio of epithelium to stroma, the presence of ER and/or AR positive cells in both epithelium and stroma, the relative percentages of each, the degree of staining intensity and the location of fluorescence in cytoplasm and/or nucleus. Specimens were interpreted as positive for ER/AR when 10 percent or more of the epithelial cells exhibited fluorescence after exposure to FITC-BSA-E₂ per T, but not after incubation with FITC-BSA, provided that there was a significant reduction of fluorescent intensity after coincubation with the respective competitors.

Biochemical assays for ER were performed by the dextran-coated charcoal assay (DCC).§ Protamine sulfate and DCC assays were used to measure AR.¶ Nuclear ER was not assayed. The lowest limit of positivity on biochemical assay was arbitrarily set at 7 fmoles per mg of deoxyribonucleic acid (DNA).

Results

After processing by the histochemical technique and upon study by incident light ultraviolet (UV) microscopy, ER and AR-positive cells were frequently detected within the glandular epithelium in prostatic hyperplasia and occasionally within the stroma. The distribution of receptor was relatively uniform throughout the specimen, but on occasion, negative and positive glands were seen side by side. ER and AR were visible in both cytoplasm and nucleus. However, one or the other

* Calbiochem-Behring, Somerville, NJ.
† CI-628, Warner-Lambert/Parke-Davis, Ann Arbor, MI.
‡ MK-316, Merck, Sharp & Dohme, Rahway, NJ.
form tended to predominate (figures 1 and 2).

In contrast to the uniform distribution of steroid receptor in hyperplasia, in prostate carcinoma there was often marked tumor cell receptor heterogeneity. Heterogeneity of receptor was evidenced by intermingling of negative with positively stained cells and by fluctuations in fluorescent intensity. The distribution of receptor in cytoplasm or nucleus was again noted to be variable with one form often predominating.

Little or no staining was seen in sections exposed to FITC-BSA. Coincubation of FITC-BSA-E₂ with excess unlabeled E₂, DES and CI-628 produced a significant diminution of staining in ER-positive cells. Similarly, coincubation with FITC-BSA-T with excess unlabeled T, cyproterone acetate and MK-316 resulted in a significant reduction of fluorescence in AR-positive cells. Excess unlabeled E₂, DES and CI-628 did not alter staining with FITC-BSA-T, nor did coincubation with excess unlabeled T, cyproterone acetate and MK-316 affect fluorescent intensity with FITC-BSA-E₂.

The results of ER/AR assay by the histochemical technique are summarized in table I. In prostatic hyperplasia, 35 specimens were both ER/AR-positive, two were ER-positive, AR-negative, four were ER-negative and AR-positive while the remaining four were ER/AR-negative. Seven of the prostatic carcinomas were both ER/AR-positive, one was ER-positive only and two were ER/AR-negative.

The location of receptor as determined histochemically in the group of carcinomas studied is shown in table II. In seven specimens, ER was predominantly nuclear and in one, primarily cytoplasmic. Nuclear AR predominated in four tumors, cytoplasmic AR predominated in one while the remaining two exhibited a mixed nuclear and cytoplasmic AR staining pattern.

Endogenous E₂ and/or T was detected in only three specimens. The effect of in vivo bound steroids upon the results of assay has yet to be determined in prostatic tissue.

Biochemical assay results were available for comparison in 17 specimens (table III). There was complete correlation in 14 cases and partial correlation in another.

**Figure 1.** Prostatic hyperplasia. Glandular epithelium showing predominantly cytoplasmic ER. Fluorescence microscopy. Original magnification (× 500).

**Figure 2.** Nuclear AR in prostatic hyperplasia. Fluorescence microscopy. Original magnification (× 100).
specimen. Biochemical assay was not possible in eight cases because of insufficient solubilized cytosol protein.

Discussion

There have been recent improvements in biochemical assay techniques for AR using the synthetic androgen methyltrienolone (R1881) which binds to AR with high affinity but not to TeBG. However, R1881 also interacts with a progesterone receptor-like component of human prostatic cytosol and therefore requires co-incubation with an excess of the synthetic progestin R5020 or with triamcinolone acetonide to distinguish androgen from progesterone receptor.

All currently available biochemical assay methods for human prostatic steroid receptor proteins are expensive, require preparation of cytosol and employ radio-labeled chemicals and sophisticated monitoring equipment. Consequently, their performance is limited to a few centers and there is little available data concerning the responsiveness of receptor-positive and receptor-negative prostatic lesions to various therapeutic modalities.

The availability of an inexpensive histochemical technique for detection of ER and AR, capable of performance and interpretation at the community hospital level, should lead to full scale clinical trials whereby the absence or presence of steroid receptor proteins could be correlated with response to different treatment regimens. Knowledge of the level of ER/AR present in an individual case would allow the clinician to select the most rational therapeutic approach as is now possible in the treatment of women with breast cancer. Men with receptor-negative tumors might best be started on non-hormonal therapy. Castration, with its psychological and physiological impact, or estrogens with their possible adverse effects on the cardiovascular system and associated thromboembolic phenomena, could then be reserved for those cases where therapeutic benefits might be reasonably expected to outweigh complications.

Transurethral prostatic resection (TURP) employing electrocautery is frequently the treatment of choice in the surgical management of patients with prostatic disease. Unfortunately, AR is heat-labile, and specimens secured by TURP are unsuitable for receptor assay by any method if the tissue to be studied is severely burned. We have begun to assay needle biopsy specimens obtained immediately prior to commencement of TURP
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and although our experience is limited at this time, such an approach is perfectly feasible. Procurement of several needle biopsies may help obviate problems of receptor heterogeneity within a given lesion.

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References


